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NDA-020638

FIRM: GILEAD

1 OF 4

TRADE NAME: VISTIDE (CIDDOFOVIR)

GENERIC NAME: CIDDOFOVIR

Summary Basis of Approval  
Cover Form

Appl #: 020638

Firm: GILEAD  
Reviewing Div: 530  
Trade Name: VISTIDE (CIDOFOVIR)  
Generic Name:

CIDOFOVIR

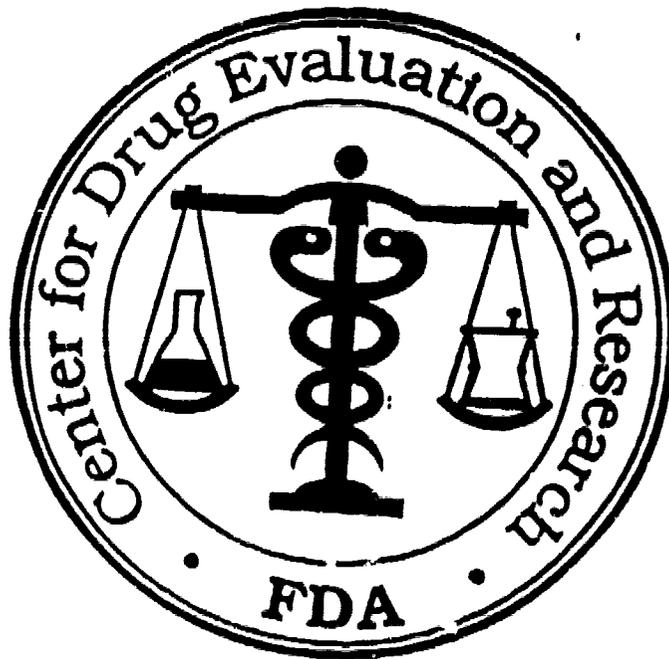
Approval Letter: Y	Statistician Review: Y
SBA Form: N	Bio/Dissolution Review: Y
Final Printed Labeling: Y	Microbiologist Review: N
Medical Officer Review: Y	NAS/NRC Review: N
Chemist Review: Y	Pharmacologist Review: Y
Federal Register Notice: N	Completion Date: 09-APR-97

20638

Approval Letter  
And Related  
Correspondence

**NDA 20-638**

**VISTIDE (CIDOFOVIR) INJECTION 75 MG/ML  
FOR THE TREATMENT OF CMV RETINITIS IN  
PATIENTS WITH AIDS**



**Regulatory Management Officer: Kimberly A. Struble, R.Ph., (301) 827-2335**

**Medical Officer: Douglas Pratt, M.D., M.P.H., (301) 827-2331**

**vol 1**

JUN 26 1996

NDA 20-638

Gilead Sciences, Inc.  
Attention: Gene D. Mason, Pharm.D.  
353 Lakeside Drive  
Foster City, CA 94404

Dear Dr. Mason:

Please refer to your October 4, 1995, new drug application submitted under 505(b) of the Federal Food, Drug, and Cosmetic Act for VISTIDE (cidofovir) injection 75 mg/ml.

We acknowledge receipt of your amendments dated:

October 17, 1995	February 12, 1996	March 28, 1996
October 31, 1995	February 15, 1996	April 2, 1996
November 10, 1995	February 16, 1996	April 3, 1996
November 27, 1995	February 20, 1996	April 4, 1996
December 6, 1995	February 23, 1996	April 6, 1996
December 13, 1995	February 26, 1996	April 8, 1996
January 5, 1996	February 27, 1996	April 9, 1996
January 9, 1996	March 4, 1996	April 11, 1996
January 16, 1996	March 8, 1996	April 12, 1996
January 17, 1996	March 13, 1996	April 16, 1996
January 24, 1996	March 20, 1996	May 7, 1996
February 7, 1996	March 21, 1996	May 10, 1996
February 8, 1996	March 25, 1996	May 20, 1996
February 9, 1996	March 27, 1996	June 10, 1996

This new drug application provides for the use of VISTIDE for the treatment of cytomegalovirus retinitis in patients with acquired immunodeficiency syndrome.

We have completed the review of this application including the submitted draft labeling and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the final printed labeling (FPL) submitted on April 9, 1996. Accordingly, this application is approved effective on the date of this letter.

Please refer to the June 26, 1996, teleconference during which you agreed to submit revised FPL that is identical to the April 9, 1996 labeling and incorporating the changes agreed upon during this teleconference. Please note your agreement

that the revised FPL would be used in all advertising and promotional materials.

Please submit twenty copies of the revised FPL as soon as it is available, in no case more than 30 days after it is printed. Please individually mount ten of the copies on heavy weight paper or similar material. For administrative purposes this section should be designated "FINAL PRINTED LABELING" for approved supplemental NDA 20-828. Approval of this FPL is not required before it is used. Should additional information relating to the safety and effectiveness of this drug become available, further revision of that labeling may be required.

We acknowledge your commitment to conduct phase 4 studies as stated in your March 25, 1996, letter.

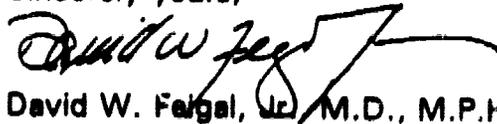
Validation of the regulatory methods has not been completed. At the present time, it is the policy of the Center not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any deficiencies that may occur.

Please submit one market package of the drug when it is available.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any further questions please contact Kimberly Struble, R.Ph., Regulatory Management Officer, at 301-827-2335.

Sincerely yours,



6.26.96

David W. Fergal, Jr. M.D., M.P.H.  
Director

Division of Antiviral Drug Products  
Office of Drug Evaluation IV  
Center for Drug Evaluation and Research

GILEAD  
SCIENTES

June 26, 1996

Via FedEx  
301/827-2335

Food and Drug Administration, CDER, ODE IV  
Division of Antiviral Drug Products (HFD-530)  
Attention: *Kimberly Struble, CSO*  
9201 Corporate Blvd., First Floor Document Room  
Rockville, MD 20850

Subject: **NDA 20-638, Vistide® (cidofovir injection), Amendment #062**  
• **General Correspondence - Letter of Understanding**

Reference is made to pending NDA 20-638 dated 09/29/95 for Vistide (cidofovir injection) and the June 26, 1996 teleconference call between representatives of Gilead Sciences and the Division of Antiviral Drug Products (Drs. S. Gitterman and D. Pratt and Ms. Struble).

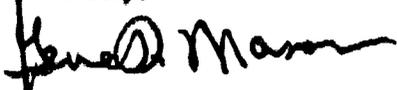
This is our letter of understanding regarding revisions to the Vistide Package Insert for incorporation into promotional materials and commercial product. The following language will be incorporated into the package insert:

**Malignancies or serious adverse reactions that occur in patients who have received VISTIDE should be reported to Gilead in writing to the Director of Clinical Research, Gilead Sciences, Inc., 353 Lakeside Drive, Foster City, CA 94404 or by calling 1-800-GILEAD-5 (445-3235), or to FDA MedWatch 1-800-FDA-1088/fax 1-800-FDA-0178.**

The revised final printed labeling will be completed by August 1, 1996 incorporating the language above. The revised final printed labeling will be used with distribution of Vistide thereafter.

If you have any questions or need further information please feel free to contact me at 415/573-4861 or Dr. Howard Jaffe, VP, Clinical Affairs, at 415/573-4702.

Sincerely,



Gene D. Mason, Pharm.D.  
Director of Regulatory Affairs

CC: FDA - archive and 3 review copies  
Gilead (cover) - M. Hitchcock, H. Jaffe, W. Lee, J. Martin, M. Perry, L. Staiger

# GILEAD SCIENTES

June 27, 1996

Food and Drug Administration  
Center for Drug Evaluation and Research, ODE IV  
Division of Antiviral Drug Products (HFD-530)  
9201 Corporate Blvd., First Floor Document Room  
Rockville, MD 20850

Attention: Kimberly Struble, CSO

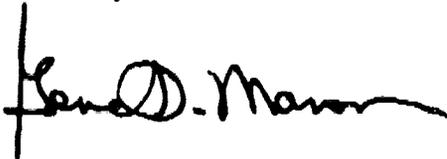
Subject: **NDA 20-638, Vistide® (cidofovir injection)**  
**•General Correspondence - Letter of Understanding**

Reference is made to NDA 20-638 approved June 26, 1996 and the June 27, 1996 teleconference calls between representative of Gilead Sciences and Dr. S. Gitterman and Dr. C.W. Chen of the Division of Antiviral Drug Products.

During the calls, we discussed the need to revise the package insert for Vistide to remove the NDC number for the 6-vial shelf-pack carton (previously assigned NDC 61958-0101-2). Because this presentation represents only an intermediate package containing 6 cartons of the identical product as NDC 61958-0101-1, it should not have a separate NDC number. Drs. Chen and Gitterman confirmed that it was acceptable to revise the package insert accordingly. This change will be incorporated into final printed labeling and submitted to the NDA per the June 26, 1996 (Amendment #062) agreement.

Should you have any questions, please contact me at 415/573-4861, or William (Bill) Lee, Ph.D., V.P. of Pharmaceutical Product Development, at 415/573-4716.

Sincerely,



Gene D. Mason, Pharm.D.  
Director, Regulatory Affairs

cc. FDA - via facsimile  
Gilead - H. Jaffe, W. Lee, J. Martin, M. Perry, L. Staiger, J. Steele

EXCLUSIVITY SUMMARY FOR NDA # 20-636 SUPPL # 000

Trade Name VISTIDE Generic Name cidofovir

Applicant Name Gilead Sciences, Inc. HFD # 530

Approval Date If Known \_\_\_\_\_

**PART I IS AN EXCLUSIVITY DETERMINATION NEEDED?**

1. An exclusivity determination will be made for all original applications, but only for certain supplements. Complete PARTS II and III of this Exclusivity Summary only if you answer "yes" to one or more of the following question about the submission.

a) Is it an original NDA?

YES /  / NO /  /

b) Is it an effectiveness supplement?

YES /  / NO /  /

If yes, what type? (SE1, SE2, etc )

c) Did it require the review of clinical data other than to support a safety claim or change in labeling related to safety? (If it required review only of bioavailability or bioequivalence data, answer "no").

YES /  / NO /  /

If your answer is "no" because you believe the study is a bioavailability study and, therefore, not eligible for exclusivity, EXPLAIN why it is a bioavailability study, including your reasons for disagreeing with any arguments made by the applicant that the study was not simply a bioavailability study.

\_\_\_\_\_  
\_\_\_\_\_

If it is a supplement requiring the review of clinical data but it is not an effectiveness supplement, describe the change or claim that is supported by the clinical data:

\_\_\_\_\_  
\_\_\_\_\_

Revised 5-90

cc: Orig NDA

Div File

HFD-85

d) Did the applicant request exclusivity?

YES // NO //

If the answer to (d) is "yes", how many years of exclusivity did the applicant request?

Five (5) years of exclusivity

IF YOU HAVE ANSWERED "NO" TO ALL OF THE ABOVE QUESTIONS, GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. Has a product with the same active ingredient(s), dosage form, strength, route of administration, and dosing schedule, previously been approved by FDA for the same use?

YES // NO //

If yes, NDA # \_\_\_\_\_ Drug Name \_\_\_\_\_

IF THE ANSWER TO QUESTION 2 IS "YES", GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

3. Is this drug product or indication a DESI upgrade?

yes // NO //

IF THE ANSWER TO QUESTION 3 IS "YES", GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8 (even if a study was required for the upgrade).

#### PART II FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES

(Answer either #1 or #2 as appropriate)

1. Single active ingredient product.

Has FDA previously approved under section 505 of the Act any drug product containing the same active moiety as the drug under consideration? Answer "yes" if the active moiety (including other esterified forms, salts, complexes, chelates or clathrates) has been previously approved, but this particular form of the active moiety, e.g., this particular ester or salt (including salts with hydrogen or coordination bonding) or other non-covalent derivative (such as a complex, chelate, or clathrate) has not been approved. Answer "no" if the compound requires metabolic conversion (other than deesterification of an esterified form of the drug) to produce an already approved active moiety.

YES // NO //

If "yes", identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA # \_\_\_\_\_

NDA # \_\_\_\_\_

NDA # \_\_\_\_\_

2. Combination product.

If the product contains more than one active moiety (as defined in Part II, #1), has FDA previously approved an application under section 505 containing any one of the active moieties in the drug product? If, for example, the combination contains one-never-before-approved active moiety and one previously approved active moiety, answer "yes". (An active moiety that is marketed under an OTC monograph, but that was never approved under an NDA, is considered not previously approved).

YES /  / NO /  /

If "yes", identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA # \_\_\_\_\_

NDA # \_\_\_\_\_

NDA # \_\_\_\_\_

IF THE ANSWER TO QUESTION 1 OR 2 UNDER PART II IS "NO", GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8. IF "YES", GO TO PART III.

**PART III THREE-YEAR EXCLUSIVITY FOR NDA'S AND SUPPLEMENTS**

To qualify for three years of exclusivity, an application or supplement must contain "reports of new clinical investigations (other than bioavailability studies) essential to the approval of the application and conducted or sponsored by the applicant". This section should be completed only if the answer to PART II, Question 1 or 2 was "yes".

1. Does the application contain reports of clinical investigations? (The Agency interprets "clinical investigations" to mean investigations conducted in humans other than bioavailability studies). If the application contains clinical investigations only by virtue of a right of reference to clinical investigations in another application, answer "yes", then skip to question 3(a). If the answer to 3(a) is "yes" for any investigation referred to in another application, do not complete remainder of summary for that investigation.

YES /\_\_\_/ NO /\_\_\_/

IF "NO", GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. A clinical investigation is "essential to the approval" if the Agency could not have approved the application or supplement without relying in that investigation. Thus, the investigation is not essential to the approval if 1) no clinical investigation is necessary to support the supplement or application in light of previously approved applications (i.e., information other than clinical trials, such as bioavailability data, would be sufficient to provide a basis for approval as an ANDA or 505(b)(2) application because of what is already known about a previously approved product), or 2) there are published reports of studies (other than those conducted or sponsored by the applicant) or other publicly available data that independently would have been sufficient to support approval of the application, without reference to the clinical investigation submitted in the application.

(a) In light of previously approved applications, is a clinical investigation (either conducted by the applicant or available from some other source, including the published literature) necessary to support approval of the application or supplement?

YES /\_\_\_/ NO /\_\_\_/

If "no", state the basis for your conclusion that a clinical trial is not necessary for approval AND GO DIRECTLY TO SIGNATURE BLOCK ON PAGE 8:

\_\_\_\_\_  
\_\_\_\_\_

(b) Did the applicant submit a list of published studies relevant to the safety and effectiveness of this drug product and a statement that the publicly available data would not independently support approval of the application?

YES /\_\_\_/ NO /\_\_\_/

(1) If the answer to 2(b) is "yes", do you personally know of any reason to disagree with the applicant's conclusion?

YES /\_\_\_/ NO /\_\_\_/

If yes, explain. \_\_\_\_\_

\_\_\_\_\_

! (2) If the answer to 2(b) is "no", are you aware of published studies not conducted or sponsored by the applicant or other publicly available data that could independently demonstrate the safety and effectiveness of this drug product?

YES /\_\_\_/ NO /\_\_\_/

If yes, explain: \_\_\_\_\_  
\_\_\_\_\_

(c) If the answers to (b)(1) and (b)(2) were both "no", identify the clinical investigations submitted in the application that are essential to the approval:

\_\_\_\_\_  
\_\_\_\_\_

Studies comparing two products with the same ingredient(s) are considered to be bioavailability studies for the purpose of this section.

3. In addition to being essential, investigations must be "new" to support exclusivity. The agency interprets "new clinical investigation" to mean an investigation that 1) has not been relied on by the agency to demonstrate the effectiveness of a previously approved drug for any indication and 2) does not duplicate the results of another investigation that was relied on by the agency to demonstrate the effectiveness of a previously approved drug product, i.e., does not redemonstrate something the agency considers to have been demonstrated in an already approved application.

a) For each investigation identified as "essential to the approval", has the investigation been relied on by the agency to demonstrate the effectiveness of a previously approved drug product? (If the investigation was relied on only to support the safety of a previously approved drug, answer "no").

Investigation #1 YES /\_\_\_/ NO /\_\_\_/

Investigation #2 YES /\_\_\_/ NO /\_\_\_/

If you have answered "yes" for one or more investigations, identify each such investigation and the NDA in which each was relied upon:

\_\_\_\_\_  
\_\_\_\_\_

b) For each investigation identified as "essential to the approval", does the investigation duplicate the results of another investigation that was relied on by the agency to support the effectiveness of a previously approved drug product?

Investigation #1            YES /\_\_\_/    NO /\_\_\_/

Investigation #2            YES /\_\_\_/    NO /\_\_\_/

If you have answered "yes" for one or more investigations, identify the NDA in which a similar investigation was relied on:

\_\_\_\_\_  
\_\_\_\_\_

c) If the answers to 3(a) and 3(b) are "no", identify each "new" investigation in the application or supplement that is essential to the approval (i.e., the investigations listed in #2(c), less any that are not "new"):

\_\_\_\_\_  
\_\_\_\_\_

4. To be eligible for exclusivity, a new investigation that is essential to approval must also have been conducted or sponsored by the applicant. An investigation was "conducted or sponsored by" the applicant if, before or during the conduct of the investigation, 1) the applicant was the sponsor of the IND named in the form FDA 1571 filed with Agency, or 2) the applicant (or its predecessor in interest) provided substantial support for the study. Ordinarily, substantial support will mean providing 50 percent or more of the cost of the study.

a) For each investigation identified in response to question 3(c): if the investigation was carried out under an IND, was the applicant identified on FDA 1571 as the sponsor?

Investigation #1

IND # \_\_\_\_\_ YES /\_\_\_/    NO /\_\_\_/    Explain: \_\_\_\_\_  
\_\_\_\_\_

Investigation #2

IND # \_\_\_\_\_ YES /\_\_\_/    NO /\_\_\_/    Explain: \_\_\_\_\_  
\_\_\_\_\_



**Debarment Statement**

Neither Gilead Sciences, Inc. nor any of its contract corporations, laboratories, or individuals involved in the development or submission of records or data regarding Vistide™ (cidofovir intravenous) has used and will not use in any capacity the services of any person debarred under subsections (a) or (b) [section 306 (a) or (b)] of the Generic Drug Enforcement Act of 1992 (21 U.S.C. 335a(k)(1)).

**NDA 20-638**

**Team Leader's Memorandum for New Drug Application 20-638**

**Date:** March 22, 1996

**Trade name:** VISTIDE®

**Generic name:** Cidofovir

**Sponsor:** Gilead Sciences

**NDA 20-638 was filed on September 30, 1995, for the treatment of CMV retinitis in HIV-infected patients. I concur with the reviews and conclusions of Medical Reviewer Dr. Douglas Pratt and Statistical Reviewer Dr. Alan Muhly. Approval of cidofovir for the indication sought by the sponsor is warranted based on the data submitted from two controlled studies conducted by the sponsor (GS-106 and GS-107).**

**Approval of cidofovir was also the unanimous recommendation of the FDA Antiviral Advisory Committee which met to discuss this application on March 15, 1996.**

**This application has posed several important medical and statistical issues. The drug is clearly a carcinogen in rats (mammary tumors appeared in female rats appeared after as few as 6 doses of cidofovir); the results of these studies were of sufficient concern to have resulted in a temporary halt of study enrollment during clinical testing. However, potent carcinogenicity was not seen in a subsequent 1 year study in monkeys (although not a full carcinogenicity study), and as yet a clear signal of carcinogenicity has not been seen in clinical studies. The sponsor has agreed to perform active surveillance for malignancies post-marketing, with specific study of women included. (Women were clearly underrepresented in clinical studies of cidofovir, a problem that has also occurred in other studies of treatments for CMV retinitis.)**

**Perhaps the most difficult aspect of this submission was appropriate estimation of the size of the treatment effect. For reasons well stated by Dr. Muhly, the estimate of median time to progression stated by the sponsor is almost certainly overestimated. The Kaplan-Meier analysis submitted by the sponsor fails to meet the assumption of uninformative censoring necessary for Kaplan-Meier estimation; further, because of the small sample size in study 106, the estimated median time to progression is substantially affected by the long shoulder (due to censoring) in the Kaplan-Meier**

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**<sup>1</sup>See transcripts of the FDA Antiviral Advisory Committee Meeting, March 15, 1996.**

curve. Review of the individual case-reports of censored patients by Dr. Pratt clearly indicates that censoring could not be considered uninformative.

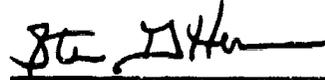
The estimate of treatment effect was discussed at great length with the sponsor, who stressed repeatedly that the agency had previously accepted similar censored analyses for estimating treatment effect size in other studies of CMV retinitis. It was agreed after substantial discussion that the sponsor would be permitted to retain this estimate of effect size in the package insert, but that a similar estimate of progression time including censored patients (i.e., 'time to progression or drug discontinuation') would also be included. It was also agreed that Kaplan-Meier curves would not be included in final package insert, consistent with other products labeled for this specific indication.

Several important clinical issues remain outstanding at this time. The sponsor has not submitted any data regarding resistance to other anti-CMV agents from clinical isolates obtained during the registrational studies of cidofovir; a major clinical concern is whether cidofovir therapy will predispose to ganciclovir resistance, thereby eliminating the utility of this other important agent. As noted by Dr. Pratt, the activity of cidofovir for systemic CMV disease is uncertain, although the specificity of the indication for retinitis alone is clearly stated in the proposed package insert.

The safety spectrum of cidofovir cannot be considered fully explored, and of particular concern is whether the incidence of metabolic acidosis will increase with more widespread clinical use. In addition, cidofovir has not been studied in patients with preexisting renal disease, and this may pose safety problems with greater clinical use. However, despite this, the risk/benefit clearly favors approval of cidofovir; the potential benefit from twice-monthly use of drug is an important quality-of-life issue that justifies allowing patients and physicians to this therapeutic option. The company has committed to post-marketing studies that should address unresolved questions regarding cidofovir, and study of patients with renal impairment is already ongoing.

I have thoroughly reviewed the proposed package insert with Dr. Pratt, and concur that it adequately represents the information available regarding the safety and efficacy of cidofovir.

**Recommendation:** Cidofovir should be approved for the indication proposed by the sponsor. Careful post-marketing surveillance of the safety of cidofovir with wider clinical use is essential, and the sponsor's compliance with phase 4 commitments should be monitored.



---

Steven Gitterman, M.D., Ph.D.  
Medical Team Leader

**concurrency:** DFeigal

**cc:** HFD-530/DPratt  
HFD-530/KStruble  
NDA 20-638

**DRUG STUDIES IN PEDIATRIC PATIENTS**  
(To be completed for all NME's recommended for approval)

**NDA # 20-638 Trade (generic) names VISTIDE (cidofovir) Injection 75 mg/ml**

Check any of the following that apply and explain, as necessary, on the next page:

- 1. A proposed claim in the draft labeling is directed toward a specific pediatric illness. The application contains adequate and well-controlled studies in pediatric patients to support that claim.
- 2. The draft labeling includes pediatric dosing information that is not based on adequate and well-controlled studies in children. The application contains a request under 21 CFR 210.58 or 314.126(C) for waiver of the requirement at 21 CFR 201.57(f) for A&WC studies in children.
  - a. The application contains data showing that the course of the disease and the effects of the drug are sufficiently similar in adults and children to permit extrapolation of the data from adults to children. The waiver request should be granted and a statement to that effect is included in the action letter.
  - b. The information included in the application does not adequately support the waiver request. The request should not be granted and a statement to that effect is included in the action letter. (Complete #3 or #4 below as appropriate).
- 3. Pediatric studies (e.g., dose-finding, pharmacokinetic, adverse reaction, adequate and well-controlled for safety and efficacy) should be done after approval. The drug product has some potential for use in children, but there is no reason to expect early widespread pediatric use (because, for example, alternative drugs are available or the condition is uncommon in children).
  - a. The applicant has committed to doing such studies as will be required.
    - (1) Studies are ongoing.
    - (2) Protocols have been submitted and approved.
    - (3) Protocols have been submitted and are under review.
    - (4) If no protocol has been submitted, on the next page explain the status of discussions.
  - b. If the sponsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request.



# PEDIATRIC PAGE

(Complete for all original applications and all efficacy supplements)

NDA/PLA # 20-638

Supplement # 000

Circle one: SE1 SE2 SE3 SE4 SE5 SE6

HFD S-30 Trade (generic) name/dosage form: Vistide (Cidofovir) injection 75mg/mL

Action:  AP  AE  NA

Applicant Gilead Sciences

Therapeutic Class Antiviral

Indication(s) previously approved \_\_\_\_\_

Pediatric labeling of approved indication(s) is adequate  inadequate

Indication in this application Treatment of CMV retinitis in Patients with AIDS

(For supplements, answer the following questions in relation to the proposed indication.)

1. **PEDIATRIC LABELING IS ADEQUATE.** Appropriate information has been submitted in this or previous applications and has been adequately summarized in the labeling to permit satisfactory labeling for all pediatric subgroups. Further information is not required.
2. **PEDIATRIC STUDIES ARE NEEDED.** There is potential for use in children, and further information is required to permit adequate labeling for this use.
  - a. A new dosing formulation is needed, and applicant has agreed to provide the appropriate formulation.
  - b. The applicant has committed to doing such studies as will be required.
    - (1) Studies are ongoing.
    - (2) Protocols were submitted and approved.
    - (3) Protocols were submitted and are under review.
    - (4) If no protocol has been submitted, explain the status of discussions on the back of this form.
  - c. If the sponsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request.
3. **PEDIATRIC STUDIES ARE NOT NEEDED.** The drug/biologic product has little potential for use in children. Explain, on the back of this form, why pediatric studies are not needed.
4. **EXPLAIN.** If none of the above apply, explain, as necessary, on the back of this form.

EXPLAIN, AS NECESSARY, ANY OF THE FOREGOING ITEMS ON THE BACK OF THIS FORM.

Kimberly A. Struble R.Ph. PM

Signature of Preparer and Title (PM, CSO, MO, other)

Date

cc: Orig NDA/PLA # 20638

HFD S-30 /Div File

NDA/PLA Action Package

HFD-510/GTrendle (plus, for CDER APs and AEs, copy of action letter and labeling)

NOTE: A new Pediatric Page must be completed at the time of each action even though one was prepared at the time of the last action.

# Final Printed Labeling





**Table 2. Colibacter Pharmacokinetics Parameters Following 3.0 and 6.0 mg/kg Single Injections, Without and With Probenecid\***

PARAMETER	VISTIDE ADMINISTRATION		VISTIDE ADMINISTRATION WITH PROBENECID	
	3 mg/kg (n = 10)	6 mg/kg (n = 10)	3 mg/kg (n = 10)	6 mg/kg (n = 10)
AUC (0-24h)	20.8 ± 3.3	28.3	27.7 ± 5.5	48.8 ± 8.9
Clearance (ml/min)	7.3 ± 1.4	11.5	8.1 ± 3.7	10.8 ± 7.2
Half-life (hr)	37 ± 12	44 ± 12	48 ± 12	44 ± 12
Volume of Distribution (L)	17 ± 2.1	18 ± 2.1	18 ± 2.1	18 ± 2.1
Peak Concentration (mg/L)	128 ± 28.9	128 ± 28.9	128 ± 28.9	128 ± 28.9
Time to Peak (hr)	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2

\* See DOSAGE AND ADMINISTRATION

In vitro, colibacter was less than 9% bound to plasma or serum proteins over the colibacter concentration range 0.25 to 25 mg/ml. CSF concentrations of colibacter following intravenous injection of VISTIDE 5 mg/kg with concomitant probenecid and intravenous injection of probenecid (0.8 g/ml, 100 mg/kg) were similar. The probability of 15 minutes after the end of 1 hour for colibacter in one patient while corresponding serum concentration was 0.7 mg/ml.

**DRUG-DRUG INTERACTIONS**

**Zidovudine**  
The pharmacokinetics of zidovudine were measured in 10 patients receiving intravenous drug in with intravenous colibacter (without probenecid). There was no evidence of an effect of colibacter on the pharmacokinetics of zidovudine.

**SPECTRA, PHARMACOKINETICS**

**Pharmacokinetics**  
Colibacter pharmacokinetics have not been investigated in patients with renal insufficiency. The data are currently available on the pharmacokinetics of colibacter in patients with creatinine clearance values below 30 ml/min. The effect of dialysis on colibacter pharmacokinetics is not known.

**Pharmacodynamics**

The effects of oral, gastric, and oral on colibacter pharmacokinetics have not been investigated.

**CONCOMITANT DRUG USE**

VISTIDE is indicated for the treatment of CMV retinitis in patients with acquired immunodeficiency syndrome (AIDS). THE SAFETY AND EFFICACY OF VISTIDE HAVE NOT BEEN ESTABLISHED FOR THE TREATMENT OF OTHER CMV INFECTIONS SUCH AS PHARYNGITIS OR GASTROENTERITIS, CONJUNCTIVITIS, OR HERPESVIRUS CMV INFECTION OR CMV DISEASE IN NON-HIV-INFECTED INDIVIDUALS.

**CONCOMITANT USE OF CALCIUM CHANNEL BLOCKERS**

The phase 2/3 controlled trials of VISTIDE have been conducted in HIV-infected patients with CMV retinitis.

**Delayed Intraocular Injections (Study 107)**  
In an open-label trial, 107 HIV-infected patients with bilateral CMV retinitis were randomized to either receive treatment with VISTIDE 5 mg/kg once a week for 2 weeks, then 5 mg/kg every other week, or to have VISTIDE (along with probenecid) administered to 23 and 21 patients in the immediate and delayed groups respectively. 23 and 21 were available for ocular photographs at baseline and follow-up photography. Based on masked readings of ocular photographs, the mean (95% confidence interval [CI]) times to relapse progression were 128 days (40, 124) and 22 days (14, 27) for the immediate and delayed therapy groups, respectively. This difference was statistically significant. However, because of the limited number of patients remaining on treatment over time (3 of 25 patients receiving VISTIDE for 128 days or longer), the median time to progression for the immediate therapy group was difficult to precisely estimate. (See Pharmacokinetics, Metabolism, & Impairment of Efficacy.)

Symptoms (CMV disease) were 52 days (37, 65) and 22 days (13, 27) for the immediate and delayed therapy groups, respectively. This difference was statistically significant. Time to progression estimates from this study may not be directly comparable to estimates reported for other therapies.

**Table 3. Patient Characteristics and Disposition (Study 107)**

Baseline Characteristics	Immediate Therapy (n = 25)		Delayed Therapy (n = 25)	
	n	%	n	%
Age (years)	38	28	38	28
Sex (M/F)	20/10	24/11	20/10	24/11
Race (C/M/O)	6	6	6	6
Ethnicity	16	16	16	16
CD4+ T-lymphocyte count (cells/mm <sup>3</sup> )	6	6	6	6
Disposition Due to Adverse Event	4	4	4	4
Withdrawn Consent	2	2	2	2
Discontinued Due to Intercurrent Disease	1	1	1	1
Discontinued Due to Inadequate Response	1	1	1	1
Discontinued Due to Other Cause	1	1	1	1
Not Evaluated at Baseline	2	2	2	2

- One patient died 2 weeks after withdrawing consent.
- Two patients in immediate therapy were hospitalized with CMV disease and discontinued from study. One patient in delayed therapy was hospitalized with CMV disease and discontinued from study. CMV disease progression not confirmed by retinal photography.

**Open-label study of VISTIDE (Study 107)**  
In an open-label trial, 107 HIV-infected patients with bilateral CMV retinitis were randomized to receive 5 mg/kg once a week for 2 weeks and then either 5 mg/kg (n = 49) or 2 mg/kg (n = 51) every other week. Enrolled patients had been hospitalized with CMV retinitis approximately 1 year prior to randomization and had received a median of 4 prior courses of systemic CMV therapy. Eighty-four of the 107 patients were considered evaluable for progression by masked ocular photographs (42 random to 5 mg/kg and 41 random to 2 mg/kg). Twenty-three and 26 patients discontinued therapy due to either an adverse event, intercurrent illness, withdrawal of medication, or withdrawal consent in the 5 mg/kg and 2 mg/kg groups, respectively. Based on masked readings of retinal photographs, the median (95% CI) times to relapse progression for the 5 mg/kg and 2 mg/kg groups were 115 days (70, 141) and 22 days (14, 27), respectively. This difference was statistically significant. Similar to Study 107, the median time to relapse progression for the 5 mg/kg group was difficult to precisely estimate due to the limited number of patients remaining on treatment over time (4 of the 49 patients in the 5 mg/kg group were treated for 115 days or longer). Median (95% CI) times to the subsequent endpoint of relapse progression in study (any discontinuation) were 49 days (28, 62) and 25 days (17, 30) for the 5 mg/kg and 2 mg/kg groups, respectively. This difference was statistically significant.

**CONCOMITANT THERAPY**

VISTIDE is contraindicated in patients with hypersensitivity to colibacter. VISTIDE is contraindicated in patients with a history of clinically severe hypersensitivity to probenecid or other sulfa-containing medications.

Direct in vitro effects of VISTIDE is concentration-dependent and dependent of region could with significant decreases in intracellular pressure and impairment of vision.

**WARNINGS**

**Retinopathy:** Dose-dependent retinopathy is the major dose-limiting toxicity related to VISTIDE administration. Dose adjustment or discontinuation is required for changes in retinal function while on therapy. Patients, as recommended by a clinical ophthalmologist, may be at early indicator of VISTIDE-related retinopathy. Continued administration of VISTIDE may lead to additional progression of retinal disease, which may result in permanent and irreversible vision loss. Patients with these adverse events occurring concomitantly and requiring a change of VISTIDE therapy should be hospitalized. Rapid function that did not return to baseline after drug discontinuation has been observed in clinical studies of VISTIDE.

Increased serum levels of VISTIDE and oral probenecid need not be accompanied by VISTIDE-related toxicity. Probenecid is known to interact with the metabolism of oral VISTIDE. Patients receiving VISTIDE should be advised that the metabolism of oral VISTIDE is altered by probenecid. The safety of VISTIDE has not been established in patients receiving other known potentially nephrotoxic agents, such as aminoglycosides, amphotericin B, foscarnet, and intravenous pyrimethamine (see DOSAGE AND ADMINISTRATION).

**Probenecid:** Probenecid is known to interact with the metabolism of oral VISTIDE. Patients receiving VISTIDE should be advised that the metabolism of oral VISTIDE is altered by probenecid. The safety of VISTIDE has not been established in patients receiving other known potentially nephrotoxic agents, such as aminoglycosides, amphotericin B, foscarnet, and intravenous pyrimethamine (see DOSAGE AND ADMINISTRATION).

**Neurological Toxicity:** Neurotoxicity may occur during VISTIDE therapy. Neurological toxicity should be monitored while receiving VISTIDE therapy.

**Metabolic Abnormalities:** Fournier's syndrome and decreases in serum bicarbonate associated with evidence of renal tubular damage have been reported in patients receiving VISTIDE (see ADVERSE EVENTS). Serum metabolic abnormalities associated with renal failure, prerenal azotemia, myoglobinuria, acute renal tubular necrosis, and progression to death occurred in 1 patient (<1%) receiving VISTIDE.

**PRECAUTIONS**

**General**  
Due to the potential for increased nephrotoxicity, doses greater than the recommended dose should not be administered and the frequency of administration should not be increased (see DOSAGE AND ADMINISTRATION).

VISTIDE is contraindicated in patients with hypersensitivity to colibacter. VISTIDE is contraindicated in patients with a history of clinically severe hypersensitivity to probenecid or other sulfa-containing medications.

**Information for Patients**

Patients should be advised that VISTIDE is used as a daily eye drop, and that they may continue to experience progression of retinitis during and following treatment. Patients receiving VISTIDE should be advised to have regular follow-up ophthalmologic examinations. Patients may also experience other retinal effects of CMV disease despite VISTIDE therapy.

HIV-infected patients may continue taking antiretroviral therapy, but these taking antiretroviral therapy should be advised to temporarily discontinue antiretroviral administration or decrease their administration dose by 50%, on days of VISTIDE administration only. VISTIDE-related adverse events may occur.

Patients should be advised that the major toxicity of VISTIDE is retinopathy, and that dose modification, including reduction, interruption, and possibly discontinuation, may be required. Close monitoring of retinal function (patient history and ophthalmologic examination) while on therapy should be complete.

The importance of completing a full course of probenecid with each VISTIDE dose should be emphasized. Patients should be advised of potential adverse effects caused by probenecid (e.g., headache, nausea, vomiting, and hypotension) and symptoms. Hypersensitivity reactions may include rash, hives, CMV disease, and anaphylaxis. Administration of probenecid after a meal or use of probenecid may decrease the toxicity. Probenecid or intravenous pyrimethamine and/or aminoglycosides can be used to probenecid hypersensitivity reactions.

Patients should be advised that colibacter causes blindness, primarily secondary to retinopathy, in HIV. VISTIDE should be discontinued if a potential cause of blindness is identified. (See Contraindications, Metabolism, & Impairment of Efficacy.) Patients should be advised of the limited availability of replacement in clinical trials of VISTIDE.

Patients should be advised that VISTIDE caused reduced body weight in HIV-infected patients. Such changes may occur in humans and other animals. However, if colibacter-related toxicity is observed, the safety of VISTIDE should be re-evaluated. Patients should be advised that colibacter is contraindicated in patients receiving other known potentially nephrotoxic agents, such as aminoglycosides, amphotericin B, foscarnet, and intravenous pyrimethamine (see DOSAGE AND ADMINISTRATION).

**Drug Interactions**

**Probenecid:** Probenecid is known to interact with the metabolism of oral VISTIDE. Patients receiving VISTIDE should be advised that the metabolism of oral VISTIDE is altered by probenecid. The safety of VISTIDE has not been established in patients receiving other known potentially nephrotoxic agents, such as aminoglycosides, amphotericin B, foscarnet, and intravenous pyrimethamine (see DOSAGE AND ADMINISTRATION).

**Neurological Toxicity:** Neurotoxicity may occur during VISTIDE therapy. Neurological toxicity should be monitored while receiving VISTIDE therapy.

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**General**  
Due to the potential for increased nephrotoxicity, doses greater than the recommended dose should not be administered and the frequency of administration should not be increased (see DOSAGE AND ADMINISTRATION).

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Patients should be advised that VISTIDE is used as a daily eye drop, and that they may continue to experience progression of retinitis during and following treatment. Patients receiving VISTIDE should be advised to have regular follow-up ophthalmologic examinations. Patients may also experience other retinal effects of CMV disease despite VISTIDE therapy.

HIV-infected patients may continue taking antiretroviral therapy, but these taking antiretroviral therapy should be advised to temporarily discontinue antiretroviral administration or decrease their administration dose by 50%, on days of VISTIDE administration only. VISTIDE-related adverse events may occur.

Patients should be advised that the major toxicity of VISTIDE is retinopathy, and that dose modification, including reduction, interruption, and possibly discontinuation, may be required. Close monitoring of retinal function (patient history and ophthalmologic examination) while on therapy should be complete.

The importance of completing a full course of probenecid with each VISTIDE dose should be emphasized. Patients should be advised of potential adverse effects caused by probenecid (e.g., headache, nausea, vomiting, and hypotension) and symptoms. Hypersensitivity reactions may include rash, hives, CMV disease, and anaphylaxis. Administration of probenecid after a meal or use of probenecid may decrease the toxicity. Probenecid or intravenous pyrimethamine and/or aminoglycosides can be used to probenecid hypersensitivity reactions.

Patients should be advised that colibacter causes blindness, primarily secondary to retinopathy, in HIV. VISTIDE should be discontinued if a potential cause of blindness is identified. (See Contraindications, Metabolism, & Impairment of Efficacy.) Patients should be advised of the limited availability of replacement in clinical trials of VISTIDE.



through day 27) precipitated (approximately 5 weeks) resulted in no effects on voluntary growth, behavior, sexual maturation, or reproductive life in the offspring.

**CL Category C**

It was embryotoxic (fetal body weights) in rats at 1.5 mg/kg/day (about 1/10 embryotoxic dose) since were also embryotoxic. Late embryotoxic effects during the period of organogenesis. The no-observed-effect levels for embryotoxicity in rats (0.5 mg/kg/day) and in rabbits (0.5 mg/kg/day) were approximately 0.04 and 0.05 times the clinical dose (5 mg/kg/day) based on AUC, respectively. An increased incidence of cleft palate and skeletal anomalies (osteopetrosis, short skull) (readily lethal) occurred in rabbits at the high dose (1.0 mg/kg/day) as also maternally toxic. There are no adequate and well-controlled studies in pregnant women. VISTIDE should be used during pregnancy only if the potential benefits justify the potential risk to the fetus.

**Warnings**

Neuroleptic malignant syndrome is described in human trials. Such a syndrome is characterized by hyperthermia, muscle rigidity, tachycardia, tachypnea, and diaphoresis. The syndrome is fatal if not treated with supportive therapy. The U.S. Public Health Service for Dexamethasone and Prednisone advises that patients receiving these drugs should be advised to avoid potential transmission of HIV to a child who may not be infected.

**Use in Children**

The use of VISTIDE in children has not been studied. The use of VISTIDE in children with AIDS warrants extreme caution due to the risk of long-term neuroleptic malignant syndrome. Administration of VISTIDE to children is contraindicated unless the potential benefits justify the potential risks.

**Use in Pregnancy**

The use of VISTIDE in patients over the age of 18 has not been studied. Since clinical studies have not been conducted, the safety of VISTIDE administration (see DOSAGE AND ADMINISTRATION).

**ADVERSE REACTIONS**

Adverse reactions, as mentioned by >1% patients, were seen in 47 of 88 (53%) patients receiving VISTIDE at a dosage of 5 mg/kg every other week. Neuroleptic malignant syndrome was reported in 12 of 47 (25%) patients who had not received prior therapy for CMV infection (Study 106) and 11 of 46 (23%) patients who had received prior therapy for CMV infection (Study 107). Prior treatment with the drug should be mentioned clearly.

**Use in Pregnancy**

In clinical trials, at the 5 mg/kg maintenance dose, neuroleptic malignant syndrome occurred in 20% of patients. Granulocyte colony-stimulating factor (G-CSF) was used in 20% of patients.

**Use in Pregnancy**

Among the studies of patients randomized for intrathecal use, neuroleptic malignant syndrome (2.5% change from baseline) was reported in 5 patients. Neuroleptic malignant syndrome with characteristic features (hyperthermia, tachycardia, tachypnea, diaphoresis) was reported in 5 patients. Risk of neuroleptic malignant syndrome may be increased in patients with other conditions.

**Use in Pregnancy**

A diagnosis of Fanconi's syndrome, as manifested by multiple abnormalities of proximal tubule function, was reported in 1 patient. Decreases in serum bicarbonate to <5 mEq/L, associated with evidence of renal tubular damage occurred in approximately 1 patient. Serum metabolic studies, as associated with liver function tests, were normal. Neuroleptic malignant syndrome, disseminated intravascular coagulation, and progression to death occurred in 1 patient receiving VISTIDE.

In clinical trials, VISTIDE was withdrawn due to adverse events in approximately 25% of patients treated with 5 mg/kg every other week at maintenance therapy.

The incidence of adverse reactions reported as serious in two controlled clinical studies in patients with CMV infection, regardless of pretreatment with drug, is listed in Table 4.

Table 4. Serious Adverse Events in Laboratory Neuroleptics: Summary to > 1% of Patients

Adverse Event	Study 106 (%)	Study 107 (%)
Proteinuria ( $\geq 100$ mg/dL)	42	48
Neutropenia ( $\leq 500$ cells/mm <sup>3</sup> )	18	20
Chemistry Abnormalities	13	15
Fever	13	15
Infection	11	12
Dyspnea	9	10
Pneumonia	8	8
Decreased Serum Bicarbonate ( $\leq 18$ mEq/L)	8	8
Chemistry Abnormalities ( $\geq 2.0$ mg/dL)	7	8
Neuropathy with Numbness	7	7
Dizziness	6	7
Agitation	6	7
Oral Cavity Infection	5	12

Patients receiving 5 mg/kg maintenance regimen in Studies 106 and 107 were treated for 42 patients receiving 5 mg/kg maintenance regimen in Study 106 and 107 with pretreatment laboratory abnormalities and follow-up evaluation > 50% of patients.

The most frequently reported adverse events, regardless of relationship to study drug (probable or possible) or severity are shown in Table 5.

Table 5. All Clinical Adverse Events, Laboratory Abnormalities or Instrumental Abnormalities (Regardless of Severity) Occurring in > 10% of Patients

Adverse Event	Study 106 (%)	Study 107 (%)
Any Adverse Event	88	100
Proteinuria	71	88
Neutropenia	58	65
Fever	51	57
Neutropenia ( $\leq 750$ cells/mm <sup>3</sup> )	41	48
Rash	27	38
Headache	24	27
Dyspnea	24	27
Agitation	22	25
Diarrhea	22	25
Chemistry Abnormalities	21	24
Agitation	20	22
Dyspnea	20	22
Abnormal PFT	18	20
Chemistry Abnormalities ( $\geq 1.5$ mg/dL)	16	18
Abnormal PFT	15	17

Patients receiving 5 mg/kg maintenance regimen in Studies 106 and 107 were treated for 42 patients receiving 5 mg/kg maintenance regimen in Study 106 and 107 with pretreatment laboratory abnormalities and follow-up evaluation > 50% of patients.

The following additional list of adverse events/subsequent diseases have been observed in clinical studies of VISTIDE and are listed below regardless of causal relationship to VISTIDE.

Body as a whole: allergic reaction, face edema, malaise, back pain, chest pain, neck pain, sore throat, sinusitis.

Cardiovascular System: hypotension, postural hypotension, palpitations, tachycardia.

Operative System: colic, constipation, loose stools, diarrhea, dyspepsia, dysphagia, flatulence, gas, indigestion, increased liver function tests, pain, rectal prolapse, rectal tenesmus, strabismic, strabismic strabismus, mouth irritation.

Genitourinary System: thrombocytopenia.

Metabolic & Nutritional System: edema, dehydration, hypoglycemia, hyperkalemia, hypokalemia, hypocalcemia, hypomagnesemia, increased alkaline phosphatase, increased SGOT, increased SGPT, weight loss.

Musculoskeletal System: arthralgia, myalgia, myositis, myopathy.

Nervous System: anxiety, confusion, depression, dizziness, dry mouth, abnormal gait, incontinence, insomnia, neuroleptic malignant syndrome, numbness.

Respiratory System: asthma, bronchitis, coughing, dyspnea, tachypnea, increased sputum, lung disease, pleuritic pain, pneumonia, rhinitis, sinusitis.

Skin & Appendages: alopecia, acne, skin discoloration, dry skin, herpes simplex, pruritus, rash, sweating, urticaria.

Special Senses: anisocoria, conjunctivitis, eye disorder, lacrimation, vision abnormality, vision abnormality, vision abnormality, vision abnormality.

Urogenital System: decreased creatinine clearance, glycosuria, hematuria, urinary incontinence, urinary tract infection.

Disturbances of Attention/Consciousness

Adverse events or serious adverse reactions that occur in patients who have received VISTIDE should be reported to the Director of Clinical Research, Glaxo Inc., 505 Linden Blvd., Suite 100, New York, NY 10036, or by calling 1-800-455-6105 (455-6105) or in NY 1-800-455-6105 (455-6105).

**PREPARATION**

Overexposure with VISTIDE has not been reported. However, hemolysis and hypotension may occur if patients receive VISTIDE in patients who receive an overdose of VISTIDE. Patients may receive the product for neuroleptic malignant syndrome in patients who receive an overdose of VISTIDE through collection of urine.

**DOSE AND ADMINISTRATION**

VISTIDE MUST NOT BE ADMINISTERED BY INTRAVENOUS INJECTION.

**Dosage**

THE RECOMMENDED DOSEAGE, FREQUENCY, OR DURATION MUST NOT BE EXCEEDED. VISTIDE MUST BE DILUTED IN 100 MILLILITERS (3.0 FL OZ) SALINE PRIOR TO ADMINISTRATION. TO MINIMIZE POTENTIAL NEPHROTOXICITY AND HYPERKALEMIA, THE RECOMMENDED DOSEAGE MUST BE ADMINISTERED WITH EACH VISTIDE INFUSION.

**Indications/Contraindications**

The recommended dose of VISTIDE is 5 mg/kg body weight given as an intravenous infusion at a constant rate over 1 hour. Indications/Contraindications: The recommended maintenance dose of VISTIDE is 5 mg/kg body weight given as an intravenous infusion at a constant rate over 1 hour administered once every two weeks.

**Contraindications**

Pretreatment must be administered only with each VISTIDE dose. Two grams must be administered 2 to 4 hours prior to the VISTIDE dose and one gram administered at 2 and again at 8 hours after completion of the 1 hour VISTIDE infusion (for a total of 4 grams).

Injection of fluid prior to each dose of pretreatment may reduce drug-related toxicity and vomiting. Administration of an antemetic may reduce the potential for nausea associated with pretreatment injection. In patients with severe allergic reactions associated with pretreatment injection, patients with severe allergic reactions associated with pretreatment injection.

hypersensitivity symptoms be pretreated the use of an appropriate antihistamine or corticosteroid administration and/or administration should be considered (see CONTRAINDICATIONS).

**Side Effects** Patients should receive a total of 100 mg (0.9%) (normal saline solution) intravenously with each infusion of VISTIDE. The saline solution should be infused over 1 to 2 hours immediately before the VISTIDE infusion. Patients who can tolerate the additional fluid load should receive a second 100 mg (0.9%) saline solution. The second hour of saline should be infused either at the end of the VISTIDE infusion or immediately thereafter, and infused over 1 to 2 hours.

**Drug Administration**

**Change in Renal Function During VISTIDE Therapy** For clinically significant changes in renal function (BUN  $\geq 0.4$  mg/dL) the VISTIDE dose should be reduced from 5 mg/kg to 3 mg/kg. VISTIDE therapy should be discontinued for an increase in serum creatinine of  $\geq 0.3$  mg/dL, or development of 2+ proteinuria. **Preventing Renal Impairment** VISTIDE has not been studied in patients with preexisting renal impairment. The most appropriate renal and electrolyte status of VISTIDE for patients with serum creatinine concentrations  $\geq 1.5$  mg/dL in creatinine clearance  $\leq 35$  mL/min are not known. When the potential benefits of therapy exceed the potential risks, dose adjustments should be made based on the following table:

Condition/Chemistry (mL/min)	Infusion (once weekly for 2 weeks)	Duration (weeks)
41-50	2.0 mg/kg	2.0 mg/kg
30-40	1.5 mg/kg	1.5 mg/kg
20-29	1.0 mg/kg	1.0 mg/kg
5-19	0.5 mg/kg	0.5 mg/kg

\* These recommended dose adjustments are based on pharmacokinetic estimates, not on actual clinical data.

Intravenous normal saline and oral pretreatment must accompany each VISTIDE infusion. VISTIDE has not been studied in patients receiving dialysis.

Because of clinical efficacy safety or pharmacokinetic data are available from patients with evidence to support renal impairment (creatinine clearance  $\leq 35$  mL/min), careful monitoring of disease progression and patient safety is required.

**Method of Collection and Administration**

Injectable vials of VISTIDE contain 100 mg (0.9%) (normal saline) and 100 mg (0.9%) (normal saline) in a 100 mL vial. The vial should not be used if the vial is damaged or if the vial is not sealed. The vial should not be used if the vial is damaged or if the vial is not sealed. The vial should not be used if the vial is damaged or if the vial is not sealed.

It is recommended that VISTIDE infusion administration be administered within 24 hours of preparation and that refrigerator or freezer storage not be used beyond the 24-hour limit.

If administration is not intended for immediate use, they may be stored under refrigeration (2-8°C) for no more than 24 hours. Refrigeration administration should be allowed to equilibrate to room temperature prior to use.

The chemical stability of VISTIDE, administered as a solution in polypropylene vials, is stable for up to 24 hours at room temperature. The chemical stability of VISTIDE, administered as a solution in polypropylene vials, is stable for up to 24 hours at room temperature. The chemical stability of VISTIDE, administered as a solution in polypropylene vials, is stable for up to 24 hours at room temperature.

# Medical Officers Review



**General Information**

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**Related Reviews:**

**Biopharmacology:** Kellie Reynolds (HFD-880)

**Chemistry:** Albinus D'Sa (HFD- 530)

**Microbiology:** Walla Dempsey (HFD-530)

**Ophthalmology Consult:** Wiley Chambers (HFD-540)

**Pharmacology/Toxicology:** Pritam Verma (HFD-530)

**Statistics:** Alan Muhly (HFD-725)

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## SECTION 1

## General Information

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## SECTION 3

**Material Reviewed**

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**3.0 Material Reviewed**

This clinical review was based primarily on the following parts of NDA 20-638:

<b><u>Section</u></b>	<b><u>Volumes</u></b>	<b><u>Contents</u></b>
1	1	Table of Contents
2	1,2	NDA Overall Summary
7	47	Microbiology (Virology) Summary
8	53-76	Clinical Data, Study Reports, Integrated Summaries
10 - 11	93-128	Case Report Forms
Information Amendment #014 (1/24/96)		Updated Study Report of GS-93-107
Information Amendment #018 (2/9/96)		Revised Integrated Summaries and Revised Package Insert
Information Amendment #025		Updated Case Report Forms of Study GS-93-107
Information Amendment #027 (2/29/96)		Revised Integrated Summaries

In addition, data files for studies GS-93-106 and GS-93-107 were submitted to FDA on computer disk. These data files were used to check tables submitted by the sponsor and to search the data for unreported events and associations.

See Section 6.5 of this review for a brief regulatory history of *cidofovir*.

## SECTION 4

**Chemistry and Manufacturing Controls**

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**4.0 Chemistry and Manufacturing Controls**

Cidofovir belongs to the class of phosphonomethylether nucleoside monophosphate analogues which the sponsor refers to as "nucleotide analogues." Cidofovir differs from the nucleotide cytidine by an acyclic sugar moiety and a chemically stable (ether) linkage to a methylphosphonate group. According to the sponsor, after entering cells cidofovir is converted by cellular enzymes to the active diphosphate form. In this respect, cidofovir differs from the approved anti-CMV drug, ganciclovir, which must be phosphorylated by a virus-encoded kinase within infected cells. Cidofovir diphosphate has a relatively long intracellular half-life (17-65 hours). The sponsor postulates that these chemical properties allow for infrequent dosing, may account for a differing viral resistance pattern (vis-a-vis ganciclovir), and may bestow on uninfected cells the ability to "prime" against subsequent infection.

Cidofovir possesses a single chiral center. Early microbiologic and toxicologic studies showed that the (R)-enantiomer held minimal antiviral activity as well as little toxicity. The sponsor elected to use the (S)-enantiomer purified to >97% in subsequent clinical studies.

Cidofovir is highly water soluble and virtually insoluble in organic solvents. It is formulated without excipients or preservatives as a sterile solution, adjusted to pH 7.5 with sodium hydroxide and hydrochloric acid. The principal degradative product is HPMPU, resulting from deamination to the uracil analogue. Each vial is intended for single use only.

For a comprehensive review of the chemistry and manufacturing controls the reader is referred to the chemistry review of Dr. Albinus D'Sa. Comments from Dr. D'Sa's review have been incorporated into the proposed labeling by the sponsor.

## SECTION 5

## Microbiology

**5.0 Microbiology**

Cidofovir demonstrates *in vitro* antiviral activity against cytomegalovirus (CMV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), herpes simplex virus types 1 and 2, adenoviruses and human papilloma viruses. The primary mechanism of anti-viral activity is believed to occur through inhibition of the virus-encoded DNA polymerase which can be measured by *in vitro* assays. (How cidofovir is able to specifically inhibit papilloma viruses is unclear as this family of viruses does not encode DNA polymerase.) Viral resistance to cidofovir, when it occurs, is thought to be due to mutations within the viral DNA polymerase gene. Cidofovir inhibits the human DNA polymerases  $\alpha$  ( $K_i= 51 \mu\text{M}$ ),  $\beta$  ( $K_i=520 \mu\text{M}$ ), and  $\gamma$  ( $K_i= 299 \mu\text{M}$ ) at substantially higher concentrations than that required to inhibit CMV DNA polymerase ( $K_i= 6.6 \mu\text{M}$ ).

According to the *in vitro* data submitted, cidofovir remains active against most ganciclovir-resistant strains of CMV. Most (95%) of ganciclovir resistance is due to mutations in the virus-encoded UL97 kinase gene of CMV, which is responsible for adding the first phosphate group to ganciclovir. Cidofovir, which structurally resembles a monophosphorylated cytosine, does not require this initial phosphorylation step. Those CMV strains which are resistant to ganciclovir due to DNA polymerase mutations (5%) are likely resistant to cidofovir.

Resistance of CMV to foscarnet is believed to be due to viral DNA polymerase mutations. The small number of foscarnet-resistant isolates tested prohibit generalizations about the likelihood of cross-resistance to cidofovir. Strains resistant to all three anti-viral drugs have been isolated.

The sponsor states that no cidofovir-resistant strain has been isolated from a patient receiving cidofovir. Cidofovir-resistant strains have been isolated from patients receiving ganciclovir or foscarnet, and under selective pressure *in vitro*.

For a complete review of the virological data, the reader is referred to the review of Dr. Walla Dempsey. Comments from Dr. Dempsey's review have been incorporated into the proposed package labeling by the sponsor.

## SECTION 6

Pharmacology and Toxicology

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**6.0 Pharmacology and Toxicology****General Remarks**

Toxicities observed in the preclinical studies were generally predictive of toxicities which have been observed in the human studies with the notable exception of a remarkably high incidence of tumors seen in rats. Fetal malformations were observed in rabbits exposed to *cidofovir in utero* at equivalent human doses well below the intended clinical dose. Renal and bone marrow toxicities identified in the preclinical studies have been born out in the clinical setting. The margin of safety for both acute and chronic toxicities is narrow after extrapolating to the human equivalent doses based on a body surface conversion.

For a detailed review of the pharmacology and toxicology data the reader is referred to the pharm/tox review of Dr. Pritam Verma.

**Acute Toxicities**

The single dose acute lethal dose in monkeys is estimated to fall between 40 and 75 mg/kg. Based on body surface conversion, this dose range corresponds to a human equivalent dose ranging from 11 to 25 mg/kg.

**Multiple Dose Toxicities**

Nephrotoxicity was the major dose-limiting toxicity observed after repeat intravenous dosing in both rats and monkeys. The kidney lesions were described as a renal tubular nephrosis characterized by karyomegaly, degeneration, and necrosis of proximal convoluted tubules. Lymphoid depletion, bone marrow hypoplasia, testicular degeneration and hepatocellular hypertrophy were also seen at higher doses. These latter findings indicate that tissues which have a cellular component which rapidly proliferates will be the target organs of toxicity.

Through pre-clinical testing it was discovered that co-administration of probenecid served to ameliorate the renal toxicity. It has been hypothesized that probenecid blocks secretion of *cidofovir* at the proximal tubule, thus sparing this tissue from local high concentration of the drug. Additional studies showed that *cidofovir* given once weekly with probenecid was less toxic than an equivalent amount of the drug given as daily injections.

## SECTION 6

**Pharmacology and Toxicology**

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In a 13-week study of cidofovir with co-administration of probenecid, animals in the highest dose, 5 mg/kg/week, showed minor nephropathic changes, while testicular toxicities were again observed. The human equivalent dose should be considered to be 1.7 mg/kg/week (3.5 mg/kg/biweekly).

At the time of this review the longest study in primates is a six-month intravenous study of cidofovir given with probenecid by weekly injection (a 1-year study in monkeys is in progress). No clinical signs were observed at the dose given, 2.5 mg/kg/week. Histopathological exam revealed no treatment-related effects according to the sponsor's assessment. It should be noted that the human equivalent dose corresponding to the dose used in the monkey experiment is about 0.8 mg/kg/week; this is well below the intended clinical maintenance dose of 5 mg/kg/biweekly (or 2.5 mg/kg/week).<sup>1</sup>

**Reproductive and Developmental Toxicities**

In segment I studies (drug administration prior to fertilization) increased fetal deaths and absorptions were seen at a maternal dose of 1.2 mg/kg/week (human equivalent dose of 0.17 mg/kg/week). A no effect level was not demonstrated.

In segment II studies (drug administration to pregnant animal during organogenesis), reduced fetal weights and reduced ossification were seen in rats, while increased fetal absorption and soft tissue and skeletal malformations were observed in rabbits at the maternal toxic dose of 1 mg/kg/d (human equivalent dose of 0.036 mg/kg/d).

Based on these findings, cidofovir should be considered a teratogen in animals and a potential teratogen in humans.

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<sup>1</sup>A draft study report of the 1 year monkey study has subsequently been submitted and reviewed. (See Dr. Verma's Pharm/Tox review.) Briefly stated, no tumors were observed in male or female monkeys at any of the doses studied

## SECTION 6

Pharmacology and Toxicology

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Carcinogenicity*In vitro*

Cidofovir produced, in a dose dependent manner, an increased number of breaks in chromosomes and chromatids in human peripheral blood lymphocytes. Cidofovir also caused an increase in polychromatic erythrocytes in the mouse micronucleus assay. These findings are consistent with interactions at the level of DNA which could result in mutations and disruption of cellular replication or differentiation.

*Tumors in rats*

In a 19-week chronic toxicity study in which rats were given cidofovir by subscapular, subcutaneous injection, 4/20 (0.6 mg/kg), 7/20 (3.0 mg/kg), and 12/30 (15 mg/kg) female rats developed palpable tumors in the head, neck, axillary, and thoracic area. Histological examination revealed most of these to be mammary gland adenocarcinomas with an inflammatory component. Palpable tumors were evident as early as day 43 and after as few as 6 doses. Five animals in the high dose group had multiple tumors. One male rat had an anaplastic sarcoma. It was the sponsor's assessment at the time that the high tumor incidence could be attributable to local high concentrations of cidofovir on rapidly dividing glandular tissue. The sponsor contended that in the clinical setting the drug would be administered by the intravenous route and would therefore be diluted in the bloodstream.

After discussions with FDA, the sponsor agreed to conduct a 6-month intravenous study in rats and to conduct 6- and 12-month studies in monkeys. These were not intended to serve as carcinogenicity studies, rather to provide a better indication of long term toxicities and to see if the drug did cause early tumors in primates.

The sponsor subsequently became aware, and reported to FDA, the finding of mammary carcinomas in two female rats administered intravenous cidofovir in a previous 13-week repeat dose toxicity study.

The 6-month study of intravenous administration of cidofovir by weekly injection to male and female rats at doses of 0.6, 3.0, and 15 mg/kg showed that at the high dose, 22/44 female rats developed mammary adenocarcinomas. Zymbal's

## SECTION 6

Pharmacology and Toxicology

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gland (an auditory tube sebaceous gland in rats) carcinomas were found in 3/44 high dose females and 6/44 high dose males. Multiple masses were present in 10/44 high dose females. Palpable tumors were observed as early as week 12.

The high dose in this study, 15 mg/kg in the rat, is roughly equivalent to a human dose of 2.1 mg/kg using a body surface conversion factor of 7-fold.

The 6-month study in cynomolgus monkeys (5 males and 5 females) of 2.5 mg cidofovir given once weekly by the intravenous route was completed and reported to FDA in September 1995. No tumors were seen on gross or histological examination of tissues. The administered dose corresponds to a human equivalent dose of 0.83 mg/kg based on a body surface conversion. Results of the 12-month study of intravenous cidofovir in monkeys are not currently available. It is estimated that the study will be completed in early 1996.

Summary

The carcinogenic risk to humans of intravenous cidofovir cannot be accurately predicted based on animal studies. Nevertheless, the rapidity with which palpable tumors appeared in rats (as early as 6-weeks), and the small number of doses leading to tumors (as few as 6 doses) is striking. Moreover, the doses causing tumors in rats were lower than the estimated human equivalent dose proposed for clinical use. Therefore, cidofovir must be considered a potent carcinogen in rats, and a potential carcinogen in humans.

These concerns are addressed in the review by Dr. Verma, whose comments have been incorporated into the proposed package labeling.

## SECTION 7

**Clinical Background**

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**7.0 Clinical Background****7.1 Relevant human experience**

Primary cytomegalovirus (CMV) infection is most frequently acquired in the first few years of life. In infancy and childhood transmission occurs through contact with infected cervical secretions, breastmilk, oropharyngeal secretions and urine. By puberty 40% to 80% of children have been infected. In adults, sexual transmission of CMV occurs commonly. The prevalence of CMV seropositivity in adults with AIDS is nearly universal (90-100%).

Neonates infected *in utero* can develop a congenital syndrome resulting in severe neurological deficits, chorioretinitis, hearing loss, hepatitis and pneumonia; auditory deficits may be progressive during the first year of life. Post-natal infection in the neonate may result in severe respiratory infection, while anicteric hepatitis typically occurs in older children. In immunocompetent adolescents and adults, primary CMV infection causes a mononucleosis-like systemic disease which is generally self-limiting.

CMV shares with other members of the Herpesviridae family a state of clinical and virological latency. A propensity to reactivate under immunosuppressive conditions results in viral shedding and possible clinical disease. In individuals with cellular immune dysfunction, reactivation of CMV may lead to severe and progressive disease in multiple organ systems.

The most common manifestation of CMV in patients with AIDS is a progressive, necrotizing retinopathy which, if untreated, may progress to blindness. Other debilitating or life-threatening manifestations of CMV disease in persons with AIDS include polyradiculopathy, colitis, pneumonitis, esophagitis, and hepatitis. CMV disease occurs much more commonly in individuals with advanced HIV infection (CD4+ cell counts less than 50/mm<sup>3</sup>). Median life-expectancy with a diagnosis of CMV retinitis and AIDS currently is 6-12 months. Available antiretroviral and CMV therapies have had little impact on survival duration of this population.

Solid organ transplants recipients often show evidence of active CMV infection, whether newly acquired or through reactivation. Typically, clinically apparent disease presents 1-3 months after transplantation. An interstitial pneumonia is the most common serious manifestation while retinitis is rare in this group.

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### Clinical Background

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Recipients of bone marrow transplants undergo a profound immunosuppression. Interstitial pneumonia due to CMV occurs in 15-25% with a mortality of 90% if untreated. CMV retinitis is also observed in bone marrow transplant patients, reflecting the severe degree of immunosuppression in this population.

In summary, antiviral therapy for CMV infections is likely to have a role in congenitally infected infants, newly infected neonates, cancer patients on chemotherapy, post-transplant patients on immunosuppressive regimens, and in persons with advanced HIV infection.

#### 7.2 Important information from related INDs and NDAs

At present, intravenous formulations of two drugs are approved for the treatment of CMV disease.

##### Ganciclovir

Ganciclovir (Cytovene<sup>®</sup>) was approved in the U.S. in 1989 for the treatment of CMV retinitis in immunocompromised individuals although it was available on a compassionate use basis from 1984. Approval was based largely on an efficacy analysis of a retrospective, non-randomized, single-center study of 41 patients with AIDS and CMV retinitis diagnosed by ophthalmologic examination between August, 1983 and April, 1988. Treatment with Cytovene-IV solution resulted in a significant delay in median time to first retinitis progression compared to untreated controls (71 days from diagnosis versus 35 days from diagnosis). Efficacy was confirmed in a controlled, randomized, study (ICM 1697), conducted between February, 1989, and December, 1990. Immediate treatment with Cytovene-IV was compared to delayed treatment in 42 patients with AIDS and peripheral CMV retinitis; 35 of 42 patients (13 in the immediate-treatment group and 22 in the delayed-treatment group) were included in the analysis of time to retinitis progression. Based on masked assessment of fundus photographs, the mean (95% CI) and median (95% CI) times to progression of retinitis were 66 days (39, 94) and 50 days (40, 84), respectively, in the immediate-treatment group compared to 19 days (11, 27) and 13.5 days (8, 18), respectively, in the delayed-treatment group. Treatment is 5 mg/kg BID for 14 to 21 days followed by maintenance treatment with either 5 mg/kg once daily, 7 days per week or 6 mg/kg once daily, 5 days per week.

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**Clinical Background**

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Intravenous ganciclovir was subsequently approved for prevention of CMV disease in transplant recipients. An oral formulation of ganciclovir was approved in 1994 for maintenance therapy after intravenous induction for treatment of retinitis in patients with AIDS. An additional indication for oral ganciclovir for prevention of CMV disease in HIV infected individuals was approved in 1995.

Ganciclovir was shown to be carcinogenic and teratogenic in animal studies. Neutropenia and thrombocytopenia are the most common dose limiting toxicities of ganciclovir therapy.

**Foscarnet**

Foscarnet (Foscavir<sup>®</sup>) was approved in 1991 for the treatment of CMV retinitis in patients with AIDS. Approval was based primarily on two studies. FOS-03 was a prospective, controlled clinical trial, in which 24 patients with AIDS and non-sight threatening CMV retinitis were randomized to immediate therapy versus no therapy until retinitis progression. All diagnoses and determinations of retinitis progression were made from retinal photographs by ophthalmologists who were masked to the patients' treatment assignment. The 13 patients randomized to treatment with foscarnet had a significant delay in progression of CMV retinitis compared to untreated controls. Median times to retinitis progression from study entry were 93 days (range 21-364) and 22 days (range 7-42), respectively. In another prospective clinical trial of CMV retinitis in patients with AIDS (ACTG-915), 33 patients were treated with two to three weeks of foscarnet induction and then randomized to two maintenance dose groups. Median times from study entry to retinitis progression were 96 (range 14-176) days and 140 (range 16-233) days, respectively (FDA analysis). The same criteria for retinitis progression were used as described above.

Safety and efficacy of foscarnet have not been established for treatment of other CMV infections or for treatment of CMV retinitis in non-immunocompromised individuals. Treatment consists of intravenous infusions over a minimum of one hour every 8 hours for 2-3 weeks as induction, followed by a daily 2 hour infusion. Electrolyte disturbances and renal impairment are the predominant toxicities.

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### Clinical Background

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#### 7.3 Foreign experience

Cidofovir is not approved or marketed in any country for any indication.

Cidofovir is a new molecular entity. No other monophosphorylated nucleoside analogue ("nucleotide analogue") has been approved for marketing in the U.S. or elsewhere.

#### 7.4 Human Pharmacology, pharmacokinetics, pharmacodynamics

The pharmacokinetics of cidofovir were examined at 5 dose levels in three Phase 1/2 studies conducted under IND                      Additional data were obtained at 2 dose levels in 10 subjects in the Phase 2/3 study, GS-93-107, with concomitant probenecid and hydration.

Serum concentrations following intravenous infusion were dose-proportional over the dose range 1-10 mg/kg. Cidofovir was excreted entirely by the kidney, unmetabolized, at a level above the glomerular filtration rate, indicating that active tubular secretion of cidofovir occurred. Probenecid, which is believed to block secretion of cidofovir by renal tubule cells, significantly lowered total clearance, renal clearance, 24 hour urine recovery, and the steady state volume of distribution of cidofovir. This latter effect probably results from a decreased tissue concentration of cidofovir in the kidney; the remaining volume of distribution reflects body water composition.

No metabolites of cidofovir were detected in any sample of serum or urine. Repeat dosing did not alter pharmacokinetics of the drug. Protein binding was negligible.

The CSF level was undetectable following infusion of cidofovir in the 1 patient in which it was measured (corresponding serum level was 8710 ng/mL).

A summary table of the pharmacokinetic parameters is reproduced below:

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## Clinical Background

**Table 1: Cumulative data for Cidofovir with or without probenecid at all dose levels (Data from protocols GS-92-101, -102, -103, and GS-93-107)<sup>1</sup>**

Probenecid	NO	YES	p-value
N	22	25	
CL <sub>tot</sub> (ml/hr/kg)	148 (24.6)	126 (36.2)	0.016
V <sub>ss</sub> (mL/kg)	490 (136)	390 (116)	0.010
T 1/2 β (hr)	2.58 (1.18)	2.51 (0.56)	0.816
CL <sub>r</sub> (mL/hr/kg)	129 (42.1)	87.3 (30.0)	0.002
CL <sub>cr</sub> (mL/hr/kg)	82.7 (20.7)	93.4 (24.7)	0.294
24 hr urine recovery (% dose)	86.0 (17.7)	73.3 (11.9)	0.020

<sup>1</sup>Reproduced from Volume 1.1, page 269, Table 8 of NDA 20-638.  
Values shown represent means (SD). (Sponsor's analysis)

Two dose levels (5 mg/kg and 3 mg/kg) have been studied in the phase 2/3 clinical trials; the intended maintenance dose will fall in this range for most individuals. Additional pharmacokinetic data for these doses accumulated across all studies are listed below:

**Table 2: Cumulative pharmacokinetic data for cidofovir at the 3 and 5 mg/kg dose levels with or without probenecid and hydration<sup>1</sup>**

Dose	3 mg/kg		p-value	5 mg/kg	
	YES	NO		YES	NO
N	10	12		2	6
C <sub>max</sub> (µg/mL)	7.34 (1.39)	9.79 (3.74)	0.075	11.6 (1.5)	19.6 (7.18)
AUC (µg.hr/mL)	20.0 (2.30)	25.7 (8.46)	0.052	28.3 (2.4)	40.8 (8.97)

<sup>1</sup>Reproduced from Volume 1.1, page 268 of NDA 20-638.  
Values shown represent means (SD). (Sponsor's analysis)

Maximum plasma levels and area under the curve increase proportionately in this dose range. Probenecid appears to increase the systemic exposure to cidofovir, as expected if renal elimination is decreased by probenecid.

## SECTION 7

**Clinical Background**

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**7.5 Other relevant background information**

The original Investigational New Drug (IND) application for **cidofovir** was submitted to FDA in March, 1992. The original indication was for the treatment of **CMV retinitis** in patients with the **AIDS**. Pre-clinical studies had defined the kidney as the organ system most likely to be the target of a dose limiting toxicity. Phase 1/2 studies confirmed this suspicion; **proteinuria** and **elevated serum creatinine** were observed, and at least 2 cases of renal failure requiring kidney dialysis occurred. In these early studies the sponsor was able to define a dosing regimen which was less nephrotoxic. The regimen consisted of less frequent dosing, concomitant intravenous hydration, and co-administration of **probenecid**, which was believed to block uptake of **cidofovir** from the plasma by renal tubular cells and therefore block renal tubular secretion of **cidofovir**. The sponsor presented evidence of the enhanced safety profile to FDA in October, 1993, at an end of phase 2 meeting. It was subsequently decided that efficacy trials could ensue, and three clinical trials believed to be sufficient to prove efficacy in an ethically acceptable manner were agreed upon. These trials were patterned in large measure after studies used in the development of **foscarnet**.

and **GS-93-106**, both randomized subjects with non-sight threatening peripheral retinopathy to either immediate treatment or to treatment deferred to that time when it became clear that the retinopathy observed at baseline had progressed a pre-determined extent. A crossover of subjects in the deferred treatment arm to **cidofovir** treatment was allowed at the time of documented progression. The third trial, **GS-93-107**, was designed as an open-label study of **cidofovir** in individuals who had failed existing therapy. It was believed that this latter study would provide additional safety data and could be supportive of efficacy claims if the treatment effect were clearly superior to historical controls.

In July, 1994, after phase 2/3 trials were underway, FDA was made aware by the sponsor of a study in rats which resulted in visible and palpable tumors in a substantial number of animals receiving **cidofovir** by the subcutaneous route. Tumors were observed primarily in the neck and thoracic area. The tumors occurred at doses in the range of the intended human dose after adjustment for

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body-surface area, and after as few as 6 doses. Following discussion between the sponsor and FDA/DAVDP, the sponsor agreed that all clinical trials would be suspended pending further investigation of the tumors.

Subsequent histological analyses revealed that the majority of the tumors were mammary adenocarcinomas. Radioactive tracer studies indicated that subcutaneous administration of cidofovir in the subscapular area in rats resulted in drainage into mammary tissue. The sponsor speculated that tumor formation in rats was the result of local high concentrations of cidofovir on rapidly dividing cells in the mammary glands, and that this situation was not relevant in the setting of intravenous administration in humans. Additional animal studies, including intravenous administration to rats and monkeys, were to be undertaken.

Discussions with the sponsor at this time focused on weighing the risks of further development of a known carcinogen against the potential for clinical benefit from cidofovir, and possible significant quality of life advantages due to its convenient dosing schedule. Considerations included: a) the expected duration of treatment of individuals with CMV retinitis and AIDS, b) tumor formation, should it occur in humans treated with cidofovir, would be a multi-step, long term process, and therefore less likely to occur in this setting, and, c) ganciclovir, an approved CMV therapy, is also a demonstrated carcinogen. Nevertheless, the apparent carcinogenic potency of cidofovir was concerning.

FDA agreed with the sponsor in October, 1994, that clinical trials of cidofovir in pursuit of an indication for the treatment of CMV retinitis in patients with AIDS could resume.

Results of an efficacy analysis of GS-93-106 were reported by the sponsor at a national scientific conference in early 1995. According to the sponsor's analysis, a highly significant treatment effect favoring immediate treatment with cidofovir was demonstrated. In order to report the findings at that time it was necessary to break the blind of assignment to treatment arms and to cease collection of additional data. The Data Safety Monitoring Board for the study disagreed with the sponsor regarding this; as a result, the board disbanded. The DSMB felt that potentially valuable additional safety and efficacy data would be lost were the study to end prematurely. The sponsor's position was that the study was near completion and

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that additional efficacy endpoints would not appreciably alter the statistical significance of the study.

**Reviewer's comment:** At the time the study was stopped, one subject in the primary analysis remained on their original treatment assignment. Stopping the study at that point had little effect on the primary efficacy analysis. Loss of follow-up safety data may have been the greater concern of the DSMB.

A phase 3 assessment meeting between the sponsor and FDA/DAVDP was held in June, 1995, at which time the sponsor stated their intention to file a NDA in the autumn of 1995. FDA expressed concern over the small safety data base. The sponsor was encouraged to provide cidofovir in an expanded access format before filing the NDA in order to enlarge the safety data base. The sponsor agreed and submitted a treatment IND proposal which FDA reviewed and allowed to proceed in early September, 1995. The NDA submission was received at FDA October 4, 1995.

In summary, concerns arising from the development of tumors in animal studies led FDA to restrict subsequent efficacy trials of the intravenous formulation of cidofovir to persons with advanced AIDS. A perception that the convenient dosing regimen could impact positively on quality of life added support for continued development of cidofovir for the population with advanced AIDS.

#### 7.6 Directions for Use

Cidofovir was formulated as a sterile solution containing 25 mg/mL or 75 mg/mL for the clinical trials. Cidofovir was infused into a peripheral vein over a 1 hour period after dilution into 100 mL of 0.9% saline. Subjects receiving hydration were given 1 liter of 0.9% saline over one hour immediately before cidofovir administration. Subjects receiving probenecid were given either 2 grams total (1 g at 3 hours before, and 500 mg at 2 and 8 hours after cidofovir infusions) or 4 grams total (2 g at 3 hours before and 1 g at 2 and 8 hours after infusions).

It is intended that patients will receive 4 gram probenecid and at least 1 liter of saline hydration with each dose of cidofovir in the post-marketing setting.

## SECTION 8

### Data Sources

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#### 8.0 Description of Clinical Data Sources

##### 8.1 Clinical studies

The clinical studies during all phases of the drug development were conducted primarily within the United States; two sites in the United Kingdom and one site in Canada participated the phase 2/3 multicentered trials.

The sponsor has provided FDA with efficacy data from a single controlled clinical trial, GS-93-106. Data from a second trial, GS-93-107, for which historical controls provide the comparative arm, were submitted as additional supportive evidence of efficacy.

The tables below outline the studies conducted under IND which were submitted by the sponsor to support this NDA. At least 8 patients have received cidofovir under investigator-held emergency INDs. In addition to the studies in the tables below, at least 100 subjects have enrolled under a treatment IND.

The safety data base consists of all subjects in the three phase 1/2 studies, all subjects in GS-93-106, and the first 100 subjects enrolled in GS-93-107.

**TABLE 3**  
**Cidofovir Intravenous Phase III Clinical Trials**

Study	Sites	Start/ Stop Dates <sup>1</sup>	Subjects enrolled/planned (through 9/1/95)	CDV-treated subjects enrolled (through 9/1/95)	CDV-treated subjects in report	CDV +P-treated subjects in report	Dosing regimens (P=probenecid)
GS-92-101	2 (UCSF, SFGH)	05-05-92/ 10-29-93	35/39	35	35	15	<ul style="list-style-type: none"> <li>• 0.5, 1.0, 3.0, or 10.0 mg/kg/week (- P)</li> <li>• 3.0 mg/kg/week + P</li> <li>• 5.0 mg/kg/week + P</li> <li>• 5.0 mg/kg q 2 weeks + P (first 2 doses weekly x2)</li> <li>• 7.5 mg/kg q 3 weeks + P</li> </ul>
GS-92-102	1 (Johns Hopkins)	06-23-92/ 11-18-92	22/21	16	16	0	<ul style="list-style-type: none"> <li>• 1.0, 3.0, or 10.0 mg/kg/dose (-P) by IV, PO, and SQ routes with 2-week washout between doses (2 placebo patients/ dose)</li> </ul>
GS-92-103	1 (NIH)	09-21-92/ 09-02-93	21/24	21	21	11	<ul style="list-style-type: none"> <li>• 0.5, 1.5, or 5.0 mg/kg twice weekly (-P)</li> <li>• 5.0 mg/kg twice weekly + 1/2P</li> <li>• 5.0 mg/kg/week + P</li> <li>• 7.5 mg/kg q 3 weeks + P</li> <li>• 5.0 mg/kg q 2 weeks + P (first 2 doses weekly x2)</li> </ul>

<sup>1</sup>Date first patient randomized (date last patient randomized). Adapted from Table 3, Volume 2, page 13 of the submission.

**TABLE 4**  
**Cidofovir Intravenous Phase III/III Clinical Trials**

Study	Sites	Start/ Stop Dates <sup>1</sup>	Subjects enrolled/p lanned (through 9/1/95)	CDV-treated subjects enrolled (through 9/1/95)	CDV-treated subjects in report	CDV +P-treated subjects in report	Dosing regimens (P=probenecid)
GS-93-106	8 (7 US, 1 UK)	12-28-93/ 11-30-94	48/48	41	41	41	<ul style="list-style-type: none"> <li>• deferred therapy</li> <li>• 5.0 mg/kg/week x2, then 5.0 mg/kg q 2 weeks (+P)</li> </ul>
GS-93-107	15 (12 US, 1 CAN, 2 UK)	07-11-94/ ongoing	120/100- 150	118	100	100	<ul style="list-style-type: none"> <li>• previously treated</li> <li>• 5.0 mg/kg/week x2, then 3.0 mg/kg q 2 weeks + P</li> <li>• 5.0 mg/kg/week x2, then 5.0 mg/kg q 2 weeks + P</li> </ul>
<b>Total<sup>2</sup></b>			<b>300</b>	<b>274</b>	<b>242</b>	<b>196<sup>3</sup></b>	

<sup>1</sup>Date first patient randomized / date last patient randomized (enrolled).

<sup>2</sup>Does not include "number of subjects planned."

<sup>3</sup>Includes miscellaneous subjects treated under emergency INDs.  
 Adapted from Table 2, Volume 2, page 14 of the submission.

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**Data Sources**

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**8.2 Post-marketing experience**

Cidofovir has not been approved for any indication in the U.S. At the time of this NDA review, cidofovir has not been licensed for marketing anywhere outside the United States.

**8.3 Literature**

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#### 9.0 Clinical trials

##### 9.1 Indication

The sponsor seeks an indication for intravenous cidofovir for the treatment of CMV retinitis in patients with the acquired immunodeficiency syndrome (AIDS).

##### 9.1.1 Clinical trial GS-93-106

###### 9.1.1.1 Objectives

The primary objective of this study was to determine if intravenous cidofovir could extend the time to progression of newly diagnosed peripheral CMV retinitis in patients with AIDS. Additional information about visual acuity, virologic effects, as well as safety and tolerance data were to be collected and analyzed.

###### 9.1.1.2 Study Design

Subjects diagnosed with non-sight-threatening, peripheral retinitis, who had not previously received therapy for retinitis, were randomized in equal numbers to receive cidofovir immediately (within 24 hours) or to receive no therapy. Cidofovir therapy consisted of an induction course of 5 mg/kg given as once weekly intravenous injections for 2 weeks, followed by a maintenance course of 5 mg/kg every other week until retinitis progression, intolerance or death. Each dose of cidofovir was given with saline hydration and probenecid.

Subjects were followed bi-weekly for the first 2 months and monthly thereafter by fundoscopic photographs which were read at a central site by readers masked to treatment assignment. An endpoint was reached when the border of an existing retinitis lesion progressed by 750  $\mu$ M, or when a new lesion of 750  $\mu$ M appeared. Subjects in the deferred arm, who had met an endpoint were then allowed to "cross-over" and receive cidofovir while being followed by regular fundus photography. These "cross-over" subjects were included in a secondary analysis. Subjects who received immediate cidofovir and who progressed were offered the best available therapy.

The primary analysis of GS-93-106 was time from baseline fundoscopy until an endpoint of retinitis progression or until the subject died. All subjects were to be followed by regular fundoscopic photographs until an endpoint had been reached. The analysis was to be based on intent-to-treat, i.e., all subjects were to be included

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in the analysis whether or not they were able to continue the assigned treatment until an endpoint of retinitis progression or death had been met.

**Reviewer's comment:** According to the protocol, subjects who went on to receive alternative therapies for any reason, including systemic CMV disease or adverse experiences while on cidofovir, were no longer followed by retinal photography. Therefore, the analysis cannot be described as "intent-to-treat".

#### 9.1.1.3 Endpoints

The protocol specified primary endpoints were time from randomization to treatment-limiting toxicity, time to progression of retinitis, or death. Secondary endpoints included mortality and changes in visual acuity.

The treatment-limiting toxicity was defined in the protocol as a grade 3 or 4 nephrotoxicity. Serum chemistries with renal function assessments and hematology profiles were obtained every two weeks.

Retinitis progression was defined in the protocol as the advancement of the edge of an existing lesion by 750  $\mu\text{M}$  or the occurrence of a new lesion 750  $\mu\text{M}$  in diameter in either eye.

#### 9.1.1.4 Results

Forty-eight subjects were randomized to receive either immediate cidofovir (25 subjects) or deferred treatment (23 subjects). All subjects were enrolled between December, 1993, and November, 1994. The study was conducted in 7 clinical centers in the U.S. and 1 center in the United Kingdom. Gary Holland, M.D., at the University of California, Los Angeles, served as the retinal photograph reader; he was masked to treatment assignment.

Most protocol violations were related to drug administration. Eleven per cent of the violations were due to subjects continuing to take concomitant acyclovir during the study (4 subjects, 2 in deferred arm and 2 in immediate arm). No subject was excluded from the safety or efficacy analysis due to a protocol violation.

##### 9.1.1.4.1 Patient Disposition and Comparability of Study groups

###### Immediate and deferred treatment groups

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Of the 25 subjects in the immediate group, 23 had discontinued therapy at the data cut-off date of March 31, 1995. CMV retinitis progression had occurred in 10 subjects, 6 stopped due to an adverse experience, 3 withdrew consent (2 in moribund condition), 2 stopped for intercurrent illness (both CMV gastrointestinal disease), 1 began ganciclovir following an ophthalmologic diagnosis of retinitis progression, and baseline photographs were non-evaluable at baseline for two subjects.

Of the 23 deferred subjects, all 23 had discontinued deferred therapy at the data cut-off. CMV retinitis progression had occurred in 19, alternative therapy was begun for 2 (1 following an ophthalmologist's diagnosis of retinitis progression later unconfirmed by retinal photographs, and 1 for systemic CMV disease), 1 withdrew consent when randomized to the deferred arm, and 1 was excluded due to zone 1 retinitis at baseline.

Baseline characteristics prior to randomization were similar in both the immediate and deferred study groups. The table below indicates similarity based on a p-value derived from a 2-way ANOVA with treatment and institution as main effects or by Cochran-Mantel-Haenzel test stratified by institution (Sponsor's analysis).

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Table 5: Baseline Characteristics (GS-106)

<u>Characteristic</u>	<u>Immediate</u>	<u>Deferred</u>	<u>p-value</u>
Age (mean)	38.4	37.7	0.736
Gender			
male	24	22	0.868
female	1	1	
Race			
White	20	20	0.655
Hispanic	4	2	
Native American	1	0	
Other	0	1	
Time from HIV diagnosis (months)	67.2	78.2	0.470
Time from diagnosis of CMV retinitis (days)	5.8	8.1	0.147
CD4 count ( / $\mu$ L) (median)	6.0	9.0	0.695
Creatinine clearance (mL/min)	99.2	102.3	0.798
Absolute neutrophils ( $\times 10^3/\mu$ L)	2.02	2.28	0.467
Number of retinitis lesions (total both eyes)	1.2	1.7	0.081
Malignancies	8	9	

Adapted from Table 2.1, volume 62, page 144 of the submission

There were no statistically significant differences in subject weight, Karnofsky score, or relevant findings on physical exam. The CD4 cell counts in both groups (6.0 and 9.0) reflects that the study population was severely immunocompromised.

Findings on ophthalmologic exam and in retinal photographs at baseline revealed no statistically significant differences in assessments of lesion activity, intraocular pressure, or visual acuity for either eye. No apparent differences were discernible in the extent of fundal involvement or lesion location. Twenty subjects in the immediate group had no lesions in the better eye versus 17 in the deferred group. No subject in either group had a retinal or foveal detachment in either eye at baseline.

**Reviewer's Comments:** Two apparent trends in the baseline characteristics deserve discussion. The deferred group had more advanced retinal disease based on the total number of retinal lesions ( $p=0.08$ ). More advanced retinitis would likely progress more rapidly, thus shortening the time to progression in the deferred group. Moreover, healthier eyes in the immediate group might be more likely to respond to an active treatment, further exaggerating a treatment effect.

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Another apparent trend may be seen in a delay in the time from diagnosis of CMV retinitis to randomization in the deferred group relative to the immediate group ( $p=0.15$ ). Presumably the delay to randomization would tend to increase the treatment effect by shortening the time to progression in the deferred group. However, the delay is of short duration (mean difference of 2.3 days).

It is unlikely that either of these differences alone would impact the effect size of cidofovir; however, taken together, the differences in treatment groups cited above should be considered if a marginal treatment effect is seen.

**Cross-over and deferred treatment groups**

Of the 16 subjects in the crossover arm, 3 had retinitis progression, 7 discontinued due to adverse events, 3 discontinued for intercurrent illness (one of which was CMV GI disease), 1 withdrew consent, and 2 remained on study drug at the data cut-off.

Baseline characteristics of the 16 cross-over subjects and the 7 deferred subjects who did not cross-over were examined. No significant or apparent differences in baseline characteristics were observed between these two treatment groups.

**Reviewer's comment:** The "baseline" evaluations in these comparisons were taken as the last assessments prior to randomization. A more accurate reflection of baseline characteristics for the cross-over group would be assessed at the time of crossover, if one wishes to validate efficacy endpoint comparisons.

**Drug exposure**

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The following table outlines the extent of cidofovir exposure in the immediate and crossover groups:

Table 6: Drug exposure

	<u>Immediate</u>	<u>Crossover</u>
N	25	16
Cumulative number of doses		
Minimum	2	4
Median	4	6.5
75%tile	6	8
Maximum	13	22
Mean	5.5	7.6
Cumulative dose (mg/kg)		
Minimum	8.1	18.0
Median	20.3	28.5
75%tile	30.5	40.5
Maximum	63.0	90.0
Mean	26.9	33.8

Adapted from Table 1.8, Volume 62, page 138 of the submission.

**Reviewer's comment:** No statistical analysis of the difference in cidofovir exposure between the two groups was provided by the sponsor. It is readily apparent, however, that the crossover group was able to remain on cidofovir for a more prolonged period; whether this is due to delay in progression or better tolerance of drug toxicities is unknown. These differences are probably best explained by "self-selection" for further study participation; healthier subjects were able and willing to remain on study and to cross over to the treatment phase. This is one reason why comparisons of treatment effects for the cross-over group relative to the deferred group who did not cross-over is not a valid comparison.

#### 9.1.1.4.2 Efficacy endpoints outcome

##### 9.1.1.4.2.1 Primary endpoint

The primary protocol-specified outcome measure of GS-93-106 was time to CMV retinitis progression or death, whichever came first. Using this endpoint, comparisons of the immediate vs. deferred group and the crossover vs. deferred group were made.

#### Immediate treatment versus deferred treatment

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The median time to CMV-retinitis progression or death in the sponsor's analysis was 86 days (95% CI 40-not reached) in the immediate treatment group versus 22 days (95% CI 13-31 days) for the deferred group. This was significant in the log-rank test at  $p < 0.001$ .

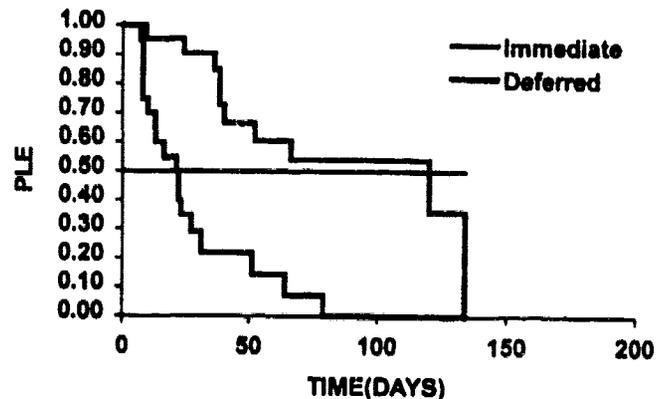
The sponsor also chose to analyze time to progression of retinitis without consideration of deaths. The estimate of the median time to retinitis progression in a Kaplan-Meier plot for the immediate group was 120 days (95% CI 40-134 days) compared to 21.5 days (95% CI 10-27 days) in the deferred group. This was statistically significant in the log-rank test ( $p < 0.0001$ , sponsor's analysis). A Kaplan-Meier plot of the sponsor's analysis follows:

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Figure 1

## Kaplan-Meier Plot of Time to CMV Retinitis Detection



**Reviewer's comment:** The protocol specifies that all subjects were to be followed by retinal photographs until retinitis progression, start of alternative therapy, or death, whichever came first. Protocol-specified endpoints were not captured after most adverse events, intercurrent illnesses, and withdrawn consents. It was the investigator's judgement in most of these cases that alternative therapy should be initiated, after which, per protocol, no subsequent photographs were obtained. The sponsor's approach to these missing data was to "censor" the data from those subjects at the time of the last retinal photography showing no progression.

In the immediate therapy group, 12 subjects lacked follow-up retinal photographs. This occurred in 5 subjects who stopped cidofovir due to adverse events, 4 who withdrew consent, 2 with intercurrent illnesses (both with systemic CMV disease). Two subjects had baseline photos which were not evaluable and were censored at baseline. One subject continuing on cidofovir was censored at the data cut-off.

In the deferred group, 4 subjects were censored for reasons stated above.

#### Treatment after cross-over versus deferred treatment

Time to retinitis progression or death for the cross-over group was not analyzed or presented by the sponsor. A time to second progression analysis was presented. The comparator group in this analysis was the same group of 15 subjects as

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participants in the deferred therapy arm prior to cross-over (one subject had a baseline photograph which was not evaluable and so was not included in the cross-over vs. deferred analysis). The sponsor used a paired Prentice-Wilcoxon Z-statistic to compute  $p=0.0002$ .

**Reviewer's comment:** Although the sponsor refers to the "cross-over group", the design of this part of the clinical trial is not a true crossover design. Subjects are not randomized to the crossover group. Moreover, because the subjects are not re-randomized, they maintain their original patient numbers and the reader of the retinal photographs is no longer blinded to the treatment assignment; in fact, the reader fills out a "new baseline" form for the subject at the time of crossover. This part of the study is truly open-label in this respect.

The sponsor has chosen a statistical test involving pairing of a subject's time to retinitis in the pre-crossover phase with the same subject's time to retinitis progression in the post-crossover period. It might be expected that because crossover subjects have documented progression of their CMV disease, further progression will occur more rapidly, thus biasing against a treatment effect. However, randomization is intended to normalize groups with respect to both expected and unexpected effects.

Interpretation of a treatment effect in the crossover group must necessarily be of a qualitative nature, rather than statistical, and assessed against a background of historical data.

#### 9.1.1.4.2.2 Secondary endpoints

##### Mortality

No subject died during the treatment phase of this study. Of the 32 subjects for whom follow-up survival information through 10/1/95 was available, 29 had died. Subjects for whom no updated information was available were censored at the date of the last follow-up date. The median time to death for subjects in the immediate therapy arm was 410 days (95% CI 239 - not reached) vs. 319 days (95%CI 263-353) in the sponsor's analysis.

For the 16 subjects in the crossover arm, the median time to death was 322 days (95%CI 277-413 days) versus 200.5 days (95%CI 146-286) for the 7 subjects who elected not to take cidofovir after the first progression.

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Visual acuity

One subject in the immediate group experienced a transient  $\geq 3$  line decrease in visual acuity on the EDTRS chart while receiving cidofovir. Two subjects in the cross-over group experienced a  $\geq 3$  line decrease in visual acuity. No analysis was attempted due to the small numbers.

Virological parameters

Two centers participated in the collection of blood and urine specimens at baseline, and at 3 and 11 weeks into the study. Specimens were examined by traditional culture methods and by the shell-vial technique. The total number of specimens was small; the sponsor did not attempt a statistical analysis. The sponsor does cite examples of positive cultures turning negative and negative cultures remaining so.

**Reviewer's Comment:** One way to look at this data is to pool the subjects receiving cidofovir from the immediate and cross-over arms. Greater numbers of specimens were cultured by traditional methods and as this technique remains the "gold standard" these results are considered in the reviewers's analysis below:

Table 7: Clinical Virology

	<u>On cidofovir</u> n positive/N (%)	<u>Deferred</u> n positive/N (%)
<u>Blood:</u>		
Baseline	6/15 (40)	2/8 (25)
Week 3	7/15 (47)	6/8 (75)
Week 11	4/8 (50)	0/0 (0)
<u>Urine:</u>		
Baseline	10/11 (91)	7/8 (88)
Week 3	3/10 (30)	3/3 (100)
Week 11	3/7 (43)	0/0 (0)

Adapted from Tables 5.1, 5.3, 5.5, and 5.7 of Volume 62, page 219-25.

When the virologic data is arranged in this way, it appears that cidofovir has little effect on viremia but an apparent effect on viruria. The proposed mechanism of renal toxicity is via active secretion of cidofovir at the renal tubule, so it may not be

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surprising that an anti-CMV effect would be seen in urine. The apparent lack of activity in blood raises concern about the ability of cidofovir to impact on systemic CMV disease.

## 9.1.1.4.3 Safety Comparisons

Mortality

No deaths occurred while subjects were receiving study drug. Three deaths occurred within 30 days of the last dose of cidofovir. Investigators did not consider cidofovir to be related to these deaths.

**Reviewer's comments:** Case report forms for each death occurring within 2 months of the last dose of cidofovir, for both the immediate and cross-over arms, were reviewed:

<u>Subject</u>	<u>Cause of Death</u>
102	Cryptococcal meningitis; Grade 3 creatinine elevation, grade 2 LFTs, metabolic acidosis; received 22 doses;
2303	CNS lymphoma present at entry; discontinued after 103 days;
2306	AIDS complications, general debilitation; received 2 doses;
2607	Brain tumor and hemorrhage; received 2 doses; multiple brain lesions present by CT on day 16.
3602	Wasting syndrome, toxoplasmosis; received 3 doses;

No pattern is apparent indicating drug-related toxicity contributing to death.

Mortality was also a secondary endpoint for efficacy; Kaplan-Meier plots for the different treatment arms with relevant comparisons appear above. For the immediate treatment group the median time to death was 410 days (95% CI 239-not reached) versus 319 days (95%CI 263-353 days) for the deferred group. The sponsor used a chi-square test with one degree of freedom to yield a p-value of 0.1488.

In the crossover arm the median time to death was 322 days (95%CI 277-413 days) while for the deferred group of 7 subjects who did not cross over it was 200.5 days (95% CI 146-286 days). The p-value for this comparison in a chi-square test was p=0.681 (Sponsor's analysis).

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**Malignancies**

Malignancies were present at study entry in 8 of 25 (32%) subjects in the immediate group, and in 9 of 23 (39%) in the deferred group. Through the follow-up period new AIDS related malignancies occurred in 5 subjects in the immediate group (Kaposi's sarcoma 2, B-cell lymphoma 1, cervical neoplasia 1, and possible brain tumor 1) and in 1 subject from the deferred group (Kaposi's sarcoma). Tumors not associated with AIDS, including adenocarcinomas, were not observed in either group throughout the treatment period.

**All adverse experiences or intercurrent illnesses**

The most common specific adverse events or intercurrent illnesses of any severity, regardless of assessed relationship to cidofovir, for all subjects receiving cidofovir are listed below in decreasing order of frequency of their occurrence:

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Table 8: All Adverse Events<sup>1</sup>

Adverse event	Deferred N=23 (%)	Immediate N=25 (%)	Immediate+Crossover N=41 (%)
Total with AE	15 (65)	25 (100)	41 (100)
Proteinuria ( $\geq 1+$ )	5 (22)	17 (68)	29 (71)
Fever	2 (9)	12 (48)	24 (59)
Asthenia	2 (9)	14 (56)	23 (56)
Nausea with vomiting	3 (13)	10 (40)	21 (51)
Neutropenia	1 (4)	9 (36)	15 (37)
Rash	1 (4)	11 (44)	15 (37)
Headache	0 (0)	10 (40)	14 (34)
Diarrhea	1 (4)	7 (28)	13 (32)
Chills	0 (0)	8 (32)	13 (32)
Alopecia	0 (0)	5 (20)	12 (29)
Abdominal pain	2 (9)	8 (32)	12 (29)
Infection	0 (0)	6 (24)	12 (29)
Nausea w/o vomiting	3 (13)	9 (36)	11 (27)
Pain	0 (0)	6 (24)	10 (24)
Insomnia	0 (0)	4 (16)	9 (22)
Anorexia	4 (17)	3 (12)	8 (20)
Sweating	0 (0)	5 (20)	7 (17)
Myalgia	0 (0)	6 (24)	7 (17)
Oral moniliasis	0 (0)	5 (20)	7 (17)
Pruritus	1 (4)	3 (12)	6 (15)
Anemia	0 (0)	1 (4)	6 (15)
Weight loss	1 (4)	3 (12)	6 (15)
Dyspnea	0 (0)	3 (12)	6 (15)
Increased cough	0 (0)	4 (16)	6 (15)
Rhinitis	0 (0)	6 (24)	6 (15)
Dizziness	0 (0)	2 (8)	5 (12)
Dehydration	0 (0)	1 (4)	5 (12)
Herpes simplex	2 (9)	2 (8)	5 (12)
Creatinine increased	0 (0)	2 (8)	5 (12)

<sup>1</sup>Includes adverse experiences that began after start of cidofovir but  $\leq$  30 days after last infusion.

Adapted from Tables 6.2.1, Volume 62, pages 236-40.

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**Reviewer's comments:** Although informative, this presentation of the adverse events is somewhat misleading. Time spent (person days) on study in the deferred arm was less than the time spent on cidofovir. This would tend to exaggerate the relative frequency of adverse events in the treated group. Nevertheless, this is the only study presented by the sponsor in which comparisons of adverse events can be made with respect to an untreated group.

Proteinuria ( $\geq$  1+ or 0.3 mg/dL) was the most common adverse event following cidofovir administration. The incidence of proteinuria presented above differs from figures presented in the tables of the NDA, which were much lower, and probably were based on the adverse event field of the case report form rather than tabulated from the laboratory data. For entry into the study, baseline urine protein was required to be <1+. At the time of cross-over this requirement was relaxed to allow patients with 1+ protein to receive cidofovir. Six patients entered the crossover arm with urine proteins of 1+; 3 of the 6 progressed to  $\geq$  3+ urine protein after receiving cidofovir.

General systemic symptoms of asthenia, fever, chills, pain and insomnia in the treated group were very common.

Gastrointestinal symptoms of nausea, vomiting, abdominal pain, diarrhea, and anorexia indicate that the GI tract may be a principal target organ of treatment toxicity. Investigator's tended to attribute these findings to probenecid.

Myalgia of mild or moderate intensity occurred in 7 (17%) of subjects taking cidofovir; in no case was the myalgia considered serious by investigators.

Alopecia occurred frequently (29%) and was considered severe in at least 1 case.

No cases of pancreatitis were recorded during the study period. Sub-clinical pancreatic inflammation might have been missed because serum amylase was not a protocol specified laboratory assessment.

No cases of liver failure were observed during the study period. Two subjects in the crossover group (subjects 111 and 3601) had grade 3 SGOT or SGPT elevations.

Findings of dyspnea, increased cough and rhinitis were unexpected; it is unclear how these symptoms may be related to drug exposure.

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Serious Adverse events

Two subjects in the deferred group had serious adverse events during or within 30 days of discontinuing the study. One subject (101) had a new diagnosis of PCP and KS; the other (3808) had CMV colitis and thrombocytopenia.

**Reviewer's comment:** It should be noted that the sponsor classified adverse events as serious in all of their tables based on the investigators' subjective assessment of seriousness. Toxicity grading scales included in the study protocols were used for dose modifications but not for adverse event classification.

Adverse events graded as serious by investigators which occurred in > 5% of subjects in the combined treated group are in the table that follows:

**Table 9: All serious adverse events<sup>1</sup>**

<u>Adverse event</u>	<u>Deferred N=23 (%)</u>	<u>Immediate N=25 (%)</u>	<u>Immediate+Crossover N=41 (%)</u>
Total with serious adverse event	2 (8)	13 (52)	22 (54)
Neutropenia	0 (0)	5 (20)	8 (20)
Proteinuria	0 (0)	2 (8)	5 (12)
Fever	0 (0)	3 (12)	5 (12)
Infection	0 (0)	2 (8)	5 (12)
Diarrhea	0 (0)	2 (8)	3 (7)
Nausea+ vomiting	0 (0)	2 (8)	3 (7)
Death	0 (0)	1 (4)	3 (7)
Pneumonia	1 (4)	3 (12)	3 (7)

<sup>1</sup>Includes adverse experiences occurring up to 30 days after last infusion.  
Adapted from Table 6.1.3, Volume 62, pages 233-4.

Using the sponsor's toxicity grading scale as objective criteria to classify abnormal laboratory assessments, where any grade 3 or 4 toxicity is considered serious yields the data in the following table:

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Table 10: Serious Adverse Events Based on Toxicity Scale<sup>1</sup>

<u>Abnormal Laboratory Assessment</u>	Deferred	Immediate	Immediate +Crossover
	<u>N (%)</u>	<u>N (%)</u>	<u>N (%)</u>
Proteinuria	5 (22)	17 (68)	26 (63)
(≥ 2+ or 100 mg/dL)			
Neutropenia (≤ 500/mm <sup>3</sup> )	0 (0)	7 (28)	10 (24)
Creatinine (≥ 2.0 mg/dL)	0 (0)	1 (4)	3 (7)
SGOT or SGPT (> 5x nl)	0 (0)	0 (0)	2 (5)

<sup>1</sup>Includes assessments within 30 days of last infusion of cidofovir for the treated groups.  
Adapted from Table E, Volume 62, pages 68-69.

When the investigator's assessment of relatedness of adverse experience to cidofovir is considered, the following frequencies were tabulated:

Table 11: Serious adverse experiences possibly or probably related to study drug<sup>1</sup>

<u>Adverse event</u>	Immediate	Crossover	Immediate + Crossover
	<u>N=25 (%)</u>	<u>N=16 (%)</u>	<u>N=41 (%)</u>
Total with serious adverse event	7 (28)	4 (25)	11 (27)
Neutropenia	4 (16)	2 (13)	6 (15)
Proteinuria	2 (8)	3 (19)	5 (12)
Creatinine increased	0 (0)	2 (13)	2 (5)
Peripheral neuritis	1 (4)	0 (0)	1 (2)
Glycosuria	0 (0)	1 (6)	1 (2)
Headache	1 (4)	0 (0)	1 (2)
Fanconi-like syndrome	0 (0)	1 (1)	1 (2)

<sup>1</sup>Includes adverse experiences occurring up to 30 days after last infusion for the treated groups.

Serious adverse events occurred in over half of the subjects receiving cidofovir, while only 2 serious adverse events occurred in the deferred group. Investigators assessed a relationship between study drug and a serious adverse experience in over 25% of subjects.

Investigators did not believe any of the 4 deaths occurring within 30 days of a dose of cidofovir were related to the drug. Investigators saw no relationship between cidofovir and serious episodes of nausea and diarrhea, or serious infections, pneumonia and fever.

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**Reviewer's comments:** Specific toxicities of greatest concern will be addressed in greater detail below.

Renal Toxicity

Conservative entry criteria and conservative criteria for dose modification were included in the study design in order to minimize serious renal toxicity. These measures were largely successful, as only 3 of 41 (7%) treated subjects developed a serum creatinine which exceeded 2 mg/dL; none of these exceeded 3 mg/dL.

The sponsor cites the incidence of proteinuria ( $\geq 2+$ ) in the deferred group (22%) as an indication that much of the renal toxicity seen in subjects treated with cidofovir is actually due to the underlying disease process. However, cidofovir treatment accounts for greater than a 3-fold excess of cases of proteinuria relative to that observed in the deferred group (68% vs 22%).

Maintenance dose reductions, from 5 mg/kg to 3 mg/kg, were required for 5 of 25 (20%) of subjects in the immediate group and for 7 of 16 (44%) in the crossover group; overall 29% of treated subjects required dose reductions based on protocol specified criteria.

Neutropenia

In the tables above, the sponsor's assessment of serious neutropenia relies on the investigator's subjective assessment of the condition, rather than on objective criteria. Absolute neutrophil counts (ANC)  $\leq 625$  /mm<sup>3</sup> ( $\geq$  grade 2 toxicity) were observed in 15 of 41 (36%) treated subjects. Absolute neutrophil counts  $\leq 500$  /mm<sup>3</sup> (i.e.  $\geq$  grade 3 toxicity) were observed in 10 of 41 (24%) treated subjects. Life-threatening, grade 4 neutropenia ( $\leq 250$  cells/mm<sup>3</sup>) occurred in 3 of 41 (7%) treated subjects (103, 113, and 2304); these latter subjects progressed or discontinued for reasons other than neutropenia.

No subject discontinued Vistide due to neutropenia; however the protocol did not specify study discontinuation for low neutrophil counts. Per protocol, if the ANC fell below 500 cells/mm<sup>3</sup>, cidofovir treatment was to be withheld until ANC  $> 500$  cell/mm<sup>3</sup> and G-CSF therapy was to be instituted. Granulocyte colony stimulating factor (G-CSF) was used by 13 (32%) of the 41 treated subjects.

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Metabolic acidosis

No cases of severe metabolic acidosis were identified by the investigators or the sponsor. However, screening the database for bicarbonate  $\leq$  16 meq/L identified 3 subjects, two of whom received *cidofovir*:

Subject 102, in the crossover group, received 22 doses of *Vistide*. The bicarbonate was 10 meq/L on 11/7/94, 13 days after the last dose of *Vistide* (10/25/94), which was discontinued for the adverse events of creatinine elevation (2.8 mg/L) and 2+ proteinuria. A diagnosis of **Fanconi's syndrome** was made at the time of his low bicarbonate level. He died of cryptococcal meningitis on 11/17/94. Grade 2 liver enzymes elevations were also present.

Subject 116, also in the cross-over group, received 7 doses of *Vistide*, which was discontinued due to creatinine elevations and proteinuria. A bicarbonate of 16 meq/L was observed on 1/10/95, 7 days after his last dose of *Vistide*. Glycosuria (3+), and low blood uric acid levels were also present, indicating that this subject also had elements of **Fanconi's syndrome** (see summary of safety for a discussion of Fanconi's syndrome). Liver enzyme elevations were mildly elevated (grade 1). The bicarbonate was 19 meq/L on follow-up 3 weeks later (1/31/95).

Peripheral neuropathy

Severe peripheral neuropathy, considered serious by the investigator, occurred in one subject who had a history of peripheral neuropathy at study entry. This subject had taken ddC and ddI in the past and was taking ZDV, ethambutol, and other medications during the *cidofovir* study. Nevertheless, the neuropathy seemed to exacerbate with *Vistide* administration, was thought to be possibly related to *Vistide* by the study investigator, and prompted study discontinuation.

**9.1.1.5 Reviewer's Comments and Conclusions**

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Time to progression of retinitis is also generally considered by ophthalmologists to be a valid indicator of a treatment effect. Changes in visual acuity usually occur late in the disease process and are largely irreversible. Assessments of disease "activity" are probably more subjective than a photographic assessment of "time to progression" of the retinitis to a pre-determined extent.

Dr. Wiley Chambers, Acting Division Director in the FDA/CDER Division of Ophthalmologic Drug Products, was consulted to assist in verification of the primary data. Slides of the retinal photographs for each patient in the primary analysis were read by Dr. Chambers and the time to retinitis progression was recorded. Differences between the readings of Dr. Chambers and Dr. Holland occurred for several cases; however under similar censoring rules the Kaplan-Meier curves were qualitatively similar. Assured of the reliability of the primary data, and given no reason to suspect bias on the part of Dr. Holland, the sponsor's primary data was used in subsequent analyses.

Statistical concerns in the analysis of GS-93-106 center around the problem of missing data. Follow-up retinal photography was incomplete for a variety of reasons. Of the 48 subjects enrolled, protocol defined endpoints were not available for 20 (42%) in the pre-crossover study period. These are discussed below:

The following subjects in the immediate arm were censored prior to a protocol-defined endpoint:

<u>Subject #</u>	<u>Day discontinued</u>	<u>Reason for discontinuation</u>
0104	117	proteinuria
0106	89	elevated creatinine
0110	85	esophageal/ colonic ulcerations (CMV)
0113	35	dehydration and electrolyte imbalance
2304	120	proteinuria
2305	57	proteinuria
2306	7	consent withdrawn; inconvenience
2402	50	retinitis progression by exam; no AE
2403	1	baseline photos non-evaluable
2606	8	CMV colitis
2607	31	consent withdrawn; neuropathy worse
3602	27	consent withdrawn; wasting and Toxo
3802	1	baseline photos non-evaluable
3803	85	felt ill, nausea, vomiting, diarrhea
3804	103	proteinuria
3811	135	remained on drug at study cut-off

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The following subjects in the deferred arm were censored prior to a protocol defined endpoint:

<u>Subjects #</u>	<u>Day discontinued</u>	<u>Reason for discontinuation</u>
0112	1	entry criteria not met (Zone 1 retinitis)
2302	1	baseline photos non-evaluable
2401	2	began alternate therapy when deferred
3806	28	alternate therapy; progression by exam
3808	24	CMV colitis; began alternate therapy

The following subjects in the cross-over arm were censored prior to a protocol-defined endpoint:

<u>Subjects #</u>	<u>Day discontinued</u>	<u>Reason for discontinuation</u>
0102	274	creatinine elevation
0105	51	proteinuria
0107	81	creatinine elevation
0111	63	proteinuria
0114	77	probenecid reaction; bilateral iritis
0115	57	presumed CMV viremia
0116	95	creatinine elevation;
2303	78	debilitation; Toxo vs lymphoma
2404	59	on therapy at study cut-off
2605	106	probenecid reaction
3805	115	consent withdrawn
3810	71	excluded medication (Ampho B)

The sponsor's approach to missing data was to "censor" the data at the time of the last funduscopy if the subject did not return for follow-up fundal photographs. This would not be a statistically valid approach if the reason for "dropping out" was related to the study medication or the disease process. This situation occurs when subjects "drop out" following adverse events likely attributable to cidofovir, such as for renal toxicity or neutropenia (and if the probability of toxicity is related to disease status), or when subjects cease cidofovir treatment due to advancement of systemic CMV disease, or when alternative therapy is initiated due to the examining physician's assessment of retinitis progression.

A more conservative and statistically more acceptable analysis would be to assign an endpoint to each subject censored for incomplete follow-up prior to the data cut-off. In most cases designation of the endpoint would be at the time of the last retinal photograph showing no progression. Such an analysis would represent a "worst case scenario" for subjects on the cidofovir arm, and would likely underestimate a

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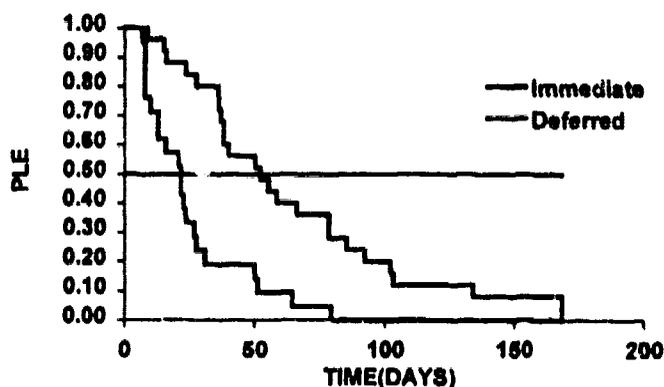
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treatment effect. Nevertheless, if such an analysis were to demonstrate statistical significance, efficacy of the drug would be demonstrated for this particular trial.

When this type of analysis was carried out, subjects treated with cidofovir had a median time to progression of 52 days vs 22 days for the deferred arm; this difference was significant in a log-rank test. Therefore, a claim of efficacy using the primary, protocol-specified endpoint is supported and confirmed by the FDA conservative analysis. A Kaplan-Meier plot of this comparison follows:

Figure 2

Kaplan-Meier Plot of Time to Event



Estimates of the median time to progression which were included in the labels of ganciclovir and foscarnet were based on "time to retinitis progression" rather than "time to retinitis progression or death." The statistical reviewers at this time believe that the more valid analysis considers death to be an endpoint. Time to retinitis progression or death was in fact specified as the primary endpoint in the protocol. This becomes a concern in the primary analysis because, although no one died on study, 3 subjects in the immediate group withdrew consent in a moribund state. The sponsor had censored these subjects at the time of the most recent photograph showing no progression prior to the withdrawn consent. Using the date of death for the endpoint lowers the estimate of the median time to progression (120 days to 86 days in the sponsor's analysis). The sponsor has argued under equal treatment they should be permitted to include the higher estimate in the drug label.

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It is recognized in the reviewing division that some clinically meaningful estimate of a treatment effect may be useful to include in the marketing label. For the treatment-naive population studied in GS-93-106 it would be important to estimate what fraction of the population of AIDS patients with CMV retinitis would be able to tolerate cidofovir. It should also be pointed out that although subjects ceased study medication for an adverse event of proteinuria of 2+ ( $\geq 100$  mg/dL) or greater, this was done on the side of caution as proteinuria was considered to be an early sign of renal toxicity. However, proteinuria is a non-specific finding and, as the sponsor points out in the safety analysis, it occurs commonly in patients with AIDS, due to HIV-induced nephropathy, or to other AIDS-related illnesses or concomitant medications. It might be expected that in the clinical setting the proportion of individuals able to tolerate cidofovir would be greater than figures which follow.

Of the 25 subjects who received cidofovir in the immediate therapy arm, 6 (24%) were able to remain on drug, retinitis progression-free, for a period of 3 months, 3 for a period of 4 months or more, and no patient tolerated the drug without progression for as long as 6 months. Of the 16 subjects who received cidofovir in the cross-over arm, the proportions able to tolerate drug, retinitis progression-free 4 of 16 (25%) at 3 months and 1 of 16 (8%) at 6 months.

For the subpopulation of patients that are able to tolerate the drug one would like to estimate an expected treatment effect. In this analysis, subjects who experienced dose limiting adverse events or who dropped out for reasons other than treatment failure would be censored at the time of the last fundal photograph showing no progression. One might justify such an analysis statistically by stating that the population of interest is that group which is able to tolerate the drug, rather than the group made up of the entire treatment-naive population. Recognizing that this is a post-hoc analysis, it may provide a clinically meaningful estimate of a treatment effect for those able to tolerate the drug. Thus "censoring" subjects who did not meet an endpoint at the time of the last photo showing no progression led to a median time to progression of 120 days (95% CI 36-134 days) for time to retinitis progression ignoring death as an endpoint. This analysis, in effect, duplicates the sponsor's efficacy analysis.

Three subjects in the primary analysis developed systemic CMV disease, two in the treated arm (subjects 0110 [biopsy proven duodenal ulcers due to CMV] and 2606 [CMV colitis]), and one in the deferred arm (subject 3808 [biopsy proven CMV colitis]). While the trial was designed to examine a therapy for retinitis, the drug's effect on systemic CMV disease is also of interest. Subjects with progressing systemic disease would require additional or alternative anti-CMV therapy. If one considers the endpoint of the study to be time to progression of CMV retinitis or a diagnosis of other systemic CMV disease, the Kaplan-Meier estimates of a median

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time to progression is 85 days in the immediate group and 21.5 days in the deferred group.

The safety analysis shows that **cidofovir** is associated with significant toxicities. Should the drug delay onset of retinitis progression but hasten the patients' demise through drug induced toxicities it would be of questionable clinical value. The survival analysis is compromised by the crossover design, use of alternative therapies after progression or adverse events, and lack of follow-up of vital status in about a third of the study participants. Qualitative comparisons may be made to the non-crossover subjects in the deferred therapy arm (N=7), and reference can be made to historical data. This type of analysis is more likely to expose lethal toxicities than to validate any true survival benefit of **cidofovir**, as subjects often go on to receive **ganciclovir** or **foscarnet**.

**Table 11a: Median Time to Death by Treatment**

	N (%) <sup>1</sup>	Median time to death (days)	95% CI
Immediate group	25 (80)	410	239-not reached
Crossover group	16 (94)	322	277-413
Deferred group	23 (87)	319	263-353
Deferred no crossover	7 (71)	200.5	146-286

<sup>1</sup>Number in parentheses indicates the % of subjects with follow-up survival data available at the time of study cut-off.

From these data it appears that treatment with **cidofovir** is not associated with excessive mortality and that, despite overlap of confidence intervals, there is a trend toward increased survival with treatment. It should be pointed out that survival follow-up information was not complete at the study cut-off (March, 1995) and that more recent survival information (January, 1996) is missing in 5 of the 48 subjects (2 from the immediate and 3 from the deferred groups).

The mortality data shown above may be compared to historical data cited by the sponsor from two studies of newly diagnosed and previously untreated subjects:

- 1) The SOCA research group and the AIDS Clinical Trials Group; Mortality in patients with acquired immunodeficiency syndrome treated with either **foscarnet** or **ganciclovir** for cytomegalovirus retinitis; *NEJM* 1992;326:213-220.

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The median survival time for those taking foscarnet was 12.6 months vs 8.5 months for those taking ganciclovir. Confounding factors included concomitant use of AZT.

- 2) Drew WL et al.; Oral ganciclovir as maintenance treatment for CMV retinitis in patients with AIDS; *NEJM* 1995;333:615-620.

Groups receiving either intravenous or oral maintenance ganciclovir had similar median survival times of 11 months.

Additional survival information for subjects with HIV infection and CD4 cell counts < 50/mm<sup>3</sup> comes from:

- 3) Apolonio EG et al.; Prognostic Factors in Human Immunodeficiency Virus-Positive Patients with a CD4+ Lymphocyte Count < 50/ $\mu$ L; *J Infect Dis* 1995;171:829-36,

For subjects with a "very severe" AIDS diagnosis (CMV disease, MAC, toxoplasmosis, or lymphoma), the median survival time from the first D4 count < 50 was 0.64 years (7.7 months) regardless of treatment.

From a study not cited by the sponsor:

- 4) Palestine AG et al. A randomized, controlled trial of foscarnet in the treatment of CMV retinitis in patients with AIDS; *Ann Int Med* 1991;115:665-673)

The median survival in previously untreated subjects given foscarnet was reported as longer than 12 months.

These historical data provide some reassurance that survival in cidofovir treated subjects is within the range of expected survival times for patients with CMV disease of similar duration and severity, who received alternative therapies. No statement can be made with respect to any survival advantage due to cidofovir over existing therapies.

#### Summary of GS-93-106

The study design and conduct of GS-93-106 were acceptable to FDA as a basis for an efficacy claim. A statistically significant positive treatment effect of cidofovir for the treatment of CMV retinitis in subjects with AIDS was demonstrated using a conservative analysis of time to progression of retinitis or death (FDA analysis). No

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definitive statement regarding an association between cidofovir administration and increased or decreased mortality in treatment-naive subjects can be made. For the treatment-naive subjects able to tolerate cidofovir (on drug analysis), a point estimate of the median time to progression is 120 days.

Adverse experiences occurred in 100% of subjects. Body as a whole and gastrointestinal symptoms were most common. Alopecia occurred in 29%. proteinuria (63%) and neutropenia (20%) were the most common serious adverse events. Metabolic acidosis and Fanconi's syndrome were also associated with Vistide administration in this trial.

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#### 9.1.2 Clinical Trial GS-93-107

##### 9.1.2.1 Objectives

The primary objectives of this study were to evaluate the safety and tolerance of cidofovir, and to determine the time to progression of relapsing CMV retinitis in AIDS patients. The impact of cidofovir on visual acuity was also evaluated.

##### 9.1.2.2 Design

This is an ongoing, open-label, multicenter study of two doses of cidofovir in a population of AIDS patients with CMV retinitis that was previously treated with ganciclovir and/or foscarnet. Subjects are eligible to participate if they have failed a course of ganciclovir and/or foscarnet therapy or if they are intolerant to the available therapies. Subjects are randomized to one of two bi-weekly maintenance doses of cidofovir (5 mg/kg or 3 mg/kg); both groups receive an induction course of 5 mg/kg/week for two weeks. All doses of cidofovir are accompanied by intravenous hydration and oral probenecid.

For the first 100 subjects enrolled in GS-93-107, time to progression of CMV retinitis was determined by reading of retinal photographs at a centralized center by readers masked to treatment assignment (subsequent subjects in GS-107 were followed by ophthalmologic exam alone, and are not included in the efficacy analysis below). Fundal photographs were obtained at baseline, at 3 weeks, and monthly thereafter. Subjects were examined by an ophthalmologist every two weeks; when lesions were suspected of having progressed, photographs were obtained at these visits. Treatment was to continue until retinitis progression, death, or a limiting toxicity occurred. A complete ophthalmologic exam with photography was scheduled at the end of treatment.

##### 9.1.2.3 Endpoints

The protocol specified primary endpoints were time from randomization to treatment-limiting toxicity, time to progression of retinitis, or death. Secondary endpoints included mortality and changes in visual acuity.

The treatment-limiting toxicity was defined in the protocol as a grade 3 or 4 nephrotoxicity. Serum chemistries with renal function assessments and hematology profiles were obtained every two weeks.

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Retinitis progression was defined in the protocol as the advancement of the edge of an existing lesion by 750  $\mu\text{M}$  or the occurrence of a new lesion of 750  $\mu\text{M}$  in diameter in either eye.

## 9.1.2.4 Results

The study is being conducted at 15 sites, 12 within the U.S., 2 in the United Kingdom, and 1 in Canada. One center, Mt. Zion Medical Center in San Francisco, accounted for 27 of the 100 subjects in the report. An interim report included in the original NDA submission used a data cut-off of May 31, 1995. The final study report was submitted to FDA January 24, 1996 and included data on 100 subjects using a data cut-off data of August 31, 1995.

## 9.1.2.4.1 Comparability of study populations

Disposition of Subjects

Forty-three subjects (43%) discontinued cidofovir prior to a study endpoint. The number of subjects followed to retinitis progression in the 5 mg/kg group was 11 subjects (22%), versus 23 subjects (45%) in the 3 mg/kg group. Twenty-three subjects (47%) in the 5 mg/kg group and 20 subjects (39%) in the 3 mg/kg group discontinued treatment for reasons which are listed below. Deaths and discontinuations due to adverse events were more common at the higher dose. The following table shows the disposition of subjects at the time of the NDA submission:

Table 12: Patient Disposition<sup>1</sup>

	5 mg/kg (N=49) (%)	3 mg/kg (N=51) (%)
Retinitis progression	11 (22)	23 (45)
Death	4 (8)	1 (2)
Adverse events/ discontinued	17 (35)	12 (24)
Discontinued/other	6 (12)	8 (16)
Still at risk	11 (22)	7 (14)

<sup>1</sup>The above classifications are mutually exclusive.

Adapted from Table 1.3, Information Amendment 14(1/24/96), page 53.

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Baseline characteristics

Selected demographic and baseline characteristics of both dose groups appear below. Notably, the study population as a whole was severely immunosuppressed (median CD4 <10/mm<sup>3</sup>), had a median time to CMV retinitis diagnosis of about a year, and had received and failed multiple courses of ganciclovir and foscarnet.

Table 13: Baseline Characteristics<sup>1</sup>

Characteristic	5 mg/kg (N=49)	3 mg/kg (N=51)	p-value <sup>2</sup>
Age (yrs)	40.3	39.5	0.48
Sex			
Male	48	51	0.44
Female	1	0	
Race			
White	39	41	0.92
Black	2	2	
Hispanic	8	8	
Weight (kg)	66.0	66.6	0.76
CD4 Count (/μL) (median)	7.0	4.5	0.165
Number of lesions (total both eyes)	2.1	2.0	0.88
Time from HIV diagnosis (months/median)	86.0	77.0	0.51
Time from 1st diagnosis of CMV retinitis (days/median)	367	355	0.61
Time on anti-CMV therapy (days/median)	270.5	290	
Previous courses GCV (median)	2	3	
Previous courses FOS (median)	1	1	
Creatinine Clearance (mL/min)	88.7	96.7	0.10
Urine protein			
0-Trace	39	41	0.60
1+	9	9	
2+	0	1	
>2+	0	0	
ANC (x10 <sup>3</sup> )	3.51	2.49	0.10

<sup>1</sup>All descriptive statistics are given as means unless otherwise indicated.

<sup>2</sup>p-values are according to the sponsor's analysis using 2-way ANOVA or Cochran-Mantel-Haenszel test stratified by institution.

Adapted from Tables 2.1 and 2.3, Information Amendment #014 (1/26/96), Pages 64-9.

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**Reviewer's comment:** Subjects in the 3 mg/kg dose group had spent more time on previous anti-CMV therapy, and had failed a greater median total number of previous courses of anti-CMV therapy. It is likely that the additional previous therapy in the low dose group indicates more advanced disease which would be less likely to respond to cidofovir if there is a real treatment effect. Therefore, any small treatment difference between the two groups should be interpreted with caution.

No appreciable differences between dose groups in extent of the retinal involvement, lesion activity, location of lesions, visual acuity, or intraocular pressure at baseline were discernible.

Drug exposure

Drug exposures for the two dose groups are summarized in the following table:

**Table 14: Drug Exposure**

	<u>5 mg/kg</u>	<u>3 mg/kg</u>
N	48	50
Cumulative number of doses		
Minimum	1	1
Maximum	17	11
75th %tile	6.0	5.0
Median	4	4
Mean	4.7	3.9
Cumulative total dose (mg/kg)		
Minimum	5.0	5.0
Maximum	76.6	37.0
Median	20.1	14.5
Mean	22.7	15.0

Adapted from Table 1.6, Information Amendment #014 (1/24/96), page 63.

**Reviewer's comment:** Median number of doses received was the same for both dose groups. The higher mean number of doses received and the higher upper limit of the range in the 5 mg/kg group is probably a result of delayed time to progression in this group (see below).

Dose reductions from 5 mg/kg to 3 mg/kg were made for 11 of 48 patients (23%) due to proteinuria or creatinine increases.

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## 9.1.2.4.2 Efficacy endpoint outcomes

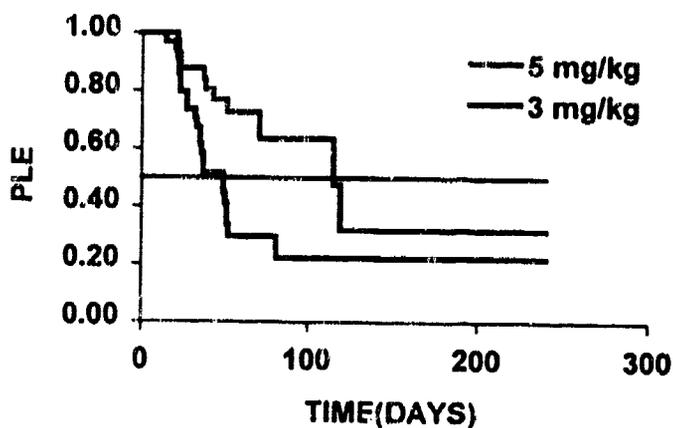
## 9.1.2.4.2.1 Primary efficacy endpoint

Time to retinitis progression

The median times to retinitis progression using Kaplan-Meier estimates were 115 days in the 5 mg/kg group, and 49 days in the 3 mg/kg group. This difference was significant in the log-rank test at a p-value of 0.0017 (Sponsor's analysis). A Kaplan-Meier plot of this comparison follows:

Figure 3

## Time to Retinitis Detection



## 9.1.2.4.2.2 Secondary efficacy endpoints

Mortality

Median survival time in the 5 mg/kg group was 167 days, and in the 3 mg/kg group 146 days. These differences were not significant in the log-rank test ( $p=0.69$ ) (Sponsor's analysis).

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Lesion Activity

As a secondary endpoint the sponsor chose to examine an assessment of "lesion activity" at the border of the retinitis lesion. Lesion activity is given as a "best response" across all visits.

**Table 15: Best CMV Retinitis Lesion Activity Response By Patient**

<u>Response</u>	<u>5 mg/kg</u> <u>(N=44) (%)</u>	<u>3 mg/kg</u> <u>(N= 42) (%)</u>
No activity	26 (59)	11 (26)
Decreased activity	7 (16)	8 (19)
Stable activity	9 (20)	9 (21)
Increased activity	2 (5)	13 (31)
Missing	0 (0)	1 (2)

Excerpted Table 4.1 from Information Amendment #014 (1/24/96), page 99.

A greater treatment effect was seen in the higher dose group in the sponsor's analysis ( $p=0.007$ , Chi-square, 1 D.F.)

**Reviewer's comment:** Assessment of lesion activity has not been of prominent interest in previous FDA approvals for this indication. Due to the subjective nature of this assessment, this has been considered an unreliable estimate of a treatment effect previously. The sponsor's use here of a "best response" across all visits is difficult to interpret and will not be considered in FDA's efficacy analysis.

Visual acuity

Ten subjects at the high dose and 13 subjects at the low dose experienced a major decline in visual acuity during the study. In a time to event analysis (log-rank comparison) this difference was not significant.

**9.1.2.4.3 Safety Comparisons**

Adverse events occurred commonly in study 107. Adverse events of greatest interest and those most clearly associated with the maintenance dose are listed below:

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Table 16: Adverse Events of All Grades<sup>1</sup>

Adverse Event	5 mg/kg		3 mg/kg	
	N=48	(%)	N=50	(%)
Any adverse event	48	(100)	50	(100)
Proteinuria	27	(55)	20	(40)
Creatinine increase	14	(29)	10	(20)
Neutropenia	13	(27)	9	(18)
Nausea +/- vomiting	26	(53)	23	(46)
Anorexia	12	(24)	8	(16)
Dyspnea	14	(29)	6	(12)
Alopecia	10	(21)	4	(8)
Ocular hypotony	5	(16)	1	(4)
Acidosis	4	(8)	1	(2)

<sup>1</sup>Events occurring within 30 days of the last infusion of cidofovir.

Adapted from Table 5.1.1, Information Amendment #014 (1/26/96), pages 131-138.

**Reviewer's comments:** Proteinuria was the most common adverse event reported. The protocol was originally written to exclude individuals with  $\geq 0.3$  mg/dL or 1+ urine protein. During the trial the entry criteria were relaxed to allow subjects with 1+ protein to enroll. It is unclear on what basis the sponsor assigned an adverse event of proteinuria to a patient when this occurred. The incidence of proteinuria was recalculated based on an increase from baseline (i.e., if a subject enters with urine protein of 1+ on visits 0 or 1, then proteinuria as an adverse event is assigned if at a later time point proteinuria increases to  $\geq 2+$ ). Using these criteria, 39 subjects (81%) in the 5 mg/kg dose group had proteinuria versus 25 (50%) in the 3 mg/kg group.

Tables of adverse events prepared by the sponsor and included in the NDA classified adverse events as serious based on the investigators' subjective assessment of seriousness. The table below was reconstructed using objective criteria for laboratory variables where serious is defined by Grade 3 or 4 toxicity as outlined in the study protocol. The most common serious adverse events as specific toxicities are shown below:

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Table 17: Serious Adverse Events<sup>1</sup>

	5 mg/kg N=49 (%)	3 mg/kg N=51 (%)	Total (%)
Total number of subjects with serious adverse experiences	37 (76)	30 (59)	67 (67)
Proteinuria ( $\geq$ 100 mg/dL)	23 (48)	21 (42)	44 (44)
Neutropenia ( $\leq$ 500/mm <sup>3</sup> )	7 (14)	9 (18)	16 (16)
Death	7 (14)	7 (14)	14 (14)
Fever	8 (16)	5 (10)	13 (13)
Asthenia	5 (10)	5 (10)	10 (10)
Pneumonia	5 (10)	5 (10)	10 (10)
Dyspnea	6 (12)	4 (8)	10 (10)
Infection	6 (12)	4 (8)	10 (10)
Creatinine ( $\geq$ 2.0 mg/dL)	4 (8)	4 (8)	8 (8)
Rash	4 (8)	4 (8)	8 (8)
Nausea with vomiting	4 (8)	2 (4)	6 (6)
Diarrhea	3 (6)	2 (4)	5 (5)
Abdominal pain	3 (6)	2 (2)	5 (5)
Sepsis	2 (4)	3 (6)	5 (5)
Anemia	3 (6)	2 (4)	5 (5)
Thrombocytopenia	2 (4)	2 (4)	4 (4)
Acidosis	3 (6)	1 (2)	4 (4)
Ocular hypotony (50% decline or $\leq$ 5 mm Hg)	5 ( )	1 ( )	6 ( )

<sup>1</sup>The adverse experiences occurred after initiation of cidofovir and within 30 days of last infusion.

Adapted from Table 5.1.3, Information Amendment #014 (1/26/96), pages 142-7, and from laboratory values derived from the electronic database.

The most frequent adverse events attributed by investigators as possibly or probably related to cidofovir are listed below:

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Table 18: Serious Adverse Events Possibly or Probably Related to Vistide<sup>1</sup>

	5 mg/kg (N=49) (%)	3 mg/kg (N=51) (%)	Total (%)
Total number of subjects with serious adverse experiences	21 (43)	16 (31)	37 (37)
Proteinuria	9 (18)	8 (16)	17 (17)
Creatinine elevation	8 (16)	3 (6)	11 (11)
Neutropenia	3 (6)	4 (8)	7 (7)
Acidosis	2 (4)	1 (2)	3 (3)
Bun increased	2 (4)	1 (2)	3 (3)
Ocular hypotony	3	0	3

<sup>1</sup>The adverse experiences occurred after initiation of cidofovir and within 30 days of last infusion.

Adapted from Table 5.1.4, Information Amendment #014 (1/26/96), pages 146-7.

None of the deaths were linked by investigators to cidofovir.

**Reviewer's comments:** Individual toxicities of greatest concern are discussed below.

### Renal toxicity

Creatinine elevations above 2.0 mg/kg ( $\geq$  Grade 3 toxicity) occurred in 4 subjects (8%) in each dose group, however creatinine elevations also exceeded 3.0 mg/dL ( $\geq$  Grade 4 toxicity) for those 4 subjects in the high dose, but only in 1 of the 4 in the low dose group. Creatinine was 8.4 for one subject in the 5 mg/kg group; he subsequently died with renal failure as a contributing cause of death. It appears that serious renal toxicity is associated with the higher maintenance dose.

The sponsor provided an analysis which demonstrated a significant association between prior foscarnet use and the development of renal toxicity as manifested by proteinuria or creatinine elevations. Four of the 8 subjects with  $\geq$  Grade 3 creatinine elevations had received prior foscarnet. The patient who developed renal failure and died had not received prior foscarnet therapy.

Based on the creatinine elevations alone, it could be estimated that at least 10% of patients previously treated for CMV retinitis will develop serious renal toxicity despite the use of probenecid and hydration at the 5 mg/kg maintenance dose proposed for the clinic.

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Ocular hypotony

Ocular hypotony as a potential toxicity of cidofovir came to the sponsor's attention following a report of intravitreal use of a cidofovir formulation by an independent investigator (Dr. William Freeman, UCSD). Higher intravitreal doses of cidofovir were associated with an increased incidence of ocular hypotony. Investigators participating in GS-107 were then asked to measure intra-ocular pressures (IOP) as part of routine visits. Subjects enrolled early in the study were not monitored for this adverse event, baseline measurements are absent for several, and baseline measurements were also low in some cases. Because of this, a clear assessment of the toxicity and its appropriate expression are problematic.

The sponsor has chosen to express a greater than 50% decrease of IOP from baseline as a serious episode of ocular hypotony. By this measure, 5 of 26 (19%) at the 5 mg/kg group and 2 of 25 (8%) in the 3 mg/kg group experienced ocular hypotony. While this is a reasonable means to express a potential drug related effect, values could decrease by 50% and still remain within the normal range.

According to FDA's ophthalmologic consultant, Dr. Wiley Chambers, IOPs  $\leq$  5 mm Hg should be considered significant and serious. This way of expressing the adverse event is complicated by findings of low baseline IOP measurements in a number of subjects. At very low pressures the measuring device is said to be less reliable. In the 5 mg/kg group, 5 subjects (2356, 2657, 0175, 6251, 4652) had at least one measurement of intraocular pressure  $\leq$  5mm Hg, out of 25 (20%) in whom baseline measurements were above 5 mm Hg. In the 3 mg/kg group, 1 subject (3855) in 23 (4%) had IOP  $\leq$  5 mm Hg following baseline values in the normal range. Median time to ocular hypotony for these cases was 64 days (range 23-105 days).

Among all subjects who had a baseline IOP measure, 4 of 56 (7%) had baseline IOPs of 5 mm Hg or less. Of the 56, 5 had baseline but no follow-up measures.

By either means of expression, it appears that ocular hypotony occurs more commonly with the higher intravenous dosage. The clinical significance of this finding is not clear.

Neutropenia

The sponsor's categorization of serious neutropenia was based on the subjective assessment of investigators. Using criteria of  $\leq$  500 cells/mm<sup>3</sup> as a basis of serious neutropenia (grade 3 or 4 neutropenia by the sponsor's toxicity grading scale), 9 subjects (19%) in the 5 mg/kg group and 9 subjects (18%) in the 3 mg/kg group had

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serious neutropenia. Totals for grade 2, 3 or 4 neutropenia ( $\leq 625$  cells/mm<sup>3</sup>) were 14 (29%) in the high dose group and 12 (24%) in the low dose group.

The study protocol did not require that subjects discontinue treatment due to neutropenia; this was left to the investigators' discretion. The protocol did provide guidelines for administration of granulocyte colony stimulating factor (G-CSF) in the event of neutropenia. No subject discontinued treatment due to neutropenia, however the incidence and severity of neutropenia was partially masked by the common use of (G-CSF). Seventeen subjects (35%) in the 5 mg/kg group and 16 (33%) subjects in the 3 mg/kg group were administered G-CSF.

Concomitant nucleoside antiretroviral usage was relatively uncommon. In the 5 mg/kg group, 8 of 17 (47%) subjects who received G-CSF began the study receiving nucleoside analogues for anti-HIV therapy, of which stavudine (6 subjects) was the most common. In the 3 mg/kg group, only 2 subjects who received G-CSF began the study on nucleoside anti-retroviral agents. It may be inferred that concomitant use of anti-retroviral nucleoside analogues alone cannot explain the frequency and severity of neutropenia observed in this study.

**Metabolic acidosis**

Metabolic acidosis was assessed as serious by investigators for 4 individuals, and not serious for 3 individuals. Case report forms for these subjects were reviewed.

Subject 6155, in the high dose group, received his last dose of Vistide on 5/22/95 following an adverse experience of profound weakness. Serious acidosis was diagnosed 5/23/95 accompanied by weakness, mild creatinine elevation, hyperglycemia, and normal liver enzymes. He had been receiving 3TC (lamivudine) and d4T (stavudine). He started foscarnet on 6/9/95 and died 6/28/95 of CMV pneumonia.

Subject 171, also in the 5 mg/kg dose group, received his last dose of Vistide on 5/18/95 after a retinitis progression endpoint. He experienced the onset of severe metabolic acidosis on 5/25/95 in conjunction with grade 3 liver enzymes elevations (onset 5/18/95), grade 4 creatinine elevations (onset 5/25/95), grade 4 hypocalcemia (onset 5/26/95), and grade 3 glycosuria (onset 6/8/95) with a normal glucose, and was described as having Fanconi's syndrome. Amylase, a marker of pancreatitis, was not a protocol specified laboratory assessment, and does not appear in the CRF. The subject had been taking 3TC but no other nucleoside analogues.

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Subject 163, in the high dose group, stopped Vistide on 12/14/94 due to renal toxicity which progressed from grade 3 proteinuria to grade 4 creatinine elevation (Cr 4.7), accompanied by metabolic acidosis on 12/27/94. No follow-up beyond this date is included in the case report forms. Liver enzymes and glucose had been essentially normal. The subject had not been receiving any nucleoside analogues.

Subject 164, in the low dose group, received his last dose of Vistide on 1/28/94 after starting a prohibited medication (Amphotericin B) for aspergillus sinusitis. He developed pancreatitis, hyperglycemia, liver failure, and died 1/23/95. This subject had received growth hormone and 3TC concurrently with didanosine but no other nucleoside analogues.

Subject 1856, in the 5 mg/kg group, discontinued Vistide on 7/17/95 due to elevated creatinine (3.5 mg/dL), elevated BUN and proteinuria. Mild metabolic acidosis (bicarbonate 19 meq/dL) was noted on 7/24/95. No follow-up was provided in the CRF.

Subject 154, in the low dose group, received a single dose of Vistide on 8/11/94, after which he developed mild metabolic acidosis (bicarbonate 15 meq/dL) in association with creatinine elevation (1.9 mg/dL) and grade 3 proteinuria (8/10/94). Metabolic acidosis was ongoing at the last follow-up visit (8/29/94). He was not taking any nucleoside analogues. The subject began ganciclovir and died 3 months later; the cause of death was not given.

Subject 1851 in the 3 mg/kg group, received his last dose of Vistide on 12/5/94 due to debilitation. He developed metabolic acidosis 6/5/95 (bicarbonate 16 meq/dL), 7 months after the last dose of Vistide. Associated conditions included peripheral neuropathy, hypoglycemia, wasting and MAC. He had been taking ganciclovir until his death, 6/9/95.

Using laboratory criteria of  $\leq 16$  meq/dL as a screen, 12 individuals (25%) in the high dose group and 7 cases (14 %) in the low dose group were identified. In most cases other abnormalities associated with proximal tubular injury also occurred (glycosuria, proteinuria, hypophosphatemia, hypouricemia, hypocalcemia).

Metabolic acidosis leading to death has been associated with nucleoside analogues (AZT and FIAU). The preponderance of evidence indicates that this toxicity is due to mitochondrial dysfunction, and may be related to incorporation of nucleoside analogues into the growing mitochondrial DNA chain. The sponsor acknowledges that didanosine can be incorporated into the growing DNA chain by the CMV DNA polymerase. Associated clinical findings with mitochondrial toxicities include

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pancreatitis, peripheral neuropathy, hepatic dysfunction, and myalgia. Serious metabolic acidosis is believed to follow hepatic dysfunction. Of the cases described above from study GS-107, subject 164 appears to have a set of conditions consistent with this syndrome.

The most plausible alternative explanation for metabolic acidosis in the other subjects is *via* bicarbonate wasting by the kidney at the level of the proximal renal tubule. This kind of renal tubular acidosis has been associated with a global injury to the proximal tubule and can be associated with glycosuria and proteinuria.

**Deaths**

Four deaths in the 5 mg/kg group (Subjects 161, 2452, 2653, 2654) and 1 death in the 3 mg/kg group (Subject 2655) were recorded as having occurred while on therapy. Kaplan-Meier survival curves for the two dose groups did not differ significantly.

**Reviewer's comment:** Classification of deaths as occurring during treatment or in the follow-up period can obscure the relationship of drug to cause of death. In the case of cidofovir, for which dosing is once every other week in the maintenance phase, this would be of particular concern. Case report forms for all subjects who died within 60 days of receiving a dose of cidofovir were examined. Causes of death and contributing factors are listed below:

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<u>Subject</u>	<u>Causes of death</u>
<u>5 mg/kg</u>	
161	generalized deterioration, pulmonary aspergillosis, grade 4 neutropenia, grade 3 anemia
1855	wasting syndrome
2452	KS, abdominal pain, diarrhea
2453	General deterioration, CMV esophagitis, abdominal pain, hepatitis
2653	Staph sepsis, myalgia, weight loss, fatigue, elevated triglycerides;
2654	Pulmonary KS, neutropenia, 3+ proteinuria
2657	Wasting, CMV colitis
3854	Acute renal failure, liver failure, CHF;
5651	CMV pneumonia
6153	Sepsis, liver abscess, GI bleed;
6155	CMV pneumonia, Staph catheter infection, neutropenia, thrombocytopenia, metabolic acidosis, electrolyte abnormalities
6253	General debilitation, Grade 4 LFTs, jaundice
<u>3 mg/kg</u>	
151	MAI, KS, anemia, weakness, weight loss, diarrhea, Cr elevation >0.4
158	CNS lymphoma, pseudomonas pneumonia
164	liver failure, pancreatitis, acidosis, hyperglycemia, aspergillus sinusitis (see above under acidosis)
169	general deterioration, fatigue, 2+ proteinuria
1251	pseudomonas septic shock, disseminated CMV, fatigue
1253	CMV colitis, myalgia, GCV and FOS started; no cause of death given
1853	aids progression; grade 3 LFT, weakness, dyspnea;
2357	KS, disseminated CMV, fever, abdominal pain
2454	General deterioration; renal dysfunction (CrCl=30), neutropenia
2655	Pneumonia, pseudomonas sepsis, PCP, neutropenia thrombocytopenia;
2656	Pulmonary coccidiomycosis, neutropenia
6252	PCP, Strep pneumonia, pneumothorax

Of those who died "on therapy", Kaposi's sarcoma was the principal contributing cause in 2, and infections in the other 3. Among all deaths in the immediate 2 months following drug discontinuation, bacterial and fungal infections were the most common contributing causes.

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Probenecid

Investigators were more likely to associate serious episodes of asthenia, fever, rash and GI toxicities with probenecid rather than with cidofovir.

Investigator's assessment of total subjects with adverse events of any severity attributable to probenecid revealed no apparent differences in frequency between the two dose groups. The frequency of adverse events attributed to probenecid for the total study population are listed below:

**Table 19: Adverse Events Attributed to Probenecid<sup>1</sup>**

<u>Adverse experience</u>	<u>% subjects</u>
Fever	18
Rash	16
Nausea with emesis	13
Nausea without emesis	13
Chills	9
Headache	8
Asthenia	7
Vasodilation	6

Adapted from Table 5.2, Information Amendment #014 (1/24/96), pages 150-1.

No other single specific toxicity attributed to probenecid occurred in greater than 5% of subjects. There was 1 case of dyspnea, 1 case of laryngeal edema, and 2 cases of hypotension attributed by investigators to probenecid.

The following adverse reactions are described in the label of probenecid with no indication of frequency of their occurrence: headache, dizziness, vomiting, nausea, anorexia, anaphylaxis, fever, pruritus, leukopenia, anemia, dermatitis, alopecia, flushing.

#### 9.1.2.5 Reviewer's Comments/Conclusions of Study Results

##### Efficacy comparisons

All retinal slides were reviewed by Dr. Chambers (FDA). Dr. Chambers felt that several of the retinal photographs which were evaluated by Dr. Holland for the sponsor's analysis were non-evaluable due to extensive retinal involvement present

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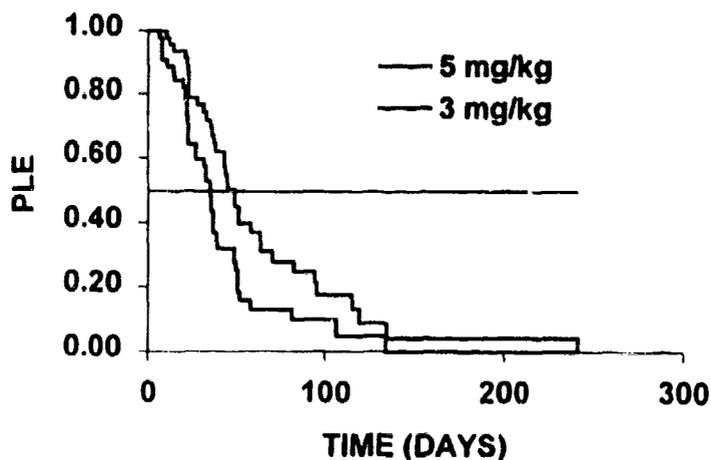
at baseline. In the sponsor's analysis 7 patients had non-evaluable photographs (1 in the 5 mg/kg group and 6 in the 3 mg/kg group) ; Dr. Chambers found that photographs for 26 patients were non-evaluable (10 in the 5 mg/kg group and 16 in the 3 mg/kg group).

According to the sponsor's analysis the 5 mg/kg dose was clearly superior to the 3 mg/kg dose in delaying the time to retinitis progression (median time to progression 115 days vs 49 days). Based on Dr. Chambers's endpoint assessments the median times to progression were 55 days (5 mg/kg) vs 33 days (3 mg/kg). This difference was also significant in a log-rank test. Kaplan-Meier plots using Dr. Chambers time to progression readings did not differ qualitatively from plots prepared using the sponsor's endpoints.

The greater number of subjects (21) experiencing severe adverse events attributable to cidofovir in the higher dose group vs the lower dose group (16) could impact significantly on a treatment difference due to censoring. In a conservative analysis (using the sponsor's endpoint determinations) in which adverse events, intercurrent illness and withdrawn consents necessitating cessation of study drug are considered endpoints, rather than censored events, the median time to progression in the 5 mg/kg group is 49 days vs 35 days in the 3 mg/kg group. This difference was significant in the log-rank test. A Kaplan-Meier plot of this comparison follows:

Figure 4

## Time to Event



Using endpoints based on Dr. Chambers's readings of the retinal photographs, in the same conservative analysis yielded a median time to progression of 33 days for the 5

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mg/kg dose group and 23 days for the 3 mg/kg group; this difference was also significant in the log-rank test.

Baseline characteristics of the two groups, though quite similar, differ in the median number of courses of systemic CMV therapy and in time from the diagnosis of CMV retinitis in a way which would favor the 5 mg/kg dose.

#### Comparison vs historic controls

This study used time to retinitis progression endpoints to compare two maintenance doses of cidofovir. A dose-response effect may be interpreted as supportive of a claim to efficacy if the magnitude of the treatment effect is greater than or comparable to appropriate historical controls. The sponsor cites the following references in which historical data could be used as the comparative arm:

- 1) Studies of the ocular complications of AIDS research group in collaboration with the AIDS clinical trials group; Foscarnet-ganciclovir cytomegalovirus retinitis trial 4. Visual outcomes; *Ophthalmology* 1994;101:1250-61.

In this study the median times to first, second, and third retinitis progressions in the combined totals of ganciclovir and foscarnet treated patients were 48, 41, and 35 days based on retinal photographs. The decreasing times to second and third progressions were said to indicate the progressive nature of CMV infection despite therapy.

Comparability of the GS-107 study population to that in the study cited above cannot be assured. However, in noting that subjects in the cidofovir trial had median CD4 counts  $< 10/\text{mm}^3$  at baseline and a record of multiple previous courses of systemic CMV therapy, it is likely that GS-107 study population was at equal or greater risk of CMV retinitis progression.

What appears to be of greater concern with respect to this comparison is the statistical treatment of drop-outs and those intolerant to the study drug in estimating the median time to retinitis progression. In the cited SOCA/ACTG study, 36% in the foscarnet treated group and 11% in the ganciclovir treated group changed treatment assignment prior to an endpoint; it is not clear from the SOCA/ACTG study report at what stage treatment assignment were changed (i.e., before or after first progression) or how this affected the Kaplan-Meier estimates of median time to progression.

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- 2) Spector AS et al. A randomized, controlled study of intravenous ganciclovir therapy for cytomegalovirus peripheral retinitis in patients with AIDS; *J Inf Dis* 1993;168:557-63.

Palestine AG et al. A randomized, controlled trial of foscarnet in the treatment of cytomegalovirus retinitis in patients with AIDS; *ANN Int Med* 1991;115:665-73.

The two studies cited above refer to a previously untreated patient population. The study groups are clearly not comparable; subjects in GS-107 should be more likely to progress based on their more advanced disease. The sponsor makes the following comparisons: Median time to first progression on ganciclovir or foscarnet was 52.5 vs. 115 days (5 mg/kg cidofovir maintenance dose;  $p=0.18$  log-rank), or 49 days (3 mg/kg cidofovir maintenance dose;  $p=0.65$  log-rank) in subjects treated under GS-107.

While the log-rank tests are inappropriate, the comparisons appear compelling. However, the statistical treatment of dropouts and of adverse events causing treatment cessation makes comparisons of treatment effects across the studies uninterpretable.

#### Comparison to deferred therapy

The sponsor refers to the deferred treatment arm of its own study, GS-93-106, as a comparative arm for GS-93-107. The subject populations are clearly not comparable, but this should bias against a treatment effect in GS-107. The sponsor provides the following figures: Median time to progression 115 days (5 mg/kg) and 49 days (3 mg/kg) vs. 22 days (deferred treatment arm from GS-106).

#### Safety comparisons

##### Mortality

Median survival times between dose groups were comparable: 167 days for the 5 mg/kg dose vs 146 days for the 3 mg/kg dose; this difference was not statistically significant in the log-rank test. The similarity of survival times is reassuring with respect to serious toxicities which might be attributed to the higher dose of cidofovir.

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The sponsor did not provide, and this reviewer is not aware of, references to historical data about survival duration in a similar population of previously treated individuals with advanced HIV and CMV disease.

#### Adverse events

Among all adverse events the specific toxicities most clearly related to dose were proteinuria, alopecia, anorexia, and ocular hypotony. Serious adverse experiences also occurred more commonly in the high dose group (76% vs 59%). Although  $\geq$  grade 3 proteinuria and creatinine elevations occurred with roughly equal frequencies in the two dose groups, creatinine elevations above 3.0 mg/dL were more common in the high dose group (4 cases vs. 1 case).

No difference in serious neutropenia between dose groups was observed. Interpretation of neutropenia as a severe adverse event is complicated by the use of G-CSF, often at lesser degrees of neutropenia than specified in the protocol. Attribution of neutropenia by investigator in an open-label study against a background of anti-retroviral drugs should be interpreted with caution.

#### Conclusions

The design and conduct of study GS-93-107 are acceptable to FDA for the purpose of extension of the safety profile in a previously treated patient population, and as supportive evidence of efficacy based on dose-response and comparisons to historical data. Nephrotoxicity was dose related and dose limiting. No survival advantage or disadvantage was associated with dose. Cases of severe metabolic acidosis was an unexpected finding in the safety analysis, and has not been specifically addressed by the sponsor in their summary of safety.

A conservative analysis of the primary endpoint (time to drug discontinuation event) yielded a median time to retinitis progression of 49 days and 35 days for the 5 mg/kg and 3 mg/kg maintenance dose groups, respectively. For those individuals able to tolerate cidofovir (on treatment analysis) the median times to progression were 115 days and 49 days for the high and low dose maintenance groups, respectively. Based on these analyses, and the references to historical data cited above, the study should be viewed as supportive though not sufficient evidence of efficacy.

It is anticipated that both sets of analyses (conservative and "on treatment) will be included in the marketing label with adequate information describing the assumptions of each analysis.

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## SECTION 10

**Overview of Efficacy**

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**10.0 Overview of Efficacy**

The patient populations studied in the two efficacy trials differed with respect to prior treatment for CMV retinitis, and as a likely correlate, extent of retinal disease at baseline. Both populations were profoundly immunocompromised based on CD4 cell counts. Concomitant anti-CMV medications were disallowed in both trials.

According to FDA's analyses, each of the studies supports a claim of efficacy. The strongest evidence comes from the study of the CMV treatment-naive population (Study 106), which was randomized and had a "no therapy" comparator arm. FDA's estimate of the treatment effect (52 vs 22 days), though substantially smaller than the sponsor's estimate (120 vs 22 days), adheres to the strict requisite assumptions of Kaplan-Meier analyses (i.e., no informative censoring). Nevertheless, both estimates of the treatment effect were statistically significant. Use of Dr. Chambers's independent endpoint determinations does not alter the finding of statistical significance favoring Vistide treatment. Results from the cross-over group provide additional qualitative support indicative of a treatment effect.

In the previously treated population with relapsing CMV retinitis, for which historical estimates of median time to progression for foscarnet and ganciclovir range from 30-45 days, the estimates provided for the 5 mg/kg and 3 mg/kg maintenance doses of cidofovir in the sponsor's analysis are 115 days and 49 days, respectively. These estimates are based on an analysis using informed censoring and are almost certainly overestimates of the treatment effect. In FDA's analysis, estimates of median time to progression were 49 days and 35 days for the 5 mg/kg and 3 mg/kg maintenance doses, respectively. The difference between estimates for the two dose groups in both analyses was statistically significant; this evidence of a dose response, although not sufficient in itself, can be considered as supportive evidence of efficacy. FDA's estimate of the treatment effect at the higher dose appears to be comparable to historical data for ganciclovir and foscarnet in this patient population, although comparisons of the magnitude of treatment effects made across studies are speculative.

For a detailed review of the statistical issues and the FDA statistical analysis, refer to the Dr. Muhly's statistical review.

## SECTION 11

**Overview of Safety**

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**11.0 Overview of Safety**

Assessing drug toxicity in studies of CMV retinitis is difficult due to the diverse manifestations of HIV disease superimposed on drug toxicity, the advanced stage of immunosuppression which accompanies CMV disease, and the multiple medications to which these individuals are exposed. Cidofovir presents particular challenges because of its infrequent dosing schedule; an investigator may be less likely to attribute a toxicity to cidofovir if the temporal association is obscured. The open-label study design and lack of a true comparator arm add further difficulty to assessment of drug toxicity. Because each dose is given with oral probenecid, it may also be difficult to distinguish which toxicity is associated with which drug. Therefore, interpretation of an investigator's assessment of relatedness to drug should be made with caution.

Cidofovir is a new molecular entity. As the sponsor notes, it is a "nucleotide analogue" and thus distinct from the family of antiviral nucleoside analogues with which the medical community has had much experience. All adverse events should therefore be examined, regardless of perceived relatedness to the study drug, in order to identify unexpected toxicities.

**11.1 Significant/Potentially Significant Events****11.1.1 Deaths**

No deaths occurred while subjects were receiving treatment in study 106; 3 deaths occurred within 30 days and 5 deaths occurred within 60 days of the last cidofovir dose. In study 107, 5 deaths occurred while receiving cidofovir, 4 in the high dose group and 1 in the low dose group. There were 24 deaths within 60 days of the last cidofovir dose in study 107. Case report forms were reviewed for all deaths occurring within 60 days of the last cidofovir dose; contributing causes of death were in general expected for this patient population. In most cases multiple contributing causes could be cited. Most common contributing causes are listed below:

NDA-020638

FIRM: GILEAD

2 OF 4

TRADE NAME: VISTIDE (CIDOFOVIR)

GENERIC NAME: CIDOFOVIR

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Table 20: Contributing Cause of Death

	N <sup>1</sup>
AIDS complications/debilitation/wasting	7
Infection/pneumonia-bacterial	6
CMV disease	4
Kaposi's sarcoma	4
Infection/pneumonia-fungal	4
Lymphoma	3
Metabolic acidosis	3
Liver failure	3

<sup>1</sup>More than one contributing cause per death for some cases.  
Compiled from review of case report forms.

The frequency of bacterial and fungal infections as contributing causes of death and as serious adverse events is concerning, given the incidence of neutropenia observed in the clinical trials. This concern was also raised by panel members at the advisory committee.

In no case of bacterial or fungal infection contributing to death were the neutrophil counts less than 500, i.e., grade 3 or 4 toxicity. Although no clear relationship of serious neutropenia to life-threatening infections was established in these trials, drug-induced neutropenia of lesser degree and/or neutrophil dysfunction cannot be ruled out as risk factors.

### 11.1.2 Other significant/potentially significant events

#### a. Renal Failure/Nephrotoxicity

In phase 1/2 studies, 2 subjects receiving **cidofovir** at doses higher than the proposed clinical dose, without supplemental hydration and without probenecid, experienced irreversible renal injury requiring dialysis. One subject in study 107 developed renal failure (Cr 8.4) which contributed to the subject's death. One case of renal failure among 120 patients was reported in the first treatment IND report.

Nephrotoxicity is clearly the most serious acute toxicity associated with **cidofovir**. This was demonstrated at the pre-clinical stage and in phase 1/2 studies when 2 patients receiving **cidofovir** at doses higher than the proposed clinical dose, without probenecid and without supplemental hydration, experienced irreversible renal injury requiring dialysis. Phase 3 protocols included entry criteria and dose modifications

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**Overview of Safety**

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based on conservative criteria in order to minimize the risk of irreversible renal toxicity. Grade 3 or 4 creatinine elevations, i.e., above 2.0 mg/dL, occurred at an incidence of < 10% in both studies. In study 106, no patient had a creatinine above 3 mg/dL. In study 107, in which subjects had received prior treatment for retinitis, 4 patients in the high dose group had a serum creatinine which also exceeded 3.0 mg/dL. One patient had a creatinine of 8.4 and died with renal failure as a contributing cause of death. One patient in the 3 mg/kg dose group of study 107 also had a creatinine which exceeded 3 mg/dL.

Additional safety data with respect to renal toxicity comes from the treatment IND. Of the 120 patients receiving Vistide in an uncontrolled clinical setting, only 1 patient was reported to have had significant renal injury. This case occurred following a dose of chemotherapy for Kaposi's accompanied by dehydration. Although the total exposure under the treatment IND is relatively short, the lack of more widespread renal toxicity indicates that cidofovir can be used in a reasonably safe manner in the clinical setting.

The sponsor has provided an analysis demonstrating an association between prior foscarnet exposure and the occurrence of renal toxicity in patients in study 107. Given the well-known renal toxicities of foscarnet, this observation is not surprising. Nevertheless, at study entry all patients had met conservative criteria based on laboratory assessments.

No information from clinical trials is currently available indicating how best to dose cidofovir when there is evidence of pre-existing renal impairment.

The nephrotoxicity of Vistide underlies the occurrence of Fanconi's syndrome and as noted previously, most cases of metabolic acidosis.

**b. Fanconi's syndrome**

Fanconi's syndrome was diagnosed for 1 subject in study 106 and 1 subject in study 107. Fanconi's syndrome is characterized by general proximal renal tubule dysfunction with impaired reabsorption of phosphate, glucose, uric acid, amino acids, and bicarbonate; systemic acidosis, hypophosphatemia, hypouricemia, glycosuria, and aminoaciduria may result. Over an extended period patients with Fanconi's syndrome may experience osteopenia, bony fractures and rickets. Fanconi's syndrome is a nephrologic diagnosis based on a constellation of findings. It is a diagnosis not likely to be made by an ophthalmologist or infectious disease specialist, and therefore likely to be underdiagnosed in clinical trials.

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**Overview of Safety**

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Laboratory assessments from both efficacy studies were reviewed in an attempt to identify other cases with evidence of proximal tubule dysfunction and which could be consistent with Fanconi's syndrome. It is recognized that a true diagnosis of Fanconi's syndrome cannot be made from listings of laboratory data alone. In some cases laboratory assessments at baseline were also abnormal. Most subjects in 107 had also received prior treatment with foscarnet, which has also been associated with Fanconi's syndrome.

Eight cases were identified, 2 in 106 (102, 116) and 6 in the 5 mg/kg group in study 107 (162, 163, 171, 173, 3854, and 6157) with a low serum bicarbonate and other laboratory evidence of proximal tubule dysfunction. An estimate of the risk of low serum bicarbonate based on these subjects (9%) is proposed for the drug label. Fanconi's syndrome will be included in the drug label as a toxicity associated with didanosine in 2% of patients.

**c. Metabolic acidosis**

One patient in the clinical trials (164 in study 107) developed pancreatitis, liver failure, metabolic acidosis, sinus mucormycosis, and subsequently died. As discussed above, this case could be consistent with a mitochondrial toxicity syndrome (in contrast to low bicarbonate associated with renal toxicity as described earlier). Mitochondrial toxicity with fatty liver and fatal metabolic and lactic acidosis has been associated with nucleoside analogues FIAU and to a much lesser degree, ZDV. For ZDV, the incidence is higher in women than men. Very few women have received didanosine in clinical trials, so although the overall risk appears low (1%), it is unknown if, as for ZDV, the incidence may be higher in women.

Serious metabolic acidosis in association with liver failure should be specifically noted in the drug label as an adverse event and this case described.

**d. Neutropenia**

In the sponsor's safety analysis neutropenia was classified as serious based on the subjective assessment of investigators. Using objective criteria of  $< 500$  cells/mm<sup>3</sup>, 10 subjects in 106 and 7 subjects in the 5 mg/kg dose group of 107 had this degree of neutropenia, an incidence of 17% of patients. Roughly 40% of patients had ANC's below 1000; however the cut-off for study entry was 750 cells/mm<sup>3</sup>.

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**Overview of Safety**

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Although no subject discontinued treatment due to neutropenia, the protocol provided no objective criteria for study discontinuation for neutropenia; discontinuation was left to the discretion of the investigator. For grade 3 or 4 neutropenia the protocol calls for the administration of G-CSF. Usage was somewhat more common, as 34% of subjects had received G-CSF during the study, thus partially obscuring the true incidence of cidofovir associated neutropenia.

The sponsor has stated in the submission that neutropenia has been asymptomatic and without clinical sequelae. However, among the list of most common serious adverse events were fever, infection, pneumonia, and sepsis. Although a clear relation between infections and neutropenia was not established, neutropenia must be considered a risk factor for infection.

**e. Ocular hypotony**

Ocular hypotony as a potential toxicity came to the sponsor's attention when a subject with diabetic retinopathy and CMV retinitis developed this condition. Hypotony has also been reported following intravitreal use of cidofovir by an independent investigator. Study investigators participating in GS-106 and 107 were asked to begin to measure intra-ocular pressures (IOP) as part of the routine visit. Subjects enrolled early in the studies were not monitored for this adverse event, baseline measurements are absent for several, and baseline measurements were also low in some cases. A clear assessment of the toxicity and its appropriate expression are problematic.

Most useful is an examination of dose-effect observed in the 107 study. The clinically significant case cited above was from the high dose group. All 3 cases considered serious by investigators were in the 5 mg/kg group. When analyzed by either a 50% decline from baseline, or by decline to < 5 mm/Hg from the normal range at baseline, most cases fall into the high dose group. The median time to ocular hypotony based on the latter criteria was 64 days.

It is unclear whether these changes are clinically significant. It is proposed that information concerning increased risk of ocular hypotony in patients with diabetes mellitus be included in the package insert.

**f. Malignancies**

In view of the pre-clinical studies showing that cidofovir is a potent carcinogen in rats, malignancy data from the clinical studies was carefully examined. The appearance of AIDS related tumors in excess of expected numbers or the appearance of any

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**Overview of Safety**


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non-AIDS related tumors would be concerning. The sponsor has assembled tables of malignancies according to whether a malignancy occurred before, during, or after didofovir therapy. Because a carcinogen may be expected to induce tumors at any time after its administration, the tables presented below were constructed for all studies broken down simply by whether a tumor was present at baseline and by its appearance at any time during the treatment or follow-up periods.

**Table 21: Malignancies in All Studies<sup>1</sup>**

Prior to didofovir	67
non-AIDS related	4 <sup>2</sup>
AIDS related	66
Kaposi's sarcoma	60
lymphoma	5
anal squamous cell	1
After starting didofovir	23
non-AIDS related	0
AIDS related	23
Kaposi's sarcoma	17
lymphoma	5
cervical neoplasia	1

<sup>1</sup>Includes 8 patients treated under emergency INDs.

<sup>2</sup>Three subjects had more than 1 malignancy.

Adapted from Tables 10.1 and 10.2 of Information Amendment #018 (2/9/96), pages 151-2.

Determining whether the onset of 23 new malignancies after initiation of didofovir is excessive against a background of 67 malignancies at baseline is difficult to evaluate. Without a comparator group one cannot determine statistical or clinical significance. In study GS-93-106, for which the deferred group might be used as a comparator, there were 5 new malignancies in the treated group versus 1 in the deferred group. It is to some degree reassuring that non-AIDS related tumors have not been observed in the patients treated with didofovir. In particular, no adenocarcinomas have been observed. However, very few women have received didofovir in these clinical trials.

This reviewer remains very concerned about the carcinogenic potential of didofovir. For the population of late-stage AIDS patients for whom life-expectancy is currently limited to 6-12 months, quality of life issues may outweigh this potential risk; however, this may be affected by improved anti-retroviral therapies and possibly increased survival times. It is likely that the risk/benefit for an individual may well need to be carefully weighed, although this remains a potential risk pending further data. The carcinogenic potential of didofovir should be clearly stated in the package insert.

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**Overview of Safety**

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**11.1.3 Overdosage exposure**

No cases of overdosage were reported in the efficacy trials. Doses higher than the proposed dose were administered in the phase 1 and 2 trials. As discussed above, renal failure occurred in 2 of these cases.

**11.2 Other safety findings**

Adverse events were very common across all studies. Overall, toxicities involving the body as a whole and the digestive system occurred most commonly (See table of All Adverse Events by Body System).

## SECTION 11

## Overview of Safety

Specific Toxicities

The most common adverse experiences for the entire database (phase 1 and 2 studies) from the sponsor's tabulations are listed below:

**Table 23: Most Common Adverse Events from All Trials**

<u>Total number of subjects</u>	<u>189</u>	<u>(%)</u>
Proteinuria	85	(45)
Fever	78	(41)
Asthenia	75	(40)
Nausea	56	(30)
Headache	55	(29)
Rash	52	(28)
Neutropenia	49	(26)
Diarrhea	44	(23)
Anorexia	32	(17)
Chills	31	(16)
Abdominal pain	31	(16)
Alopecia	29	(15)
Creatinine elevation	26	(14)
Anemia	26	(14)
Weight loss	24	(13)
Myalgia	23	(12)
Herpes simplex	19	(10)

Adapted from Tables 6.1 and 7.1 in Information Amendment #018 (2/9/98), pages 87-92 and 101-108.

Diarrhea, abdominal pain, asthenia, anorexia, and weight loss are common in an advanced AIDS population, but may also be attributable to CMV infection of the GI tract in some patients. Although investigators were reluctant to attribute GI symptoms to zidovudine, the impression remains that the GI symptoms may reflect toxicity of zidovudine to the rapidly dividing tissue of the GI tract. However, direct attribution to zidovudine of these adverse events on the background of advanced HIV disease and concomitant probenecid administration is not clearly established.

Alopecia occurred frequently, particularly in the controlled pivotal study, GS-93-106, in which it occurred in 24%, and in the phase 1/2 study GS-92-101, in which it occurred in 20% of subjects receiving zidovudine. Alopecia was not reported in studies GS-92-102 and GS-92-103. This may be due to insufficient time on drug, or a failure to ascertain cases of alopecia in these latter studies. The overwhelming predominance of male subjects in all studies may have contributed to under-reporting as some hair loss in this population might be expected, while only unusual cases of hair loss would be recorded. The true incidence of drug-

## SECTION 11

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**Overview of Safety**

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associated of alopecia is likely higher than the frequency reported in the overall summary. In only one case, however, was the hair loss considered to be severe. Alopecia is a known, though rare, side effect effect of probenecid. It is of concern that alopecia may also reflect non-specific effects on rapidly dividing cells found in the hair follicle, as occurs with some anti-neoplastic agents.

Myalgia was seen in 17% of subjects in GS-93-106 and in 12% of subjects overall. Myalgia was considered severe in 2 cases. Cardiomyopathy occurred in 2 subjects (1%). Peripheral neuropathy occurred in 2% of all subjects and was judged severe in 1%. Seizures occurred in 4 (2%) individuals. Liver function abnormalities (3%) and pancreatitis (1%) occurred uncommonly.

It is somewhat of concern that Herpes simplex occurred with a frequency of 10%. The sponsor has noted that cidofovir has *in vitro* activity against HSV-1 and HSV-2, and topical preparations of cidofovir are in clinical trials for Herpes simplex in patients with AIDS. However, at the regimen of every other week dosing in these studies, clinical HSV activity may be lost.

Allergic reactions were diagnosed in 7 (4%) and rash in 52 (28%). Hypotension occurred in 6 individuals (3%). These findings may be attributed to probenecid.

The following adverse reactions are described in the label of probenecid in decreasing order of their frequency of: headache, dizziness, vomiting, nausea, anorexia, anaphylaxis, fever, pruritus, leukopenia, anemia, dermatitis, alopecia, flushing. No numerical estimates of the incidence of each adverse reaction are given. Little data is available in the medical literature concerning the relative frequencies of individual toxicities seen with probenecid. In one study of hypertensive medications (Bag MA; Ragland R Postgrad-Med-J. 1979; 55 Suppl 3: 127-32), mild adverse reactions occurred in 25% of individuals taking probenecid. The relatively high incidence of these adverse events seen in studies of cidofovir make it unlikely that all can be attributed probenecid.

Based on the results of the dose ranging 107 study, the sponsor has proposed that 5 mg/kg biweekly be the indicated maintenance dose. For the purpose of estimating risk of toxicities at this dose, tables were constructed using a denominator of 89 subjects who received this dose in clinical trials (25 subjects in the immediate and 16 subjects in the cross-over treatment groups from study 106, and the 48 subjects in study 107).

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**Table 23: All Clinical Adverse Events, Laboratory Abnormalities or Intercurrent Illnesses Regardless of Severity or Investigators' Assessment of Relatedness to Cidofovir Occurring in >15% of Patients Receiving 5 mg/kg Maintenance Dose**

Any adverse event	N=89	(%)
Proteinuria (> 1+ or 0.3 mg/dL)	75	84
Nausea and/or Vomiting	58	65
Fever	51	57
Proteinuria	45	51
Asthenia	41	46
Neutropenia (<750/mm <sup>3</sup> )	28	31
Rash	27	30
Headache	24	27
Diarrhea	24	27
Alopecia	22	25
Infections	22	25
Chills	21	24
Anorexia	20	22
Dyspnea	20	22
Creatinine elevation (>1.5 mg/dL)	19	21
Anemia	18	20
Abdominal pain	15	17

Compiled from electronic database and from Tables 8 and 16 of this review.

## SECTION 11

## Overview of Safety

**Table 24: Serious Clinical Adverse Events or Severe Laboratory Abnormalities Occurring in >5% of Patients Receiving 5 mg/kg Maintenance Dose**

Adverse Event/Laboratory Abnormality	N=89 <sup>1</sup>	(%)
Proteinuria ( $\geq$ 2+ or 100 mg/dL)	24	27
Neutropenia ( $<$ 500/mm <sup>3</sup> )	17	19
Fever	13	15
Infection	11	12
Dyspnea	9	10
Bicarbonate decreased ( $<$ 16 meq/L)	8	9
Pneumonia	8	9
Creatinine elevation ( $\geq$ 2.0 mg/dL)	7	8
Nausea with vomiting	7	8
Diarrhea	6	7
Asthenia	6	7
Ocular hypotension (50% decline from baseline or $\leq$ 5 mm Hg)	6 <sup>2</sup>	17

<sup>1</sup>Includes all patients receiving 5 mg/kg maintenance dose in clinical trials.

<sup>2</sup>Intra-ocular pressures were monitored for 35 patients.

Compiled from electronic database and from Tables 9 and 17 of this review.

It is proposed that the table of all adverse events and all serious adverse events occurring in the 89 subjects receiving the 5 mg/kg maintenance dose be incorporated into the package insert.

### 11.2.1 Special Studies: Patients with Renal Compromise

No information is currently available on which to base dosing in patients with pre-existing renal compromise. The sponsor has submitted a protocol for studying pharmacokinetic parameters in patients with renal compromise and has committed to completing this study in the post-marketing setting.

## SECTION 11

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**Overview of Safety**

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**11.2.2 Drug Demographic Interactions**

Safety data for women from the controlled clinical trials at the intended dose is minimal; only two women have received cidofovir in studies 106 and 107. Two particular concerns with respect to gender interactions deserve comment.

- In pre-clinical studies mammary adenocarcinomas were observed in a substantial number of rats at doses below the intended clinical dose. All mammary carcinomas occurred in female rats.

- Metabolic acidosis associated with liver failure occurred in one male subject in the 107 study. Metabolic acidosis has been associated with the nucleoside analogue FIAU and to a much lesser degree with ZDV, for which cases of serious and fatal metabolic acidosis occur more frequently in women.

The existing data base for cidofovir is much too small to provide useful information with regard to these two potential toxicities or to any toxicity which may be gender related. It is recommended that the package insert clearly state the paucity of safety data in women.

**11.2.3 Drug-Disease Interactions**

No drug disease interactions have been identified. Proteinuria, the most common toxicity associated with cidofovir, can also occur in association with HIV nephropathy; whether cidofovir potentiates HIV nephropathy is not known.

**11.2.4 Drug-Drug Interactions**

Cidofovir is excreted unmetabolized in the urine. No interactions between cidofovir and other drugs have been identified, although the sponsor has performed very limited drug interaction studies. Use of other known nephrotoxic agents was excluded during the clinical trials. It is recommended that the package insert include a warning with respect to concomitant use of other drugs with nephrotoxic potential.

Probenecid interacts extensively with several drugs through the secretory mechanism at the proximal renal tubule; in particular for this patient population, probenecid inhibits the excretion of zidovudine. The sponsor has proposed that the drug label include a precaution to reduce the zidovudine dose by 50% on the days when probenecid is administered. Although it is unclear whether ZDV dose

## SECTION 12

### Labeling Review

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reduction is necessary to assure safety of patients, inclusion of this precaution in the label is reasonable.

#### 11.2.5 Abuse potential

Cidofovir presents no potential for abuse.

#### 11.2.6 Human Reproductive Data

No data are available to assess the effects of cidofovir on human reproduction. No pregnancies were reported during the clinical studies. A negative pregnancy test for women and use of birth control were entry requirements for study participation. Only 2 women received cidofovir in clinical trials.

### 12.0 Labeling Review

The original label proposed by the sponsor underwent extensive revisions to incorporate FDA concerns and concerns expressed by advisory committee members. The final draft package insert submitted on March 28, 1996, is acceptable and addresses FDA concerns.

### 13.0 Advisory Committee Discussion and Recommendations

An FDA advisory committee consisting of members of the antiviral and ophthalmologic advisory committees convened on March 15, 1996, to discuss this NDA submission. FDA presentations were made by Dr. Douglas Pratt (Regulatory history and safety review), Dr. James Farrelly (toxicology issues and carcinogenicity in rats), Dr. Alan Muhly (review of efficacy) and Dr. Wiley Chambers (review and verification of retinal photography).

## SECTION 13

**Advisory Committee**

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independent investigative body added weight to FDA's decision to allow presentation of the data prior to FDA review.

Issues posed by FDA for the committee's deliberation were:

1. Are the safety and efficacy data provided by the sponsor sufficient to warrant approval of Vistide for the proposed indication?
2. Please comment on the sponsor's phase 4 commitments.

Concerns raised by the committee included:

- The lack of data regarding cross-resistance of CMV strains to ganciclovir and foscarnet and how this might affect sequential or combination therapies?
- The paucity of data for women was commented upon. FDA was advised to assure that this be made clear in the package insert.
- Impact of Vistide on systemic CMV disease was questioned.
- The criteria for designating cidofovir a potent carcinogen was questioned. Based on comments by committee members, the risk of malignancy in humans posed by cidofovir did not evoke a level of concern that approval of the drug should be affected.
- The frequency of pneumonia and infections in the clinical trials was commented on, and possible relationship to drug-associated neutropenia questioned.
- In the context of Dr. Chamber's subjective assessment of a less rapid response relative to other therapies, it was commented that it would be desirable to conduct direct comparative trials in the future.
- With respect to the efficacy review, Dr. Kilpatrick clearly preferred the more conservative "time to treatment limiting event" endpoint described by Dr. Muhly and questioned whether in the open label trial whether one could be assured of statistical significance.

The committee voted unanimously (8-0) in favor of approval of Vistide for the proposed indication.

## SECTION 14

**Conclusions**

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**14.0 Conclusions**

1. FDA accepts that efficacy for the treatment of CMV retinitis has been demonstrated in a randomized controlled trial (GS-106) using comparisons to a deferred therapy group. However, the magnitude of the treatment effect is not robust. The dose ranging study in patients with relapsing CMV retinitis (GS-107) provides supportive evidence of efficacy based on a dose-response effect and by comparisons to historical data.

No comparative studies to other agents have been conducted; therefore, relative efficacy to other therapies is not known. Concern has been expressed by FDA's consulting ophthalmologist that the rapidity of response may be delayed with respect to other therapies, although this was admittedly a subjective impression.

2. The utility of cidofovir for the treatment of systemic CMV disease has not been studied and is uncertain based on the lack of an effect of viremia in clinical studies to date.
3. The most serious acute toxicity associated with cidofovir is nephrotoxicity. A dosing regimen has been defined which reduces renal toxicity to a degree which appears to be clinically acceptable. No data from clinical trials is currently available on which to base dosing recommendations in patients with pre-existing renal impairment.
4. Serious neutropenia, modified by the use of G-CSF, has also been associated with cidofovir in clinical trials. Although no clear association between severe neutropenia and increased incidence of infection was observed, infections were a contributing cause of death in several cases. Neutropenia occurring with cidofovir therapy should be considered a risk factor for fungal and bacterial infections.
5. Cidofovir is clearly a potent carcinogen in rats, and therefore should be considered a potential carcinogen in humans. In order to be able to assess the carcinogenic potential of cidofovir in humans, continued follow-up of all study subjects, and surveillance for malignancies in cidofovir treated patients in the post-marketing setting, is essential.

**SECTION 15****Recommendations**

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6. Little safety data is available for women at this time.
7. The sponsor has presented a list of phase 4 commitments which are acceptable to FDA. In particular, the sponsor has agreed to continue to collect follow-up data on the incidence of malignancies among patients treated with cidofovir.

**15.0 Recommendations****15.1 Approval**

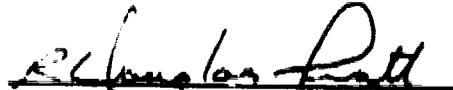
It is recommended that Vistide be approved for the treatment of CMV retinitis in patients with HIV-1 infection.

**15.2 Phase 4 Studies****15.3 Labeling**

The sponsor and FDA have agreed upon wording for a package insert which addresses FDA concerns regarding efficacy claims and safety issues. The proposed Final Product Labeling is included in the NDA package and is acceptable.

Recommendations

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R. Douglas Pratt, M.D., M.P.H.  
Medical Officer

concurrency:

DFeigal  6.20.96  
SGitterman  6.20.96

cc:

NDA 20-638  
HFD-530/KStruble  
HFD-530/DPratt

# Statistical Review

**DRAFT**

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**STATISTICAL REVIEW AND EVALUATION**

**NDA#:** 20-638

**APPLICANT:** Gilead Sciences, Inc.

**NAME OF DRUG:** Vistide (Cidofovir IV)

**INDICATION:** Treatment of cytomegalovirus retinitis in patients with AIDS

**DOCUMENTS REVIEWED:** Volume 1 of IND  
Volume 1 of IND  
Pre-meeting information submitted by FAX dated 9 June 1995.  
Volume 1 of IND  
Volume 1 of IND  
Volume 1 of IND  
Volume 1 of IND  
Volumes 1 and 2 of the submission dated 4 October 1995.  
Volumes 77 through 92 of the submission dated 4 October 1995.  
Volume 1 of Amendment #002 dated 17 October 1995.  
Volume 1 of Amendment #004 dated 31 October 1995.  
Volume 1 of Amendment #006 dated 10 November 1995.  
Volume 1 of Amendment #007 dated 27 November 1995.  
Volume 1 of Amendment #012 dated 16 January 1996.  
Volumes 1 through 7 of Amendment #014 dated 24 January 1996.  
Volumes 1 through 4 of Amendment #018 dated 9 February 1996.  
Volume 1 of Amendment #019 dated 12 February 1996.  
Volume 1 of Amendment #024 dated 22 February 1996.  
Volumes 1 and 2 of Amendment #027 dated 29 February 1996.  
Volume 1 of Amendment #028 dated 4 March 1996.

**MEDICAL INPUT:** Douglas Pratt, MD (HFD-530)

**1. BACKGROUND:**

The sponsor has provided the FDA with efficacy and safety data from the following phase II/III clinical trials.

**Trial GS-93-106: an open label, multi center, randomized study of a cidofovir treatment regimen versus delayed therapy. Patient enrollment commenced on 28 December 1993 and the study was closed on 31 March 1995. The patient population for this study consisted of HIV-infected patients with previously untreated peripheral (not immediately sight threatening) cytomegalovirus (CMV) retinitis. The primary objective of this study was to determine whether cidofovir therapy can extend the time to progression of peripheral CMV retinitis in patients with AIDS. Additionally, the safety and tolerance of cidofovir therapy in patients with AIDS and CMV retinitis was considered. Secondary endpoints of interest included the virologic effects of cidofovir therapy on CMV shedding in urine, blood, and/or semen, and the impact of cidofovir therapy on visual acuity when administered by intravenous infusion.**

**Trial GS-93-107: an open label, multi center, randomized dose comparison of two cidofovir treatment regimens. Patient enrollment commenced on 11 July 1994 and is still ongoing. Data on the first 100 patients through 31 August 1995 was submitted with Amendment #014 dated 24 January 1996. The patient population for this study consists of HIV-infected patients with relapsing CMV retinitis. The stated study objectives are to evaluate the safety and tolerance of cidofovir therapy in AIDS patients with relapsing CMV retinitis and to determine the time to progression of relapsing CMV retinitis. A secondary endpoint mentioned is the impact of cidofovir therapy on visual acuity when administered by intravenous infusion.**

**All submitted analyses of efficacy from studies GS-93-106 and GS-93-107 were provided by statisticians at Corning-Besselaar, Inc.**

**Based on the submitted results from the above studies, the sponsor is seeking an indication for the treatment of CMV retinitis disease in patients with AIDS.**

**2. STUDY GS-93-106: The pivotal trial in support of the sponsor's application.**

**2.1 Design:**

**2.1.1 Purpose** As stated in the protocol, the objectives of this trial included the following:

- To determine whether cidofovir therapy can extend the time to progression of peripheral CMV retinitis in AIDS patients when administered by intravenous infusion.**
- To evaluate the safety and tolerance of cidofovir therapy in AIDS patients with CMV retinitis when administered by intravenous infusion.**

- To evaluate the virologic effects of cidofovir therapy on CMV shedding in urine, blood, and/or semen when administered by intravenous infusion.
- To evaluate the impact of cidofovir therapy on visual acuity.

**2.1.2 Treatments** Patients receiving cidofovir were to have submitted to the following treatment regimen:

- two consecutive weekly doses of 5 mg/kg of cidofovir (induction) followed by 5 mg/kg doses of cidofovir every other week (maintenance).
- cidofovir was to be administered by IV infusion over a sixty minute period.
- prehydration with 1 liter of 0.9 percent (normal) saline solution over an approximate 60 minute period immediately prior to cidofovir treatment was stipulated.
- oral administration of four 500 mg tablets of probenecid three hours prior cidofovir administration and two 500 mg tablets each at two hours and eight hours following the completion of cidofovir administration was also stipulated.

Patients randomized to immediate therapy commenced with this regimen at the time they were randomized to treatment. Patients randomized to deferred therapy were permitted to follow this regimen once progression of non-sight threatening CMV retinitis had been documented by masked readings of retinal photographs.

**2.1.3 Endpoints** As stated in the protocol, the primary outcome measure was to have been the time to progression of CMV retinitis or death, whichever occurred first. Secondary outcome measures stipulated by the protocol included mortality, changes in visual acuity, and the effect of cidofovir therapy on CMV shedding in urine, blood, and/or semen.

Time to retinitis progression was defined as the time from randomization to the next retinitis progression. Progression of retinitis was assessed by comparing retinal photographs from each visit with those taken at the beginning of the study (obtained at baseline, within 24 hours of randomization). The reader at the central Fundus Photography Reading Center was masked and unaware of the treatment assignment of the patient being evaluated. Progression of retinitis progression was defined according to the following criteria:

- advancement of the edge of an existing lesion by one-half the diameter of the optic disc (0.5 disc diameters = 750  $\mu$ ) perpendicularly from the edge and along  $\geq$  750  $\mu$  of it; or
- occurrence of a new lesion  $\geq$  one-quarter disc area in size (a circle,  $\geq$  750  $\mu$  in diameter), separate from the previous lesion in the same eye or in a previously uninvolved eye.

As indicated by Table 1<sup>1</sup> of the protocol, patients were to have received complete ophthalmological examinations (including fundus photography) at baseline, at weeks 1, 3, 5, and 7, and every four weeks thereafter.

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<sup>1</sup> Page 033 of Volume 81 from the original submission.

As described in the study report, the primary outcome measure was the time to progression of CMV retinitis. This deviation from the protocol was explained as follows: "... in the absence of drug-related mortality, time to CMV retinitis progression without considering death more accurately reflects the impact of the drug on CMV retinitis progression".<sup>2</sup>

**2.1.4 Eligibility** As stated in the protocol, to be eligible for this study patients must have satisfied the following requirements:

- been diagnosed with AIDS according to current Centers for Disease Control criteria.
- been diagnosed with peripheral CMV retinitis as determined by an experienced ophthalmologist based on the presence of characteristic necrotizing retinitis consisting of white, fluffy, or granular retinal infiltrates with or without hemorrhage. Lesions were defined as peripheral, or not immediately sight threatening, if they were located at least 1500  $\mu\text{m}$  from the margin of the optic disc and 3000  $\mu\text{m}$  from the center of the fovea. Additionally, it was required that one lesion be at least one-quarter disc area such that it could be photographed.
- visual acuity in the affected eye had to be at least three lines on the ETDRS chart at a distance of one meter.

Additional criteria required preserved renal function, preserved hepatic function, and preserved hematologic function.

Patients were to be excluded from this study for any of the following reasons:

- evidence of CMV retinitis lesion(s) within zone 1. Lesion(s) < 1500  $\mu\text{m}$  from the margin of the optic disc or < 3000  $\mu\text{m}$  from the center of the fovea in either eye excluded patients.
- previous or ongoing therapy for CMV disease with ganciclovir, foscarnate, CMV hyperimmune immunoglobulin, or any investigational agents with anti-CMV activity. Patients who had received previous treatment with ganciclovir, foscarnate, or CMV hyperimmune immunoglobulin solely as prophylaxis were considered eligible.
- receipt of therapy within the previous one week with any of the following:
  - Amphotericin B
  - Aminoglycoside antibiotics
  - Vidarabine
  - Intravenous pentamidine
  - Other nephrotoxic agentsPatients receiving any of these drugs must have discontinued at least one week prior to the time of baseline evaluation and for the duration of the study period.
- receipt of on-going therapy with acyclovir at the time of randomization.
- a known clinically significant allergy to probenecid.

Further exclusion criteria included media opacity precluding visualization of the fundus of both eyes, retinal detachment, patients with clinically significant heart disease, and patients with active medical problems including drug or alcohol abuse.

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<sup>2</sup> Page 065 of Volume 77 from the original submission.

No incentives or disincentives were adopted to encourage or discourage the enrollment of women or minority patients.

**2.1.5 Toxicity Criteria and Dose Modifications** As stipulated in the protocol<sup>3</sup> a Graded Toxicity Scale was to be used to define toxicity criteria. For the different toxicity grades, the following actions were indicated:

- For Grade I toxicity, the patient was allowed to continue cidofovir treatment at the discretion of the Investigator.
- For confirmed Grade II nephrotoxicity (as indicated by serum creatinine [1.7 - 1.9 mg/dl], proteinuria [1+ or 30-99 mg/dl], or calculated creatinine clearance [41-50 mL/min]), the patient's dose of cidofovir was to be reduced to 3 mg/kg for the remainder of study treatment.
- For other confirmed Grade II toxicities, patients were permitted to continue cidofovir treatment at the discretion of the Investigator after consultation with the Sponsor.
- For confirmed Grade III or IV toxicity considered to be either remotely or not cidofovir related, the patient was allowed to continue cidofovir treatment at the discretion of the Investigator after consultation with the Sponsor.
- For confirmed Grade III or IV toxicity considered to be possibly or probably related to cidofovir, the patient was to have been removed from cidofovir treatment (except for neutropenia).
- For confirmed Grade III or IV neutropenia, cidofovir therapy was to be held until the ANC exceeded 500 cells/ $\mu$ L and filgrastim (G-CSF) was to have been instituted.
- For a confirmed increase in serum creatinine of at least 0.5 mg/dL from baseline, the patient was to have been removed from cidofovir treatment.

**2.1.6 Patient Withdrawal** As stated in the protocol<sup>4</sup>, patients were to have been discontinued from cidofovir therapy for either of the following reasons:

- documentation of retinitis progression,
- occurrence of treatment-limiting toxicity,

whichever occurred first. Further, the protocol stipulated that patients were allowed to withdraw or be removed from study treatment assignment in the following instances<sup>5</sup>:

- Intercurrent illness which would, in the judgement of the Investigator, affect assessments of clinical status to a significant degree.
- Unacceptable toxicity (see section 2.1.5).
- Disease progression (see section 2.1.3).
- Development of biopsy-documented extraocular CMV disease.

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<sup>3</sup> Page 038 of Volume 81 from the original submission.

<sup>4</sup> Page 003 of Volume 81 from the original submission.

<sup>5</sup> Page 043 of Volume 81 from the original submission.

- Patient noncompliance or request to withdraw.
- Request of the Sponsor following consultation with the Principal Investigator(s).

**2.1.7 Randomization** The randomization scheme<sup>6</sup> adopted for this study was complicated and evolved during the course of the trial. What follows is a description of this scheme and a chronology of the changes made. The initial randomization scheme, dated 1 December 1993, randomized patients centrally in blocks of four. The first three patients enrolled in the trial were so randomized. At that point, the chair of the Data Safety and Monitoring Board (Dr. T. Fleming) expressed concern with regard to apparent within site imbalances and proposed a revision. The second randomization scheme adopted, dated 17 January 1994, randomized patients to therapy in blocks of four within center. However, the following adaptation was implemented. Before considering the within site block, the study wide balance between therapies was calculated. If this imbalance was 2 or more, the patient was automatically randomized to the under represented therapy provided that therapy was still available in the block. The next five patients encountered this scheme. However, for the first two of these patients the consideration of the study wide balance was not yet in place. Thus, after the first 8 patients had been randomized to therapy, the first three were randomized centrally in blocks of four, the second two were randomized within site in blocks of four, and the last three were randomized within site in blocks of four with the study wide balance influencing the actual blocks selected.

In discussions between the sponsor, the Data and Safety monitoring Board (DSMB) chair (Dr. T. Fleming) and the Corning-Besselaar statistician, dated 14 February 1994, the concern was expressed that randomization within site might theoretically provide some degree of unmasking of the next assignment to the investigators. At this point the following biased-coin adaptive randomization scheme was adopted. Counts were kept of the current within site and across study treatment assignments. For each patient assignment, the current balance at the site was checked. If an imbalance was seen, the patient was assigned to the under represented therapy with a probability of 0.6. On the other hand, if the site was perfectly balanced, then study wide balance was assessed. If the study wide imbalance was two or more, the next assignment was to the treatment diminishing the imbalance. If the within site imbalance was less than two, the next assignment was to either treatment with equal probability. Sixteen patients were randomized to therapy by this scheme.

Following a meeting with the DSMB, dated 31 May 1994, the randomization scheme was again changed to strengthen the balancing element of the biased coin procedure. Specifically, the following scheme was adopted. If a site was out of balance by one or two patients, the patient was assigned to the under represented therapy with a probability of 0.6. If a site was out of balance by more than two patients, the patient was assigned to the under represented therapy with a probability of 0.8. Finally, if the site was perfectly balanced, the study wide balance was assessed. If the across study imbalance was two or more, then the next assignment was to the treatment diminishing the imbalance. If the site imbalance was less than two, the next assignment was to either treatment with equal probability. The final 24 patients enrolled in study GS-93-106 were randomized to therapy according to this design.

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<sup>6</sup> Page 256 of Volume 1 of Amendment #018 dated 9 February 1996.

2.1.8 Centers As noted above, study GS-93-106 was a multi center trial. The following table records the centers involved, their location, and the number of patients enrolled.

Institution	Location	Immediate	Deferred	Total
Beth Israel Hospital Joslin Diabetes Center Harvard Medical School	Boston, MA	4	2	6
Mount Zion Medical Center University of California	San Francisco, CA	6	10	16
Rochester Eye Center University of Rochester Medical Center	Rochester, NY	1	1	2
University of California, Irvine	Irvine, CA	6	5	11
University of California, San Francisco	San Francisco, CA	1	0	1
University of North Carolina	Chapel Hill, NC	1	0	1
University of Southern California Doheny Eye Institute	Los Angeles, CA	4	3	7
St. Stephen's Clinic Moorfields Eye Hospital	London, UK	2	2	4
Total		25	23	48

<sup>1</sup> Source: Page 231, Volume 77 of the original submission.

2.1.9 Follow Up As stipulated by the protocol<sup>7</sup>, Investigators were to have applied best medical judgement with regard to the initiation of standard anti-CMV treatment (e.g., ganciclovir or foscarnate) for those patients whose cidofovir treatment was discontinued for any reason other than progression of their CMV retinitis. Further, consideration was to have been given to delaying the initiation of ganciclovir or foscarnate until documentation of retinitis progression. Those patients who discontinued cidofovir treatment for any reason other than progression of their CMV retinitis **AND** who remained off standard anti-CMV treatment until documentation of retinitis progression were to undergo complete ophthalmologic examination, including fundus photography, every two weeks until documentation of retinitis progression or initiation of standard anti-CMV treatment, whichever occurred first. Regardless of whether the patient initiated standard anti-CMV treatment, a complete ophthalmologic examination, including fundus photography, was to have been done at the

<sup>7</sup> Page 015 and 035 of Volume 81 from the original submission.

time that cidofovir was discontinued.

**2.1.10 The Cross Over Segment** The protocol stipulated<sup>8</sup> that data obtained from patients receiving cidofovir following demonstration of retinitis progression while receiving no treatment (the deferred group), were to be included in safety analyses and in a secondary analysis of efficacy. For this secondary analysis<sup>9</sup> of efficacy, the primary outcome measure was the time from the first (while on deferred therapy) progression to the second progression (following crossover to cidofovir therapy). The time to the second progression was calculated relative to the date of the crossover baseline (i.e., the last fundus photograph taken prior to the first infusion of cidofovir).

**2.1.11 Interim Analysis** As stipulated in the protocol<sup>10</sup>, efficacy was to be reviewed once by the Data Safety and Monitoring Board when 24 patients had been enrolled in the study for one month. The Lan-DeMets implementation of the O'Brien - Fleming Group Sequential Boundaries were to be used as guidelines. Treatment differences were to be considered significant if the nominal p-value associated with the logrank statistic was at most 0.005. As a result of the interim analysis, the study was allowed to proceed with the second cohort of 24 patients. At the point of the final analyses of efficacy, the adjustment required to accommodate the interim analysis was that any p-value be at most 0.048 to be declared significant.

## 2.2 Enrollment:

**2.2.1 Patient Demographics** As a result of the randomization scheme described in section 2.1.7, 48 patients were enrolled in study GS-93-106; 25 received immediate therapy and 23 received deferred therapy. The demographic characteristics of the enrolled population are described in Table 2 below.

		Immediate	Deferred
Age (Range):		27 - 56	30 - 46
Sex:	Male	24	22
	Female	1	1
Race:	White	20	20
	Other <sup>2</sup>	5	3

1 Source: Table 2.1 page 246 of Volume 77 of the original submission.  
2 One Native American and 7 patients of Hispanic origin.

<sup>8</sup> Page 046 of Volume 81 from the original submission.

<sup>9</sup> Page 071 of Volume 77 from the original submission.

<sup>10</sup> Page 045 of Volume 81 from the original submission.

**2.2.2 Baseline Characteristics** With respect to selected baseline characteristics, the patients enrolled in study GS-93-106 can be described as follows.

Table 3: GS-93-106 Selected Mean Baseline Characteristics <sup>1</sup>			
Characteristic	Immediate	Deferred	p-value <sup>2</sup>
CD4 Count	13.3	16.2	0.695
Time From HIV Diagnosis (months)	67.2	78.2	0.470
Number of Lesions	1.2	1.7	0.081
Time From First CMV Diagnosis (days)	5.1	8.1	0.147

<sup>1</sup> Source: Table 2.1 page 246 of Volume 77 from the original submission.  
<sup>2</sup> P-values provided by the sponsor were based on a two-way analysis of variance with treatment and institution as main effects or on a Cochran-Mantel-Haenszel test stratified by institution.

### 2.3 Methods of Analysis:

**2.3.1 Censoring Rules** As noted in section 2.1.3, the primary endpoint adopted by the sponsor in the study report was time to CMV retinitis progression. However, since the protocol stipulated that the primary endpoint should be the time to CMV retinitis progression or death, this analysis was also included in the study report. Finally, the primary endpoint for the crossover segment of study GS-93-106 was the time from the first (while on deferred therapy) progression to the second progression (following crossover to cidofovir therapy). Because the possibility existed that a patient might discontinue the study before reaching any of these endpoints, the sponsor specified the following censoring rules<sup>11</sup>:

For the comparison between the immediate and deferred groups with respect to Time to CMV Retinitis Progression:

If, at the time of the efficacy analyses, progression had not yet occurred, the time of event was considered censored and the time of the last fundus photograph just prior to the earliest of (i) the most recent non-missing evaluation demonstrating no progression performed before the data were frozen for analysis (31 March 1995) or (ii) the time of initiation of standard anti-CMV treatment (i.e., ganciclovir or foscarnate).

Patients with missing or inadequate baseline fundus examinations were censored at randomization. Patients with a baseline fundus examination but no follow up examinations were censored at the baseline examination.

<sup>11</sup> Page 070 of Volume 77 from the original submission.

- For the comparison between the deferred and crossover groups with respect to Time to CMV Retinitis Progression:

If, at the time of the efficacy analyses, the second progression had not yet occurred, the time of event was considered censored and time of censoring was the time of the last fundus photograph just prior to the earliest of (i) the most recent non-missing evaluation demonstrating no progression performed before the data were frozen for analysis or (ii) the time of initiation of standard anti-CMV treatment (i.e., ganciclovir or foscarnate).

If a crossover patient had a baseline photograph just prior to cidofovir initiation but did not have any follow up fundus exams during crossover, then the patient was considered censored at study Day 1 of the crossover period.

- For the comparison between the immediate and deferred groups with respect to Time to CMV Retinitis Progression or Death:

If, at the time of the efficacy analyses, progression or death had not yet occurred, the time of event was considered censored and the time of censoring was the time of the last follow up.

Patients with missing or inadequate baseline fundus examinations were censored at randomization.

- For the comparison between the deferred and crossover groups with respect to Time to CMV Retinitis Progression or Death:

If, at the time of the efficacy analyses, second progression or death had not yet occurred, the time of event was considered censored and time of censoring was the time of last follow up.

- Deferred patients who were censored for any reason during the initial assignment period were not included as crossover patients in the deferred versus crossover time to progression as well as time to progression or death analyses.

**2.3.2 Significance of Treatment Effect** To measure the significance of the difference between the immediate and deferred groups with respect to the endpoints of CMV retinitis progression and CMV retinitis progression or death, the logrank statistic as calculated by PROC LIFETEST (SAS Institute, Inc.) was adopted. For the primary analyses of these endpoints, p-values associated with the observed value of the logrank statistic were not adjusted by institution. However, as supplementary analyses, p-values adjusted by institution were included. It should be noted that when adjusting for institutions, data from sites with fewer than three patients in either treatment group were pooled as one institution.

To measure the significance of the difference between the time to the first and second progression in deferred patients who crossed over, the paired data version of the Prentice-Wilcoxon statistic was



1 Source: Table 1.6 page 240 of Volume 77 from the original submission.

2.4.2 *Patient Outcomes* As reported by the sponsor, the outcomes for the patients enrolled in this trial are recorded in Table 5.

**Table 5: GS-93-106 Disposition of Patients During Initial Treatment Assignment<sup>1</sup>**

Result	Immediate		Deferred	
	n	(%)	n	(%)
Progression <sup>1</sup>	10	(40)	19	(83)
Death <sup>2</sup>	0	(0)	0	(0)
Discontinued - Adverse Experience(s) /Intercurrent Event(s) <sup>3</sup>	8	(32)	0	(0)
Discontinued - Other <sup>3,1</sup>	5	(20)	4	(17)
Lost to Follow Up <sup>1</sup>	0	(0)	0	(0)
Still at Risk (at initial treatment assignment) <sup>3</sup>	2	(8)	0	(0)

1 Source: Page 238 of Volume 77 from the original submission.  
2 These categories are mutually exclusive and exhaustive with respect to all patients randomized.  
3 'Discontinued - Other' includes patients who discontinued for reasons other than those shown above (e.g., due to mental incapacity, generalized debilitation, withdrawal of consent, etc.).

**2.4.3 Significance of Treatment Effect** The value of the unstratified logrank statistic obtained by the sponsor for the endpoint of time to CMV retinitis progression was 20.934<sup>13</sup>. The associated asymptotic p-value was reported as < 0.0001. In a footnote, the asymptotic p-value adjusted for institution was reported as 0.0003.

The value of the unstratified logrank statistic obtained by the sponsor for the endpoint of time to CMV retinitis or death was 12.219<sup>14</sup>. The associated asymptotic p-value was reported as 0.0005. In a footnote, the asymptotic p-value adjusted for institution was reported as 0.0069.

**2.4.4 Magnitude of Treatment Effect** Tables 6 and 7 below record portions of the Kaplan - Meier curves reported by the sponsor for patients randomized to immediate and deferred therapy. The portions of the Kaplan - Meier plots recorded here highlight the reported estimates of medians. Table 6 focuses on the endpoint of the progression of CMV retinitis while Table 7 focuses on the combined endpoint of the progression of CMV retinitis or death, whichever comes first.

Day of Event	Treatment with Event	# of Events	Immediate (at risk)	Deferred (at risk)
16	Deferred	1	0.048 (20)	0.450 (12)
21	Deferred	1	0.048 (20)	0.500 (11)

<sup>13</sup> Page 271 of Volume 77 from the original submission.

<sup>14</sup> Page 271 of Volume 80 from the original submission.

22	Deferred	2	0.048 (20)	0.600 (10)
66	Immediate	1	0.461 (9)	0.927 (2)
79	Deferred	1	0.461 (8)	1.000 (1)
120	Immediate	1	0.641 (3)	1.000 (0)
134	Immediate	1	1.000 (1)	1.000 (0)

1 Source: Condensed from Table 3.1.1 page 268 of Volume 77 from the original submission.

For patients randomized to immediate therapy, PROC LIFETEST returned 120 days as an estimate of the median time to the progression of CMV retinitis with a 95% confidence interval ranging from 40 days to 134 days.<sup>15</sup> For patients randomized to deferred therapy, the corresponding estimate of the median time to the progression of CMV retinitis was 21.5 days with a 95% confidence interval ranging from 10 to 27 days.<sup>16</sup>

Day of Event	Treatment with Event	# of Events	Immediate (at risk)	Deferred (at risk)
21	Deferred	1	0.043 (22)	0.469 (12)
22	Deferred	2	0.043 (22)	0.565 (11)
66	Immediate	1	0.478 (13)	0.826 (3)
79	Deferred	1	0.478 (10)	0.884 (3)
86	Immediate	1	0.530 (10)	0.884 (2)
120	Immediate	1	0.589 (8)	0.884 (2)

1 Source: Condensed from Appended Table 8.1.1 page 271 of Volume 80 from the original submission.

For patients randomized to immediate therapy, PROC LIFETEST returned 86 days as an estimate of the median time to the progression of CMV retinitis or death with a 95% confidence interval ranging from 40 days to upper limit not reached<sup>17</sup>. For patients randomized to deferred therapy, the corresponding estimate of the median time to the progression of CMV retinitis or death was 22 days with a 95% confidence interval ranging from 13 to 31 days<sup>18</sup>.

*2.4.5 The Cross Over Segment Details pending.*

*2.4.5 Safety - Survival Details pending.*

<sup>15</sup> Page 271 of Volume 77 from the original submission.

<sup>16</sup> Ibid.

<sup>17</sup> Page 275 of Volume 80 from the original submission.

<sup>18</sup> Ibid.

## 2.5 Summary: The sponsor concludes the following<sup>19</sup>:

- In describing the trial, it is stated that: "As designed, 48 patients were randomized to receive either immediate (n=25) or deferred treatment (n = 23) with IV cidofovir 5 mg/kg once weekly for 2 weeks (induction), then 5 mg/kg once every other week (maintenance), until photographic documentation of CMV retinitis progression, the occurrence of treatment-limiting toxicity, or death." [emphasis added]
- "No significant differences were observed between the immediate and deferred groups in demographic and baseline characteristics."
- "The median time to CMV retinitis progression as assessed by protocol-specified, bilateral, full-field retinal photographs evaluated by an ophthalmologist masked to treatment assignment was 120 days (95% confidence interval: 40 to 134 days) in the immediate group and 21.5 days (95% confidence interval: 10 to 27 days) in the deferred group (p < 0.0001; log-rank test)".
- "Additional support for a cidofovir treatment effect was evident from consideration of the 16 deferred patients who crossed-over to cidofovir therapy following CMV retinitis progression. Median time to CMV retinitis progression for these crossover patients on cidofovir was not reached compared with a median time to progression of 21 days (95% confidence interval: 13 to 23 days) for these same patients while on the deferred arm (p < 0.001; paired Prentice-Wilcoxon Z-statistic)."
- "Neutropenia (15%) and proteinuria (12%), both asymptomatic, were the most common serious adverse experiences felt by the Investigators to be possibly or probably related to cidofovir."
- "Mild to moderate transient probenecid reactions, including fever, rash, headache, and/or nausea, occurred in 23 of 41 (56%) patients and were treatment-limiting in 3 (7%) patients."
- "Mortality in the two randomization groups was similar [7 of 25 (28%) in the immediate group and 8 of 23 (35%) in the deferred group] and not related to cidofovir; Kaplan Meier estimates of median survival had not yet been reached in the immediate group and was 9.4 months in the deferred group (p = 0.58; log-rank test)."
- "These data demonstrate that cidofovir is efficacious in delaying progression of CMV retinitis in patients with AIDS and previously untreated peripheral CMV retinitis. Treatment was associated with manageable side effects; pre-dose monitoring of renal function and concomitant administration of saline hydration and probenecid appeared to minimize the potential for significant drug-related toxicity."

**3. STUDY GS-93-107: A supportive trial for the sponsor's application. Because this trial is similar to study GS-93-106, and because it is supportive in nature, only the differences with study GS-93-106 will be highlighted.**

### 3.1 Design:

**3.1.1 Purpose** As stated in the protocol, the objectives of this trial included the following:

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<sup>19</sup> Page 154 from Volume 77 of the original submission.

- To evaluate the safety and tolerance of cidofovir therapy in AIDS patients with relapsing CMV retinitis when administered by intravenous infusion.
- To determine the time to progression of relapsing CMV retinitis in AIDS patients treated with cidofovir administered by intravenous infusion.
- To evaluate the impact of cidofovir therapy on visual acuity.

**3.1.2 Treatments** All patients enrolling in this study will receive cidofovir. The common elements of the treatment regimens include the following:

- Cidofovir will be administered by IV infusion over a 60 minute period.
- All patients will undergo prehydration with one liter of 0.9 percent (normal) saline solution over an approximate 60 minute period immediately prior to each cidofovir treatment. Additionally, all patients will receive a second liter of 0.9 percent (normal) saline solution to be given either during each cidofovir infusion period or immediately after, over an approximate 60 - 180 minute period. The second liter may be piggybacked with cidofovir infusion.
- All patients will receive probenecid with each treatment. Probenecid will be administered orally as 500 mg tablets; four tablets (2 grams) 3 hours prior to cidofovir administration and two tablets each (1 gram) 2 hours and 8 hours following the completion of cidofovir administration (four grams total).

The treatment regimens differed according to the dose of cidofovir:

Group A: 5 mg/kg/dose (induction and maintenance)

Group B: 5 mg/kg/dose (induction) and 3 mg/kg/dose (maintenance)

Treatment was to be administered as two consecutive weekly doses (induction) followed by every other week cidofovir administration (maintenance).

**3.1.3 Endpoints** The first endpoint listed in the protocol was the time to treatment-limiting toxicity<sup>20</sup>. Time to treatment-limiting toxicity was defined as the time from randomization to the documentation of toxicity. Treatment-limiting nephrotoxicity was defined as an increase in serum creatinine  $\geq 0.5$  mg/dL,  $\geq 3+$  proteinuria, or calculated creatinine clearance  $\leq 26$  mL/min. It was required that episodes of treatment-limiting toxicity be confirmed by immediately repeating abnormal laboratory parameters. Further, it was stipulated that treatment-limiting toxicities necessitated discontinuation of cidofovir treatment.

The second primary endpoint described in the protocol was the time to the progression of CMV retinitis or death, whichever occurred first. The definition of the progression of CMV retinitis was

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<sup>20</sup> Page 025 of Volume 87 from the original submission.

identical to that used in GS-93-106. As indicated by Table 1<sup>21</sup> of the protocol, patients were to have received complete ophthalmologic examinations at weeks 1, 3, 5, and 7, and every four weeks thereafter.

As with study GS-93-106, the study report argues for the time to progression of CMV retinitis as the primary outcome measure. It was argued that "... in the absence of drug-related mortality, time to CMV retinitis progression without considering death more accurately reflects the impact of the drug on CMV retinitis progression."<sup>22</sup> However, it was acknowledged that the protocol called for the combined endpoint of CMV retinitis progression or death and both endpoints were analyzed.

**3.1.4 Eligibility** As stated in the protocol, the primary eligibility criteria were the following<sup>23</sup>:

- The diagnosis of AIDS according to current Centers for Disease Control criteria.
- Diagnosis of CMV retinitis as determined by an experienced ophthalmologist based on the presence of characteristic necrotizing retinitis consisting of white, fluffy, or granular retinal infiltrates with or without hemorrhage.
- Patients with CMV retinitis involving zone 1 of the retina must have had:
  - progression of their retinitis while receiving at least 4 weeks (with at least 2 weeks at induction doses) of systemic ganciclovir and at least 4 weeks (with at least 2 weeks at induction doses) of systemic foscarnate; or
  - progression of their retinitis while receiving at least 4 weeks (with at least 2 weeks at induction doses) of systemic ganciclovir and systemic foscarnate as combination therapy; or
  - progression of their retinitis while receiving at least 4 weeks (with at least 2 weeks at induction doses) of systemic ganciclovir or foscarnate and intolerance of the other agent (e.g., ganciclovir or foscarnate).
- Patients with CMV retinitis which does not involve zone 1 (e.g., involving only zone 2 and/or zone 3) must have progression of their retinitis while receiving at least 4 weeks (with at least 2 weeks at induction doses) of systemic ganciclovir or foscarnate.

For the purpose of eligibility, CMV retinitis progression was defined as in section 2.1.3 with the following modification. In addition to the advancement of the border of an existing lesion or the occurrence of a new lesion, the definition of progression includes the persistence of active retinitis at the border of the lesion despite at least 4 weeks (with at least 2 weeks at induction doses) of systemic therapy, based upon ophthalmologic examination.

The remaining eligibility criteria for this study were identical with study GS-93-106.

Patients were to be excluded from study GS-93-107 for any of the following reasons:

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<sup>21</sup> Page 029 of Volume 87 from the original submission.

<sup>22</sup> Page 026 of Volume 84 from the original submission.

<sup>23</sup> Page 015 of Volume 87 from the original submission.

- receipt of other systemic or local anti-CMV therapy. All other anti-CMV therapy must have been discontinued within two days of randomization.
- receipt of therapy within the previous one week with any of the following:
  - Vidarabine
  - Amphotericin B
  - Other nephrotoxic agents
  - CMV hyperimmune immunoglobulin
  - Aminoglycoside antibiotics
  - Other investigational agents with anti-CMV activity.Patients receiving any of these drugs were to have discontinued the drug at least one week prior to the time of baseline evaluation and for the balance of the study period.

The remaining exclusion criteria were identical to those employed in study GS-93-106 (see section 2.1.4).

No incentives or disincentives were adopted to encourage or discourage the enrollment of women or minority patients.

**3.1.5 Toxicity Criteria and Dose Modifications** The toxicity grades and implied actions were similar to those employed in study GS-93-106 (see section 2.1.5). The primary differences follow<sup>24</sup>:

- For grade II nephrotoxicity, as indicated by an increase in serum creatinine (0.3 - 0.4 mg/dL) above screening or baseline lab (whichever was higher) or confirmed calculated creatinine clearance (41 - 50 mL/min), the patient's dose was to be reduced from 5 mg/kg/dose to 3 mg/kg/dose (Group A) or from 3 mg/kg/dose to 1.5 mg/kg/dose (Group B) for the remainder of study treatment.
- The patient was to have been removed from cidofovir treatment if there was  $\geq 3+$  proteinuria or an increase in serum creatinine of  $\geq 0.5$  mg/dL from baseline.

**3.1.6 Patient Withdrawal** As stated in the protocol<sup>25</sup>, enrolled patients were to receive cidofovir treatment until documentation of retinitis progression, the occurrence of treatment-limiting toxicity, or death, whichever occurred first. Additionally, it was stipulated that patients could be withdrawn from study treatment assignment in the following instances<sup>26</sup>:

- Intercurrent illness which would, in the judgement of the Investigator, affect assessments of clinical status to a significant degree.
- Unacceptable toxicity (see section 3.1.5).
- Disease progression as defined in section 2.1.3.
- Development of biopsy-documented extraocular CMV disease.
- Patient non-compliance or request to withdraw.
- Discontinuation of study at request of Sponsor, following consultation with the Principal Investigator.

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<sup>24</sup> Page 034 of Volume 87 from the original submission.

<sup>25</sup> Page 003 of Volume 87 from the original submission.

<sup>26</sup> Page 040 of Volume 87 from the original submission.

**3.1.7 Randomization** The randomization scheme<sup>27</sup> adopted for this study was used throughout. It consisted of the following variation on a biased-coin adaptive randomization design. If the site enrolling the patient was out of balance by one or 2 patients, the patient was assigned to the under represented therapy with probability 0.6. If the site was out of balance by more than two patients, then the patient was assigned to the under represented therapy with a probability of 0.8. Finally, if the enrolling site was perfectly balanced, then the study wide balance of the trial was considered. If the study wide imbalance was at least two, the patient was assigned to the under represented therapy. If the study wide imbalance was less than two, the patient was assigned to either therapy with equal probability.

**3.1.8 Centers** As noted above, study GS-93-107 was a multi center trial. The following table records the centers involved, their location, and the number of patients enrolled.

Institution	Location	Immediate	Deferred	Total
Cullen Eye Institute Baylor College of Medicine	Houston, TX	2	4	6
Beth Israel Hospital Joslin Diabetes Center Harvard Medical School	Boston, MA	3	6	9
George Washington University Medical Center	Washington, DC	1	0	1
Mount Zion Medical Center University of California, San Francisco	San Francisco, CA	14	13	27
Northwestern University	Chicago, IL	4	2	6
Royal Free Hospital	London, UK	2	2	4
Santa Clara Valley Medical Center	San Jose, CA	5	5	10
St. Luke's-Roosevelt Hospital Center	New York, NY	2	3	5
St. Stephen's Clinic Moorfields Eye Hospital	London, UK	3	3	6
University of California, Irvine	Irvine, CA	3	4	7
University of California, San Francisco	San Francisco, CA	4	4	8

<sup>27</sup> Page 311 of Volume 1 from Amendment #018 dated 9 February 1996.

Jules Stein Eye Institute University of California, Los Angeles	Los Angeles, CA	0	2	2
University of Southern California Doheny Eye Institute	Los Angeles, CA	5	3	8
University of South Florida College of Medicine	Tampa, FL	1	0	1
<b>Total</b>		<b>49</b>	<b>51</b>	<b>100</b>
1	Source: Page 046 of Volume 1 of Amendment #014 dated 24 January 1996.			

**3.1.9 Follow Up** As stipulated by the protocol<sup>28</sup>, following discontinuation of cidofovir therapy and/or progression to sight-threatening retinitis, **all patients** were to be evaluated (medical history, physical examination, and laboratory tests [renal function tests, CBC, and chemistry profile]) every three months for one year. The first follow up visit was to be performed two weeks following discontinuation of cidofovir therapy and/or progression to sight-threatening retinitis.

If there was clinical suspicion of retinitis progression, based upon ophthalmologic examination, at any week that retinal photography was not scheduled, retinal photographs were indicated.

A complete ophthalmologic examination, including fundus photography, was to be done at the time that cidofovir was discontinued. Additionally, those patients that discontinued cidofovir treatment for any reason other than progression of their CMV retinitis were to undergo complete ophthalmologic examination (all patients), including fundus photography (first 100 patients), every four weeks until documentation of CMV retinitis progression regardless of whether the patient initiated other anti-CMV treatment.

### 3.2 Enrollment:

**3.2.1 Patient Demographics** As a result of the randomization scheme described in section 3.1.7, 100 patients were enrolled in study GS-93-107; 49 received the 5 mg/kg dose and 51 received the 3 mg/kg dose. The demographic characteristics of the enrolled population are described in Table 9 below.

		5 mg/kg	3 mg/kg
Age (Range):		29 - 54	24 - 65

<sup>28</sup> Page 030 of Volume 87 from the original submission.

Gender:	Male	48	51
	Female	1	0
Race:	White	39	41
	Other <sup>2</sup>	10	10
1 Source: Table B page 007 of Volume 1 from Amendment #014 dated 24 January 1996.			
2 Other includes 4 African Americans and 16 patients of Hispanic origin.			

**3.2.2 Baseline Characteristics** With respect to selected baseline characteristics, the patients enrolled in study GS-93-107 can be described as follows.

Table 10: GS-93-107 Selected Mean Baseline Characteristics <sup>1</sup>			
Characteristic	5 mg/kg	3 mg/kg	p-value <sup>2</sup>
CD4 Count	19	9	0.165
Time from HIV Diagnosis (months)	85	79	0.510
Number of Lesions	2.1	2.0	0.876
Time from First CMV Diagnosis (days)	406	423	0.612
Number of Courses of IV-Ganciclovir or IV-Foscarnate	179	221	NR <sup>3</sup>
1 Source: Table B page 007 of Volume 1 from Amendment #014 dated 24 January 1996.			
2 P-values provided by the sponsor were based on a two-way analysis of variance with treatment and institution as main effects or on a Cochran-Mantel-Haenszel test stratified by institution.			
3 NR = Not reported.			

### 3.3 Methods of Analysis:

**3.3.1 Censoring Rules** As noted in section 3.1.3, the primary endpoint adopted in the study report was the time to the progression of CMV retinitis. As indicated in the study report<sup>29</sup>, the endpoint of the time to treatment limiting toxicity was considered a safety endpoint, not an efficacy endpoint and the endpoint of the time to the progression of CMV retinitis or death was considered a secondary endpoint even though the protocol defined these endpoints as primary.

For the endpoints of time to progression of CMV retinitis and the time to progression of CMV retinitis or death, the censoring rules adopted were the same as for study GS-93-106 (see section

<sup>29</sup> Pages 021 and 037 of Volume 1 from Amendment #014 Dated 24 January 1996.

2.3.1). For the endpoint of the time to treatment limiting toxicity, the following rule was adopted<sup>30</sup>: If, at the date of data cutoff for the report (which was 30 April 1995 for the first 60 patients enrolled and was later updated to 31 August 1995 for the first 100 patients enrolled), treatment-limiting toxicity had not occurred, the event was considered censored, and the time of censoring was the time of discontinuation of cidofovir treatment for causes other than adverse experience, or the date of progression as evidenced by fundus photograph, or the date of last follow up, whichever occurred first.

3.3.2 *Significance of Treatment Effect* To measure the significance of the difference between the 5 mg/kg group and the 3 mg/kg groups with respect to the endpoints of time to CMV retinitis progression, time to CMV retinitis progression or death, and time to treatment-limiting toxicity, the same methods as used in study GS-93-106 were adopted. Specifically, the logrank statistic, as calculated by PROC LIFETEST (SAS Institute, Inc.), was employed to measure the significance of the difference between the two groups. For primary analyses, p-values, unadjusted by institution, were reported. As supplementary analyses, p-values adjusted by institution were recorded. It should be noted that when adjusting for institutions, data from sites with fewer than three patients in either treatment group were pooled as one institution.

As described in the protocol<sup>31</sup>, "... p-values will be presented with the classical statistical critical values without adjustment for multiple comparisons."

3.3.3 *Magnitude of Treatment Effect* As with study GS-93-106, the measure adopted to assess the magnitude of the treatment effect involved computing the median times to the endpoint in question from the Kaplan - Meier product limit estimates. Median times and confidence intervals were computed using PROC LIFETEST (SAS Institute, Inc.). Confidence intervals were then compared and significant differences were declared when there was no overlap in the confidence intervals for the medians of the two groups being compared.

3.3.4 *Survival* Details pending.

### 3.4 Results of Analysis:

3.4.1 *Protocol Violations* A list of all protocol violations reported by the sponsor is recorded in Table 11 below. In the study report submitted with the NDA (when data from only the first 60 patients enrolled in study GS-93-107 were available), it is stated that no patients were excluded from efficacy or safety analyses due to protocol violations<sup>32</sup>.

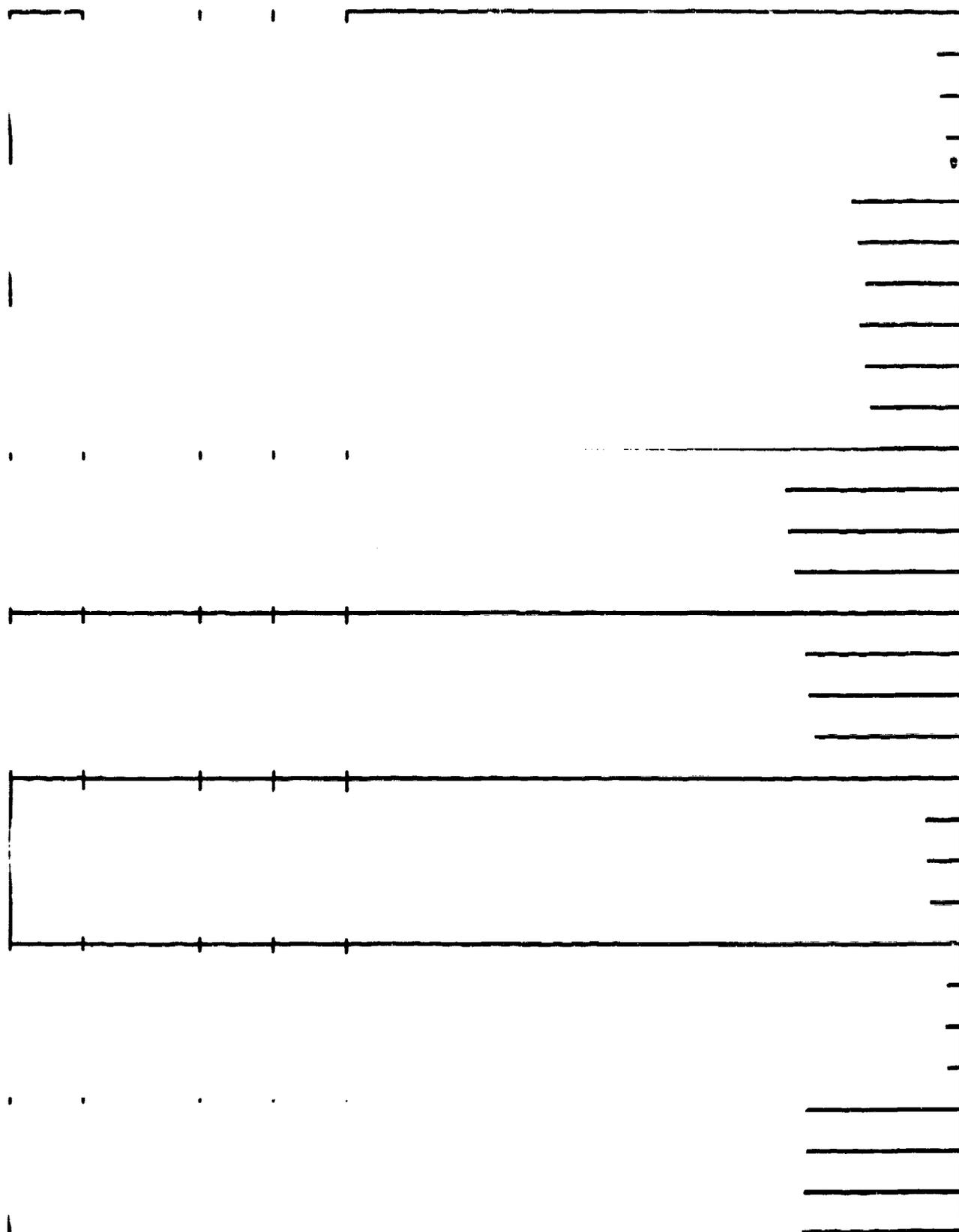
Table 11: GS-93-107 Protocol Violations <sup>1</sup>
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<sup>30</sup> Page 093 of Volume 77 from the original submission.

<sup>31</sup> Page 042 of Volume 87 from the original submission.

<sup>32</sup> Page 040 of Volume 84 from the original submission.






<sup>1</sup> Source: Table 1.4 page 054 of Volume 1 from Amendment #014 Dated 24 January 1996.

3.4.2 Patient Outcomes As reported by the sponsor, the outcomes for the patients enrolled in this trial are recorded in Table 12.

Table 12: GS-93-107 Disposition of Patients During Treatment with Cidofovir <sup>1</sup>				
Result	5 mg/kg		3 mg/kg	
	n	(%)	n	(%)
Progression <sup>2</sup>	11	(22)	23	(45)
Death <sup>2</sup>	4	(8)	1	(2)
Discontinued - Adverse Experience(s) / Intercurrent Event(s) <sup>2</sup>	17	(35)	12	(24)
Discontinued - Other <sup>2,3</sup>	6	(12)	8	(16)
Lost to Follow Up <sup>2</sup>	0	(0)	0	(0)
Still at Risk (at initial treatment assignment) <sup>2</sup>	11	(22)	7	(14)

<sup>1</sup> Source: Table 1.3 page 053 of Volume 1 from Amendment #014 Dated 24 January 1996  
<sup>2</sup> These categories are mutually exclusive and exhaustive with respect to all patients randomized  
<sup>3</sup> 'Discontinued - Other' includes patients who discontinued for reasons other than those shown above (e.g., due to mental incapacity, generalized debilitation, withdrawal of consent, etc.).

**3.4.3 Significance of Treatment Effect** The value of the unstratified logrank statistic obtained by the sponsor for the endpoint of the time to the progression of CMV retinitis or death was 4.836<sup>33</sup>. The associated asymptotic p-value was reported as 0.0279. In a footnote, the asymptotic p-value adjusted for institution was reported as 0.0668.

The value of the unstratified logrank statistic obtained by the sponsor for the endpoint of the time to treatment-limiting toxicity was 0.084<sup>34</sup>. The associated asymptotic p-value was reported as 0.7717. In a footnote, the asymptotic p-value adjusted for institution was reported as 0.3913.

The value of the unstratified logrank statistic obtained by the sponsor for the endpoint of the time to the progression of CMV retinitis was 9.847<sup>35</sup>. The associated asymptotic p-value was reported as 0.0017. In a footnote, the asymptotic p-value adjusted for institution was reported as 0.0069.

**3.4.4 Magnitude of Treatment Effect** For patients randomized to the 5 mg/kg dose, PROC LIFETEST returned 71 days as an estimate of the median time to the progression of CMV retinitis or death with a 95% confidence interval ranging from 63 days to 115 days<sup>36</sup>. For patients randomized to the 3 mg/kg dose, the corresponding estimate of the median time to the progression of CMV retinitis or death was 50 days with a 95% confidence interval ranging from 36 days to 78 days<sup>37</sup>.

For patients randomized to the 5 mg/kg dose, PROC LIFETEST returned 123 days as an estimate of the median time to treatment-limiting toxicity with a 95% confidence interval ranging from 101 days to 164 days<sup>38</sup>. For patients randomized to the 3 mg/kg dose, the corresponding estimate of the median time to treatment-limiting was 135 days with a 95% confidence interval with neither limit achieved<sup>39</sup>.

For patients randomized to the 5 mg/kg dose, PROC LIFETEST returned 115 days as an estimate of the median time to the progression of CMV retinitis with a 95% confidence interval ranging from 70 days to upper limit not reached<sup>40</sup>. For patients randomized to the 3 mg/kg dose, the corresponding estimate of the median time to the progression of CMV retinitis was 49 days with a 95% confidence interval ranging from 35 days to 52 days<sup>41</sup>.

**3.4.5 Survival** Details pending.

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<sup>33</sup> Page 177 of Volume 3 from Amendment #014 Dated 24 January 1996.

<sup>34</sup> Page 160 of Volume 1 from Amendment #014 Dated 24 January 1996.

<sup>35</sup> Page 086 of Volume 1 from Amendment #014 Dated 24 January 1996.

<sup>36</sup> Page 177 of Volume 3 from Amendment #014 Dated 24 January 1996.

<sup>37</sup> Ibid.

<sup>38</sup> Page 160 of Volume 1 from Amendment #014 Dated 24 January 1996.

<sup>39</sup> Ibid.

<sup>40</sup> Page 086 of Volume 1 from Amendment #014 Dated 24 January 1996.

<sup>41</sup> Ibid.

**3.5 Summary:** In the study report submitted with the NDA, the following conclusions are stated<sup>42</sup>:

- "Sixty patients with evidence of CMV retinitis progression while receiving ganciclovir and/or foscarnate therapy were randomized to one of two groups: 5 mg/kg group (n = 29) - IV cidofovir 5 mg/kg once weekly for 2 weeks (induction), then 5 mg/kg once every other week (maintenance) or 3 mg/kg group (n = 31) - IV cidofovir 5 mg/kg once weekly for 2 weeks (induction), then 3 mg/kg once every other week (maintenance) until photographic documentation of CMV retinitis progression, the occurrence of treatment-limiting toxicity, or death." [emphasis added]
- "No significant differences were observed between the 5 mg/kg and 3 mg/kg groups in demographic and baseline characteristics."
- "The median time to CMV retinitis progression as assessed by protocol-specified, bilateral, full-field retinal photographs evaluated by an ophthalmologist masked to treatment assignment was 115 days (95% confidence interval: 38 days to upper limit not yet reached) in the 5 mg/kg group and 49 days (95% confidence interval: 32 days to upper limit not yet reached) in the 3 mg/kg group (p = 0.12; log-rank test)".
- "Asymptomatic proteinuria (18%) was the most common serious adverse experience felt by Investigators to be possibly or probably related to cidofovir. Two of 29 (7%) patients in the 5 mg/kg group and 3 of 31 (10%) patients in the 3 mg/kg group developed a serum creatinine  $\geq$  2 mg/dL. Proteinuria and serum creatinine elevations were both partially reversible."
- "Transient, mild to moderate probenecid reactions, including fever, rash, headache, and/or nausea, occurred in 32 of 60 (53%) patients and were treatment-limiting in 2 of 60 (3%) patients."
- "Mortality in the two randomization groups was similar and not related to cidofovir; Kaplan Meier estimates of median survival time were 130 days (95% confidence interval: 95 to 242 days) for the 5 mg/kg group and 128 days (95% confidence interval: 64 to 146 days) for the 3 mg/kg group (p = 0.24; log-rank test)."
- "When compared to historical data from the Studies of Ocular Complications of AIDS Research Group, which reported median third time to CMV retinitis progression of 35 days for patients treated with ganciclovir or foscarnate (28)<sup>43</sup>, these interim data suggest that cidofovir is efficacious in delaying progression of CMV retinitis in patients with AIDS and relapsing CMV retinitis. Treatment was associated with manageable side effects; pre-dose monitoring of renal function and concomitant administration of saline hydration and probenecid appeared to minimize the potential for significant drug-related toxicity."

In the "Brief Interim Report"<sup>44</sup> submitted with the updated data on the 100 patients enrolled in study GS-93-107, the following amendments to the above conclusions were recorded:

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<sup>42</sup> Page 055 of Volume 84 from the original submission.

<sup>43</sup> The reference cited here is "Studies of the Ocular Complications of AIDS Research Group, AIDS Clinical Trials Group. Foscarnate - ganciclovir cytomegalovirus retinitis trial 4: visual outcomes. *Ophthalmol.* 101; 1250-61, 1994. (see page 060 of Volume 84 from the original submission).

<sup>44</sup> Page 003 of Volume 1 of Amendment #014 Dated 24 January 1996.

- The number randomized to the 5 mg/kg group increased to 49, the number randomized to the 3 mg/kg group increased to 51.
- The median time to CMV retinitis progression was 115 days (95% confidence interval: 70 days to upper limit not yet reached) for the 5 mg/kg group and 49 days (95% confidence interval: 35 days to 52 days) in the 3 mg/kg group ( $p = 0.0017$ ; log-rank test).
- The percentage of patients reporting asymptomatic proteinuria was updated to 17%.
- The percentages of patients who developed a serum creatinine  $\geq 2$  mg/dL were updated to 4 of 49 (8%) of the patients in the 5 mg/kg group and 4 of 51 (8%) of the patients in the 3 mg/kg group.
- The percentage of patients experiencing transient probenecid reactions was updated to 47 of 100 (47%) of the patients enrolled. The percentage of patients with treatment-limiting probenecid reactions remained at 3% (3 of 100 patients).
- The updated Kaplan-Meier estimates of the median survival time were updated to 167 days (95% confidence interval: 109 days to 242 days) for the 5 mg/kg group and 146 days (95% confidence interval: 107 to 218 days) for the 3 mg/kg group ( $p = 0.69$ ; log-rank test).
- The following claim was added: "In addition, these data demonstrate that 5 mg/kg maintenance therapy has greater efficacy than 3 mg/kg maintenance therapy; thereby confirming and extending the observed cidofovir treatment effect noted in the interim report of study GS-93-107 and the final report of study GS-93-106 ... "

#### 4. REVIEWER COMMENTS: MAJOR ISSUES

##### 4.1 General Design Comments

**4.1.1 Open Label Design** Both trials GS-93-106 and GS-93-107 employed an open label design. While readings of retinal photographs were masked, patients and physicians knew treatment assignments. As a result, biases could have entered these studies either consciously or unconsciously. For example, the grading of the severity of treatment limiting toxicities relied, in part, on the subjective judgement of the physician (as opposed to objective laboratory data). As a result, the assessment of the severity of a treatment limiting toxicities might have been influenced by a desire (either of the patient or the physician) to stay on assigned therapy or to switch to an alternative therapy. Because such biases tend to be subtle, and because the direction of the bias may not be discernible, the results from open label trials require cautious interpretation. As these trials are discussed in turn, evidence suggesting the possibility of such bias will be discussed.

**4.1.2 Randomization** While the level of complexity differed, both trials GS-93-106 and GS-93-107 employed complex randomization schemes to assign patients to therapy (see sections 2.1.7 and 3.1.7). For these complex designs, it was possible to generate appropriate reference distributions for the purpose of assessing the significance of any one of several test statistics. However, the complexity of the adopted designs rendered the estimation of variances of point estimates (reflecting the design employed) intractable. Because sample sizes were small for both trials, appeals to asymptotic variances where the design might be ignored is questionable. Thus, a consequence of the complex designs adopted is that the confidence to be placed in confidence intervals of point estimates is

unknown.

**4.1.3 Follow Up** The protocols of studies GS-93-106 and GS-93-107 mandated that unless the patient switched to an alternative proscribed therapy (e.g., ganciclovir or foscarnate), they were to be followed every two weeks with a complete ophthalmologic examination including retinal photographs (see sections 2.1.9 and 3.1.9). However, because patients presented with CMV retinitis when they enrolled in these trials, withdrawal from the trial frequently meant switching to a proscribed therapy. Thus, complete follow up on many patients is lacking and this is a major problem. Using the sponsor's readings of the retinal photographs (with the FDA's readings of the photographs, the situation is worse), 56% of the patients assigned to immediate therapy in study GS-93-106, 49% of the patients assigned to the 5 mg/kg dose in study GS-93-107, and 35% of the patients assigned to the 3 mg/kg dose in study GS-93-107 (see sections 6.2.2 and 7.2.2 below) discontinued therapy prematurely. Since many of these patients went on to treatment regimens employing proscribed therapies, their status with respect the progression of CMV retinitis was not recorded. Absent follow up on these patients, there is little that can be done but to proceed cautiously.

**4.1.4 Sample Size** The numbers of patients enrolled in studies GS-93-106 and GS-93-107 were small. For the pivotal trial, GS-93-106, total enrollment was 48 with 25 patients exposed to cidofovir. While this sample size was sufficient to assess the statistical significance of a treatment effect, it precluded a precise estimate of the magnitude of the treatment effect. Typically, when sample sizes are small, observations occur which have undue influence on the final assessment of results. When studies GS-93-106 and GS-93-107 are discussed below, evidence of this phenomenon will be discussed.

**4.2 Informative Censoring** When evaluating an endpoint which is defined by the time to an event, it is very important to examine carefully the mechanism by which patients are censored.

**4.2.1 An Example** If an omniscient investigator wanted to maximize the distinction between an experimental therapy and a control, he would simply withdraw each patient receiving experimental therapy from the trial an instant before they would have presented with an event. In this way, no events would occur on the treatment arm and the Kaplan - Meier estimate of the survival probability would remain at one throughout the trial. On the other hand, when the endpoint and treatment assignment are independent of the censoring mechanism, the reasons for a patients withdrawal from a trial are independent of the question of interest and can be ignored. Evidently, informative censoring lies somewhere between the extreme of perfect informative censoring and uninformative censoring. But, precisely where is unknown and given the nature of the follow up present in trials GS-93-106 and GS-93-107, unknowable.

**4.2.2 Consequences of Informative Censoring** The lack of independence between the censoring mechanism and the outcome event has the following consequences. First, as discussed below, the interpretation of the Kaplan-Meier (or product limit (PL)) estimator as an estimator of the survival curve is questionable. Second, absent the independence of the censoring mechanism and the outcome

event, what is tested by the logrank, Wilcoxon, and like tests is unknown. Finally, attempts to estimate the relative risk by means of Cox models is of questionable utility because the appropriate likelihood to be maximized by estimates from such a model cannot be described. The remainder of this section discusses each of these points in turn.

Absent the assumption of independence between the event of interest and the censoring mechanism, it is not possible to interpret the PL estimator as a maximum likelihood estimator of the survival curve. The reason is that to describe the appropriate likelihood would entail a knowledge of the joint distribution of the event of interest and the censoring mechanism. Given the lack of follow up beyond a patients withdrawal from one of these trials, a competing risks formulation of the time to progression of CMV retinitis or treatment limiting condition would be appropriate. In this context, the joint distribution of the time to progression of CMV retinitis and the time to a treatment limiting condition is unobservable<sup>45</sup>. Thus, the appropriate likelihood to be maximized by the PL estimator is also unobservable.

Another consideration when the outcome event and censoring mechanism are dependent is this. While the product limit estimator may cease to be a maximum likelihood estimator of the survival curve, it may still estimate a relevant quantity. To discuss this point further, suppose that  $t_i$  and  $t_{i+1}$  are adjacent event times. Let  $n_i$  and  $n_{i+1}$  denote the number of patients at risk at these event times, let  $d_i$  and  $d_{i+1}$  denote the number of events that occur at these event times, and let  $c_i$  and  $c_{i+1}$  denote the number of patients censored at these event times. Then this information contributes the following product to the PL estimate of the survival curve:

$$\left(1 - \frac{d_i}{n_i}\right) \left(1 - \frac{d_{i+1}}{n_{i+1}}\right) \text{ with } n_{i+1} = n_i - d_i - c_i.$$

When the outcome event and censoring mechanism are independent,  $d_i/n_i$  is an estimate of the instantaneous hazard at time  $t_i$ . When the censoring mechanism and the outcome event are dependent, this is no longer the case. Should it happen that the outcome event and the censoring mechanism are positively correlated, it might be argued that one or more of the patients censored at time  $t_i$  should be classified as events. In this case, the lack of independence between the censoring mechanism and outcome event suggests that the PL estimator overestimates the survival curve at time  $t_i$ . On the other hand, if the outcome event and censoring mechanism are negatively correlated, it might be argued that patients censored at time  $t_i$  were never really at risk in the first place and that such patients should not now be removed from the risk set at time  $t_{i+1}$ . If this argument is appropriate, then the PL estimator yields an underestimate of the survival curve at time  $t_{i+1}$ . Absent any attempt to assess the nature of the joint distribution of the censoring mechanism and outcome event, whether the PL estimator yields an over estimate of the survival curve or an underestimate is

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<sup>45</sup> Teistis, A. (1975) A Nonidentifiability Aspect of the Problem of Competing Risks. Proceedings of the National Academy of Sciences, 72, 20 - 22.

unknown.<sup>46</sup>

A second consequence of the lack of independence between the outcome event and censoring mechanism is this. As has already been noted, the lack of independence calls into question what the PL estimator estimates. Thus, it is not surprising to find that authors who discuss the large sample theory of the product limit estimator require independence between the censoring mechanism and the event of interest<sup>47</sup>. This reviewer is unaware of any results concerning the large sample theory of the PL estimator absent the independence assumption. In view of the fact that the logrank, Wilcoxon, and like test statistics can be expressed as functionals of the PL estimator<sup>48</sup>, the appropriate large sample theory for these tests flows from the large sample theory for the PL estimator. If the large sample theory for the PL estimator is unknown, then so too is the large sample theory for these test statistics. Thus, when the censoring mechanism and outcome event are dependent, it is not at all clear what the logrank, Wilcoxon, and like tests test.

The final consequence mentioned above is this. As noted, absent knowledge of the joint distribution of the censoring mechanism and the event of interest, it is not possible to write down the likelihood appropriate to describe the sample. Above, this hypothetical likelihood was envisaged in terms of the survivor function to provide a maximum likelihood interpretation for the PL estimator. Here, the formulation is envisaged in terms of cause specific hazard functions<sup>49</sup>. Thus, as a corollary to the problems associated with the interpretation of the PL estimator, we have that absent knowledge of the joint distribution of the censoring mechanism and the outcome event, it is not possible to write down the likelihood that a Cox model would maximize.

**4.2.3 Death and Informative Censoring** While the sponsor can argue that any of the reasons for premature patient withdrawal from trials GS-93-106 and GS-93-107 are independent of the endpoint in question and the treatment assignment, the one reason for patient withdrawal that is objectively dependent is death. An intuitive argument highlighting the dominant thread of this observation follows. Suppose that the time to CMV retinitis progression and the time to death are two random variables with a continuous joint distribution. For these two random variables to be independent, it is required that the joint probability density factor as the product of the marginal densities. A necessary condition for this to be possible is that the region where the joint density function is positive be rectangular (otherwise, the region where one of the marginal densities is positive would depend

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<sup>46</sup> This point is further elaborated in Cox and Oakes (1984) *Analysis of Survival Data*. Chapman & Hall, London. These authors discuss the possibility of bounding the survival curve by making assumptions about the joint distribution of the outcome event and censoring mechanism as discussed above. However, they note that such bounds are frequently too wide to be of any practical use.

<sup>47</sup> See, for example, Breslow, N. And Crowley, J. (1974) A Large Sample Study of the Life Table and Product Limit Estimates Under Random Censorship. *The Annals of Statistics*, 2, 437 - 453.

<sup>48</sup> Harrington, D. P. And Fleming, T. R. (1982) A Class of Rank Test Procedures for Censored Survival Data. *Biometrika*, 69, 553-66.

<sup>49</sup> See Kalbfleisch, J. D. And Prentice, R. L. (1980) *The Statistical Analysis of Failure Time Data*. John Wiley & Sons, New York. Also see Cox, D. R. (1972) Regression Models and Life-Tables. *Journal of the Royal Statistical Society, Series B*, 34, 187 - 220.

on the value specified for the other variable). However, the region where the joint density of the time to CMV retinitis and the time to death random variables is positive is, necessarily triangular. Once it is known that the patient has died, it is known that the CMV retinitis will not progress. Thus, death and the time to progression of CMV retinitis can not be independent. This is true regardless of the relationship between the cause of death and the progression of CMV retinitis. Even imagining a sequence of events leading to death that would amaze Rube Goldberg, it would still be the case that the occurrence of death and the progression of CMV retinitis are not independent.

As each trial is discussed in detail, it will be noted that the sponsor seems to acknowledge that there might be some relation between some of the reasons for premature patient withdrawal from the trials and the treatment assignment and/or endpoint of progression to CMV retinitis.

**4.3 The Median Time to an Event** The purpose of this discussion is to raise some issues concerning the estimate and use of the median time to an event as a means for quantifying the magnitude of a treatment effect.

*4.3.1. Complete Information and Large Samples* Consider the case where the underlying survival distribution is continuous and strictly increasing. If the distribution function for this population were known, then a unique estimate of the median would be provided by projecting on to the time axis, the point where the survival curve and the horizontal line with height 0.5 intersect. Next, suppose that the distribution function is not known but is estimated by a large sample without censoring. In this event, the strong law of large numbers ensures that the empirical distribution function provides a good estimate of the underlying survival curve. Thus, while the empirical distribution function is a step function, with large samples and without censoring the steps involved are generally small (in fact they can be made as small as desired by increasing the sample size sufficiently). However, the situation changes in the presence of small samples and censoring.

*4.3.2. Medians of Discrete Distributions* One way to think of the product limit estimate is as the distribution function for the events actually observed in the trial. Under this interpretation, the product limit estimate is, in fact, the distribution function for the discrete distribution given by the population of patients enrolled. And here is the problem. Typically, for discrete distributions, the median is not uniquely defined. Thus, two distinct approaches to describing the median present themselves. On the one hand, acknowledge that the median is not defined and simply report the percentiles that bracket what would be the median if it existed. On the other hand, compute a weighted average of the bracketing percentiles and call this average the median.

*4.3.3 The Linear Interpolate / Weighted Average* Translating the above comments into the case of the Kaplan - Meier survival curve the situation is this. Suppose that the median is bracketed by the event times  $t_1 < t_2$ . By this is meant the following. The event times  $t_1$  and  $t_2$  are adjacent and the corresponding probabilities given by the Kaplan - Meier Survival curve satisfy  $p_1 > 1/2 > p_2$ . Let  $p$  denote any probability in the interval  $(p_2, p_1)$ . Then the linear interpolate for the  $p^{\text{th}}$  percentile is given by

$$t = \frac{(p - p_2)}{(p_1 - p_2)} \cdot t_1 + \frac{(p_1 - p)}{(p_1 - p_2)} \cdot t_2.$$

Thus, it is seen that not only is  $t$  the linear interpolate for the  $p^{\text{th}}$  percentile, it is also a weighted average of the percentiles bracketing the median. Setting  $p$  equal to  $1/2$  in this equation gives the linear interpolate for the median.

**4.3.4. PROC LIFETEST (SAS Institute, Inc.)** The procedure PROC LIFETEST (SAS Institute, Inc.) estimates the median from a Kaplan - Meier survival curve by reporting the value of the percentile that brackets the median from above. However, because the linear interpolate appears to be a natural alternative to this procedure, why hasn't it been adopted? A potential is this. The asymptotic variance<sup>50</sup> of the linear interpolate of the median is given by

$$AVar(\hat{\theta}) = \frac{AVar(\hat{S}(\theta))}{f^2(\theta)}.$$

Here,  $\hat{\theta}$  is the linear interpolate for the median  $\theta$ ,  $\hat{S}(\cdot)$  is the product limit estimator of the survival function, and  $f(\cdot)$  denotes the probability density of the distribution for which  $S$  is the survival function. The numerator of the right hand side can be estimated by Greenwood's formula. The denominator, on the other hand, introduces a whole new set of problems. Thus, while the linear interpolate makes sense as a point estimate for the median, computing its variance (and, thus, confidence intervals) presents problems. One final comment, however, is in order. Given the small samples observed in studies GS-93-106 and GS-93-107 and given the complicated nature of the designs adopted, neither the SAS estimate of the variance of the estimate of the median nor the expression above truly represents a good assessment of the variability of the estimate.

## 5. DATA ISSUES

**5.1 FDA Assessment of the Raw Data:** To assess the quality of the data submitted with this NDA, Dr. Wiley Chambers, Acting Division Director of the Division of Anti-inflammatory, Analgesic, and Ophthalmologic Drug Products, HFD-550, was consulted. He read all of the slides submitted with the NDA to check the raw data for accuracy. There were some differences between these readings of the retinal photographs and the readings provided by the sponsor. Explicit patient by patient differences are recorded in Appendix A below. Summary descriptions of differences between the sponsor and the FDA consultant are recorded with the description of each study.

**5.2 Time Line of Events:** Throughout the course of this NDA review, new data was arriving.

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<sup>50</sup> See page 76 of Miller, R. G. Jr. (1981) Survival Analysis. John Wiley & Sons, Inc. New York.

The purpose of this section is to chronicle the timing of the arrival of this data.

Table 13: Selected Chronology of Events in the Review of NDA 20-638	
Date	Event
25 July 1995	Preliminary data from GS-93-106 arrived filed under IND 39,192 #158.
14 August 1995	The derived data from what the sponsor labeled a worst case scenario for study GS-93-106 arrived filed under IND 162.
4 October 1995	NDA # 20-638 arrived along with data. The data diskettes were unreadable.
17 October 1995	A second set of data diskettes arrived filed as Amendment #002. These diskettes were still unreadable.
31 October 1995	A third set of data diskettes arrived filed as Amendment #004. These diskettes were readable.
27 November 1995	Updated survival data through 4 October 1995 arrived for GS-93-106 filed as Amendment #007.
16 January 1996	Derived data files culling information on adverse events arrived for both studies GS-93-106 and GS-93-107. This submission was filed as Amendment #012.
24 January 1996	An analysis of GS-93-107 updated to encompass 100 enrolled patients arrived along with updated data diskettes for GS-93-106 and GS-93-107. This submission was filed as Amendment #014.
9 February 1996	Detailed information on the randomization schemes adopted for GS-93-106 and GS-93-107 arrived, filed as Amendment #018.
22 February 1996	An updated analysis of survival for GS-93-106 and GS-93-107 through 31 January arrived without data diskettes. This submission was filed as Amendment #024.
29 February 1996	The background information that the sponsor was submitting to the upcoming advisory committee meeting arrived, filed as Amendment #027.
4 March 1996	Received Dr. Chamber's assessment of the data from GS-93-106 in the form of a print out of a spread sheet page.
11 March 1996	Received Dr. Chamber's assessment of the data from GS-93-107 in the form of a print out of a spread sheet page.
15 March 1996	The sponsor presented their case before a joint session of the Anti-virals and Ophthalmologic advisory committees.

## 6. STUDY GS-93-106

**6.1 Patient Characteristics** The patient demographics and baseline characteristics have not changed since they were recorded in section 2.2. They are repeated below to emphasize two points. The first is that only two women were enrolled in this trial and, of these, only one was exposed to cidofovir. The second point is this. While the sponsor dismisses all baseline differences between patients

differences in the number of lesions and in the time from first diagnosis of CMV retinitis are sufficiently large to merit comment. If there is any bias in this trial with respect to these characteristics, it is apparently in the direction that would benefit a favorable assessment of cidofovir therapy.

		Immediate	Deferred
Age (Range):		27 - 56	30 - 46
Sex:	Male	24	22
	Female	1	1
Race:	White	20	20
	Other <sup>2</sup>	5	3

1 Source: Table 2.1 page 246 of Volume 77 of the original submission.  
2 One Native American and 7 patients of Hispanic origin.

Characteristic	Immediate	Deferred	p-value <sup>2</sup>
CD4 Count	13.3	16.2	0.695
Time From HIV Diagnosis (months)	67.2	78.2	0.470
Number of Lesions	1.2	1.7	0.081
Time From First CMV Diagnosis (days)	5.1	8.1	0.147

1 Source: Table 2.1 page 246 of Volume 77 from the original submission.  
2 P-values provided by the sponsor were based on a two-way analysis of variance with treatment and institution as main effects or on a Cochran-Mantel-Haenszel test stratified by institution.

**6.2 Patient Outcomes** Before proceeding, it should be pointed out that the endpoint actually observed in this study is not time to the progression of CMV retinitis. Rather, what is observed is the time to the detection of the progression of CMV retinitis. With this in mind, then, how patients fared on this trial is recorded in tables 16 and 17 below. There is some disagreement between Table 5 above and table 16. This reviewer is not aware of the reason for this difference. The entries in Table 5 do not match the data submitted with the NDA on diskette nor with the paragraph narratives

of the reasons for premature discontinuation for patients who discontinued therapy prematurely<sup>51</sup>. Additionally, when Dr. Chamber's assessments of the retinal photographs are considered, there are further differences. In Table 16, how patients responded is partitioned into mutually exclusive and exhaustive categories. For each category, those patients who belong are recorded by number. For verification, these patient numbers were checked against the paragraph narratives previously cited. Table 17 repeats Table 16 using the data as assessed by Dr. Chambers. One new category is included in this table. It consists of those patients who were labeled as progressors by the sponsor's readings of the retinal photographs who were not labeled as progressors as a result of Dr. Chamber's reading of the retinal photographs.

Result	Immediate		Deferred	
	#	Patients	#	Patients
<b>Progressed</b>	<b>10</b>		<b>18</b>	
<b>Excluded Events</b>	<b>15</b>		<b>5</b>	
<b>Possible Progression</b>	<b>3</b>		<b>2</b>	
<b>Progression by Examination</b>	<b>(1)</b>		<b>(1)</b>	
<b>With Respect to Second Photograph</b>	<b>(2)</b>		<b>(1)</b>	
<b>Systemic CMV Disease</b>	<b>2</b>		<b>1</b>	
<b>Withdrawal Consent (Moribund State)</b>	<b>2</b>			
<b>Adverse Reaction:</b>	<b>7</b>			
<b>Proteinuria</b>	<b>(4)</b>			
<b>Probenecid Reaction / Peripheral Neuropathy</b>	<b>(3)</b>			
<b>Administrative</b>	<b>1</b>		<b>2</b>	
<b>Baseline Zone 1 CMV Retinitis</b>			<b>(1)</b>	
<b>Enrolled at Treatment Assignment</b>			<b>(1)</b>	
<b>Completed Trial</b>	<b>(1)</b>			
<b>Total</b>	<b>25</b>		<b>23</b>	

\* Patient 2304 was diagnosed with grade III proteinuria, was taken off study treatment, remained off alternative CMV therapy, and continued to be photographed. The patient subsequently progressed and was so scored in the Sponsor's analysis.

<sup>51</sup> See page 173 of Volume 77 from the original submission.

The following explanations of the above categories is in order. Possible progression (progression by examination) includes all patients who were diagnosed as having progressed by ophthalmologic examination who were not subsequently confirmed as having progressed on the basis of masked readings of the retinal photographs. Possible progression (with respect to second photograph) includes all patients whose baseline photograph was not evaluable and yet they showed progression when subsequent photographs were compared. Whether this was their first progression could not be determined. Systemic CMV disease includes all patients who presented with CMV disease (primarily colitis and gastroenteritis). Withdrawn consent (Moribund State) includes all patients who withdrew consent and either died soon after or were assessed as being debilitated. Adverse Reaction (Proteinuria) includes all patients who were discontinued prematurely after presenting with grade III or IV proteinuria. The remaining categories are self explanatory.

When looking at this table, the first point to notice is that only one of the 48 enrolled patients actually completed the trial. All others were discontinued either prematurely or because they progressed. Eighteen patients on deferred therapy and 10 patients on immediate therapy progressed. On the surface, this would suggest a treatment effect in favor of cidofovir therapy. Of concern, however, are the 14 patients on immediate therapy who are described as having been discontinued prematurely. A question is whether these patients can be ignored when describing the overall impact of cidofovir therapy.

Table 17: GS-93-106 Patient Outcomes (FDA Readings)				
Result	Immediate		Deferred	
	#	Patients	#	Patients
<b>Progressed</b>	13		19	
<b>Excluded Events</b>	12		4	
<b>Possible Progression</b>	3		2	
<b>Progression by Examination</b>	(1)			
<b>With Respect to Second Photograph</b>	(1)		(1)	
<b>Rescored as a Nonprogressor</b>	(1)		(1)	
<b>Withdraws Consent in Moribund State</b>	2			
<b>Adverse Reaction:</b>	6			
<b>Proteinuria</b>	(4)			
<b>Probable Reaction / Peripheral Neuropathy</b>	(2)			
<b>Administrative</b>	1		2	
<b>Baseline Zone 1 CMV Retinitis</b>			(1)	112

Based on Treatment Assignment		(1)
Completed Trial	(1)	

As is evident from this table, there were some differences between the Sponsor's assessment of the retinal photographs and the FDA's assessment of the retinal photographs. However, the general picture is still the same: (1) there is an apparent treatment effect in favor of cidofovir, and (2) the number of patients randomized to immediate therapy who discontinued prematurely is still large.

A notable difference between Tables 16 and 17 is the following. The category of Systemic CMV Disease has disappeared. The patients previously placed in this category by the sponsor's assessment of the retinal photographs were scored as having progressed on the basis of the FDA's assessment of the retinal photographs. This fact suggests that the assumption of noninformative censoring required of the sponsor's estimates of the median times to the detection of CMV retinitis progression is optimistic.

**6.3 The Combined Endpoint** Because it is difficult to suppose that any of the reasons for premature patient discontinuation are independent of the endpoint of CMV retinitis progression and/or treatment assignment, a combined endpoint that can be interpreted as the time to the detection of CMV retinitis progression or treatment limiting condition was constructed. How this was done will be described presently. What is important to note here is that all estimates of medians and all Kaplan - Meier plots presented by the sponsor require the assumption that all of the patients who discontinued therapy prematurely did so independently of the endpoint and/or treatment assignment. As a counter balance to this optimistic assumption, the combined endpoint assumes that all reasons for premature discontinuation from study therapy are not independent of the endpoint and/or treatment assignment. Evidently, these two extremes bracket the true state of affairs.

With respect to the applicant's assessment of the retinal photographs, the following procedure was used to generate the combined endpoint of the time to the detection of CMV retinitis or treatment limiting condition (whichever comes first). First, all patients who were scored as having progressed continued to be so scored. (This leads to one inconsistency that favors cidofovir therapy. As noted in the footnote to Table 16, patient 2304 withdrew after presenting with grade III proteinuria and, since he did not go onto alternate proscribed therapy, he was followed until he progressed. Both the sponsor's endpoint of the time to the detection of CMV retinitis progression and the construction of the combined endpoint scored this patient as a progressor at the time his progression was detected.) To determine the appropriate dates to give to patients who discontinued therapy prematurely, there were three sources. First, was a variable supplied in the sponsor's data base called DISCDAT. This variable was supposed to record when a patient discontinued therapy. However, there were sufficient inconsistencies that this variable proved to be unreliable. Second, were the variables AESTART and AETEXT. AETEXT described the adverse event the patient experienced and AESTART recorded the date of the occurrence. Except for patients who withdrew consent, this pair of variables was used to determine the time when a patient discontinued therapy prematurely. Finally, to establish the

reason for discontinuation and to more accurately determine when a patient's consent was withdrawn, the source of last resort was the listing of paragraph narratives cited previously.

With respect to the definition of the combined endpoint when the FDA's assessment of the retinal photographs was employed, the following algorithm was adopted. If the patient was scored as a progressor, he continued to be scored as a progressor. If the patient had been scored as a progressor by the sponsor's reading of the retinal photographs but not by the FDA's reading of the retinal photographs, the patient was treated as if he had been scored a progressor on the basis of an ophthalmologic examination, i.e., he was treated as an event at the time his photograph confirmed progression (sponsor's reading). Finally, all other patients who discontinued therapy prematurely were scored as events at the time they discontinued (determined as described above).

One further endpoint will be considered in this review. It simply focuses on the progression of CMV disease, whether retinitis or systemic. While it is still difficult to presume that all reasons for patient discontinuation are independent of the combined endpoint of the time to progression of CMV disease, this simple modification of the sponsor's endpoint serves to point out just how sensitive their reported estimates of treatment effect are.

**6.4 Magnitude of Treatment Effect** Recorded below are estimates of the median times to detection for the endpoints included in this table (the endpoint of CMV disease will be treated separately later). Beside each estimate in parentheses is the linear interpolate that was discussed in section 4 above.

Table 18: GS-93-106 Median Time to Detection Summary								
	Sponsor Assessments				FDA Assessments			
	CMV Retinitis		Combined		CMV Retinitis		Combined	
Immediate	120.0	(78)	52	(51)	78.0	(49.5)	40.0	(39)
Deferred	21.5	(21.5)	22	(21.2)	21.0	(20.2)	21.0	(20.1)
Difference	98.5	(56.5)	30	(29.8)	57.0	(29.3)	19.0	(18.9)

Before describing the general story told by this table, a few words about the sponsor's estimate of 120 for the median time to the detection of CMV retinitis progression. In the context of bracketing the median, as described in section 4.3.3, Table 6 reveals that, in fact, 66 is the 48<sup>th</sup> percentile and 120 is the 64<sup>th</sup> percentile. As an estimate of the median or 50<sup>th</sup> percentile, SAS and the sponsor advocate choosing 120. Another point to notice is this. By the time of 120 days is reached, only three of the patients randomized to the immediate therapy are left on trial. Thus, aside from estimating the wrong percentile, the estimate of 120 days relies on very little information and is very imprecise.

Turning to the general message contained in this table it is seen that the estimate of treatment effect is highly variable - quite apart from any sampling error. The estimates of the treatment effect range

anywhere from 19 to 98.5 days depending on the endpoint adopted and the assumptions granted. It should be noted that on the deferred arm there is little variation in the estimated medians across the endpoint categories. This is simply a reflection of the fact that on the deferred arm of the trial, few patients discontinued therapy prematurely. Further, there is considerably less variability in the interpolated estimates of the median on the immediate arm of the trial. This reflects the fact that the linear interpolate is less influenced by one or two influential observations. On the other hand, because of extensive loss to follow up on the immediate therapy arm and because of the sensitivity of the estimate of the median time to detection of CMV retinitis progression, there is wide variation in the estimate of a potential treatment effect.

One further observation illustrates this point further. If the appropriate endpoint for the evaluation of this drug is deemed to be better represented by the combined endpoint of CMV disease (retinitis or systemic), if the assumption that all other reasons for the premature discontinuation of patients from therapy are independent of this endpoint and treatment assignments is granted, if the sponsor's assessment of the retinal photographs are accepted (so the FDA's assessment of the photographs can be ignored), and if the approach taken by the sponsor and SAS to estimate the median time to detection of CMV disease progression is adopted, then the following results obtain. On the immediate arm of the trial, the estimate of the median time to detection of CMV disease progression drops from 120 to 85 days while on the deferred arm the estimate remains unchanged at 21.5 days.

**6.5 Significance of Treatment Effect** With respect to the assessment of the statistical significance of a treatment effect, the situation is dramatically different. Before describing results, there are two points to comment upon. The first involves the choice of statistic to measure the differences between the survival curves for the two arms of the trial. Both the logrank and generalized Wilcoxon test statistics can be viewed as computing weighted averages of the deviation between the two curves. The generalized Wilcoxon test tends to give more weight to earlier observations. The log rank test, on the other hand, gives all observations equal weight. In between these two tests are the Tarone and Ware test and the Prentice test both of which weight observations differently. For the purpose of assessing the statistical significance of the difference between the immediate and deferred survival curves, each of these statistics was evaluated.

The more difficult issue relates to the complexity of the randomization scheme adopted in the design of this trial. To assess the significance of a test statistic, the following approach was taken. Under the null hypothesis there is no distinction between the immediate and deferred therapy. Thus, when the null hypothesis is true, treatment assignment is ancillary - it merely acts as a noninformative label. Because of this, when the null hypothesis is true relabeling patients (regardless of their original treatment assignment), according to the randomization scheme of the design, will not materially affect the magnitude of the test statistic. By reassigning labels a large number of times (in the present case, 1,000,000 times), a set of reference values is generated against which to compare the observed value of the test statistic. Again, when the null hypothesis is true, the observed value of the test statistic will not be unusual with respect to this set of reference values. On the other hand, if the observed value of the test statistic is unusual, this casts doubt on the truth of the null hypothesis. In this manner, the significance of the treatment effect was established. Also employed were asymptotic p-

values. However, because the sample size is small and the design is complex, a simple appeal to the asymptotic p-values seemed ill advised *a priori*. In Tables 19 and 20 below, both randomized and asymptotic p-values associated with the logrank, generalized Wilcoxon, Tarone & Ware and Prentice statistics are recorded under a variety of assumptions concerning how patients who discontinued the trial prematurely should be handled and concerning which set of retinal photography readings is more accurate.

Table 19: GS-93-106 Significance of Treatment Effect - detection of CMV retinitis progression								
	Sponsor Assessment				FDA Assessment			
	L'	W	T	P	L	W	T	P
Randomized	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Asymptotic	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
1	L = the Logrank test, W = the generalized Wilcoxon test, T = the Tarone and Ware test, P = the Prentice test.							

Table 20: GS-93-106 Significance of Treatment effect - detection of the Combined endpoint.								
	Sponsor Assessment				FDA Assessment			
	L'	W	T	P	L	W	T	P
Randomized	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Asymptotic	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
1	L = the Logrank test, W = the generalized Wilcoxon test, T = the Tarone and Ware test, P = the Prentice test.							

Clearly, what these tables show is that regardless of the assumptions describing how patients who prematurely discontinued therapy should be handled in the analysis, there is a significant treatment effect in favor of cidofovir therapy.

#### 6.6 The Crossover Group Details Pending.

#### 6.7 Survival Details pending.

### 7. STUDY GS-93-107

#### 7.1 Evidence of Open Label Bias Details pending.

#### 7.2 Patient Characteristics Details pending.

**7.4 The Combined Endpoint Details pending.**

**7.5 Magnitude of Treatment Effect Details pending.**

**7.6 Significance of Treatment Effect Details pending.**

**7.7 Survival Details pending.**

## **8. CONCLUSIONS**

In summary, then, the following observations have been made. Study GS-93-106 provided the following conclusions:

1) There is a significant treatment effect in favor of **cidofovir** therapy. This was observed under a variety of assumptions describing how patients who prematurely discontinued therapy should be handled in the analysis. In each instance, the data supported the conclusion that there is a treatment advantage in favor of **cidofovir** therapy at least the 0.001 level of significance.

2) On the other hand, the magnitude of the treatment effect is hard to assess. The estimate of this effect provided by the median time to detection is not robust with respect to the underlying assumptions granted. Further, as it happened for this data set, when the assumption of noninformative censoring was entertained the estimate of the treatment effect was, in large measure, determined by one or two influential observations.

3) Thus, the conclusion that there is a statistically significant treatment effect in favor of **cidofovir** therapy is robust. The estimate of the magnitude of the treatment effect, however, is not.

Study GS-93-107 provided similar conclusions:

1) There is a significant dose response favoring the high dose of **cidofovir**. While this conclusion is not as strong as the conclusion reached with respect to the comparison of the immediate and deferred therapies in study GS-93-106, it was still seen to be the case that at the 5% level of significance, a dose response was observed.

2) However, as with the size of the treatment effect in study GS-93-106, the estimate of the size of the dose response is problematic.

3) Thus, the conclusion that there is a statistically significant dose response is robust with respect to a number of possible contingencies. The estimate of the dose response is not.

**Alan Muhly, Ph.D.  
Mathematical Statistician**

**Concur: Dr. Kammerman**

**cc:**

**Archival. NDA# 20-638**

**HFD-530**

**HFD-530/Dr. Pratt**

**HFD-530/Dr. Feigal (via Team Links)**

**HFD-530/Ms. Struble**

**HFD-725/Dr. Muhly**

**HFD-725/Dr. Kammerman**

**HFD-725/Ms. Shores**

**HFD-725/Dr. Harkins**

**AM/29 February 1996/WP6.1/C:\P90\_HD\WPF\ .ES\CIDOF\OV\REVIEW\GILEAD.REV**

# BIO Review

**CLINICAL PHARMACOLOGY/BIOPHARMACEUTICS REVIEW**

**NDA:** 20-638  
**DRUG:** Cidofovir intravenous  
**TRADE NAME:** VISTIDE™  
**APPLICANT:** Gilead Sciences  
**TYPE OF SUBMISSION:** 1P

**REVIEWER:** Kellie Schoolar Reynolds, Pharm.D.  
**SUBMISSION DATES:** 09-29-95, 12-06-95,  
01-05-96, 02-07-96, 02-08-96, 02-09-96  
**DRAFT REVIEW:** 01-17-96, 03-22-96  
**FINAL REVIEW:** 03-27-96

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**SYNOPSIS:****Background:**

Cidofovir is a nucleotide analog with *in vitro* and *in vivo* activity against a broad spectrum of herpes viruses. The applicant has submitted this application in support of the use of cidofovir for the treatment of CMV retinitis in patients with AIDS.

Results from Phase I/II studies provided evidence of dose-dependent anti-CMV activity and nephrotoxicity. Based on Phase I/II results, the applicant chose the dosing regimens for investigation in Phase II/III studies: 5 mg/kg cidofovir once a week for two weeks, followed by either 3 mg/kg or 5 mg/kg every other week. All cidofovir infusions in Phase II/III studies were administered with prehydration (intravenous normal saline) and concomitant oral probenecid.

**Pharmacokinetics****Cidofovir intravenous**

The pharmacokinetics of cidofovir appeared dose independent after IV doses of 1 to 10 mg/kg. Following a single IV infusion, cidofovir serum concentrations declined in a bioexponential manner. The mean  $\pm$  SD volume of distribution was  $429 \pm 138$  mL/kg. Cidofovir is believed to accumulate in the kidneys. Renal clearance accounted for  $88 \pm 28\%$  of total clearance. Over 24 hours,  $90 \pm 26\%$  of the cidofovir IV dose was recovered unchanged in urine. Cidofovir demonstrated very low protein binding ( $\leq 10\%$ ) in human serum and plasma across the concentration range of 0.6 to 25.6  $\mu$ g/mL.

No cidofovir accumulation was observed when 3 mg/kg cidofovir IV was administered on a once weekly schedule. Week 1 and week 4 pharmacokinetic parameters were similar.

**Subcutaneous bioavailability**

Cidofovir was well absorbed after subcutaneous administration; bioavailability was  $95.3 \pm 7.9\%$  based on serum data. This route of administration was not pursued due to poor patient tolerability.

**Oral bioavailability**

Bioavailability was poor after oral administration of cidofovir. Following oral administration at 10 mg/kg, cidofovir serum concentrations reached quantifiable levels in only two of five patients.

### Cidofovir intravenous administered with probenecid

**Note:** To reduce nephrotoxicity, cidofovir IV should always be administered with oral probenecid (2 grams 3 hours prior to cidofovir infusion, 1 gram at 2 hours and 8 hours after the completion of the infusion) and saline hydration (IV infusion of 1 L normal saline over 1 hour just prior to cidofovir administration).

The effects of various combinations of hydration and oral probenecid (total dose of 2 or 4 grams) were investigated in a small number of patients (n = 2 or 3 per treatment) administered 5 mg/kg cidofovir IV. The 2 gram probenecid regimen did not appear to affect the pharmacokinetics of cidofovir. The 4 gram regimen appeared to increase AUC and C<sub>max</sub> and decrease CLT, CLR, and V<sub>dss</sub>. However, these comparisons were based on a very small number of patients.

The pharmacokinetic parameters from all patients administered cidofovir IV without concomitant probenecid were compared to those from all patients administered cidofovir IV with the 4 gram regimen of probenecid (with or without hydration). Patients administered 4 grams of probenecid with their cidofovir regimen had significantly higher AUC and C<sub>max</sub> values (normalized to dose), significantly slower total clearance and significantly smaller volume of distribution compared to patients administered cidofovir without probenecid.

It should be noted that the individual effects of probenecid and prehydration on the pharmacokinetics of cidofovir were not investigated thoroughly. Also, the rationale for choosing the 4 gram regimen of probenecid was not addressed. However, the current combination of probenecid and prehydration was investigated clinically and resulted in less cidofovir related nephrotoxicity than early studies using cidofovir alone or cidofovir plus prehydration.

### **Special Populations**

Cidofovir was not investigated in pediatric or elderly patient populations. The numbers of female and minority patients were very low.

No data are currently available on the pharmacokinetics of cidofovir in patients with creatinine clearance values below 60 mL/min. A protocol describing a study designed to determine the pharmacokinetic profile of cidofovir in subjects with varying degrees of renal function has been submitted to the FDA. Subjects on dialysis will be included in this study.

In the absence of clinical data, the applicant has proposed a dosing nomogram based on the index of renal function (ratio of calculated creatinine clearance in the impaired subject to that in the normal subject) and predicted AUC values. The applicant designed the nomogram to provide doses that should produce similar AUC values in patients with varying degrees of renal function.

### **Drug Interactions**

Other than the effect of probenecid on the pharmacokinetics of cidofovir, formal drug interaction studies were not performed. The applicant states that in light of the intermittent administration of cidofovir, potential interactions will be minimized.

Concomitant administration of antiretroviral agents was permitted in most clinical studies. In one phase I/II study, the applicant evaluated the effect of cidofovir on zidovudine pharmacokinetics in a few patients. There was no evidence that cidofovir altered zidovudine pharmacokinetics.

The proposed labeling contains information concerning interactions with probenecid. Probenecid increases serum zidovudine concentrations by inhibiting glucuronidation and also decreasing renal excretion. It is recommended that patients decrease their daily zidovudine dose by 50% on the day of cidofovir and probenecid administration.

#### **Pharmacokinetic/Pharmacodynamic evaluations**

The applicant did not perform any pharmacokinetic/pharmacodynamic evaluations.

#### **CONCLUSIONS:**

The applicant has examined the pharmacokinetics of cidofovir in HIV-infected patients with and without asymptomatic CMV retinitis, including patients with relapsing CMV retinitis. They have described the pharmacokinetics after intravenous infusion, both with and without concomitant oral probenecid. The pharmacokinetics of cidofovir are dose independent after IV doses of 1 to 10 mg/kg. To decrease nephrotoxicity, cidofovir must be administered with prehydration and a 4 gram regimen of probenecid. Probenecid appears to inhibit the renal tubular secretion of cidofovir. Pharmacokinetics in special populations (pediatric, elderly, women, minorities, renal dysfunction) have not been (fully) addressed. A study in patients with varying degrees of renal function is planned.

#### **Phase IV Commitments:**

The phase IV commitments include a commitment to complete the pharmacokinetic study in patients with renal impairment. No other commitments regarding pharmacokinetics were deemed necessary.

#### **Label**

A copy of the approved label is on file in the Division of Pharmaceutical Evaluation III.

#### **RECOMMENDATION:**

The pharmacokinetic studies provided in section 6 of NDA 20-638 (Cidofovir Intravenous) submitted to the Division of Anti-Viral drug Products to fulfill section 320 of the code of federal regulations (21 CFR) provided an understanding of the pharmacokinetics of cidofovir in adults. The information on the pharmacokinetics of cidofovir (VISTIDE™) provided is adequate to support approval.

**Advisory Committee- March 15, 1996**

**Biopharm Day- January 22, 1996**

Participants: Drs. N. Fleischer, J. Lazor, M-L. Chen, B. Gillespie, J. Collins,  
J. Jenkins, K.S. Reynolds.

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**I. SUBMISSION CONTENTS:**

- A. Pharmacokinetics of Single Dose Intravenous HPMPC in HIV Infected Patients with Asymptomatic CMV Infection (A report on data from protocol GS-92-101). Volume 45, pages 69-134.
- B. Pharmacokinetics and Bioavailability of HPMPC in HIV-Infected Patients (A report on data from protocol GS-92-102). Volume 45, pages 135-198.
- C. Pharmacokinetics of HPMPC in HIV-Infected patients with Asymptomatic CMV Infection (A report on data from protocol GS-92-103). Volume 45, pages 199-277.
- D. Pharmacokinetics of Cidofovir (HPMPC) in Patients with AIDS and Relapsing CMV Retinitis (An interim report on data from protocol GS-93-107). Volume 46, pages 1-35.
- E. Protein Binding of Cidofovir, Cyclic HPMPC, PMEA, and PMPA in Human Plasma and Serum. (Study 95-DDM-XXXX-001). Volume 46, pages 52-75.
- F. Summary Report: Effect of HPMPC on Pharmacokinetics of AZT- Johns Hopkins Data. Volume 46, pages 76-89.
- G. An Open-Label Study of the Pharmacokinetics of Cidofovir in Subjects with Renal Insufficiency (Protocol GS-95-118 Summary). Volume 46, pages 101-102.
- H. Effect of Cidofovir and its Deamination Product, HPMPU, on the Activity of Dihydropyrimidine Dehydrogenase (Study 94-PM-0504-001). Volume 46, pages 103-107.
- I. Validation of a Modified Assay for Determination of HPMPC in Human Serum (Validation Report VR-0504-002). Volume 46, pages 108-140.
- J. Documentation of the Analytical Procedures for the Determination of HPMPC in Human serum (SOP #01-385SM005). Volume 46, pages 141-161.
- K. Validation of HPLC Analysis of HPMPC in Serum : Final Report (Validation Report 01-385-SM004). Volume 46, pages 162-180.
- L. Analysis of HPMPC in Urine by (SOP #01-385SM008). Volume 46, 191-208.
- M. Validation of Analysis of HPMPC in Urine (Validation Report #01-385SM009). Volume 46, pages 209-235.
- N. Response to FDA Request: Demographic and laboratory data (hard copy and diskette), pharmacokinetic data (diskette), complete protocols for pharmacokinetic studies. 2 volumes. (Amendment 008, Submitted 12-08-95).
- O. Response to FDA Request: PCNONLIN Output and Patient Dosing Histories. (Amendment 010, Submitted 01-05-96)
- P. Response to FDA Request: Renal dysfunction dosing calculations. (Amendment 015, Submitted 02-07-96)
- Q. Response to FDA Request: Assay standard curve data and Assay QA data. (Amendment 017, Submitted 02-08-96)
- R. Vol 1: Integrated Safety Summary, Integrated Efficacy Summary. Vol 2: Response to FDA Request: Assay standard curve data and chromatograms. (Amendment 018, Submitted 02-09-96)

## **II. BACKGROUND/RATIONALE:**

Cidofovir is a nucleotide analog with *in vitro* and *in vivo* activity against a broad spectrum of herpes viruses. The applicant has submitted this application in support of the use of cidofovir for the treatment of CMV retinitis in patients with AIDS.

Cidofovir's mechanism of action is attributed to its intracellular active metabolite, cidofovir diphosphate, which inhibits CMV DNA polymerase. Cidofovir diphosphate persists in cells ( $T_{1/2} = 17-30$  hours) after cidofovir is removed from the medium.

Results from two Phase I/II (101 and 103) studies provided evidence of dose-dependent anti-CMV activity and nephrotoxicity. Anti-CMV activity (urine and semen) was observed in the majority of patients with asymptomatic CMV infection treated at doses  $\geq 3$  mg/kg. The relevance of an anti-CMV effect, with regard to either CMV retinitis or systemic disease, is not known. While administration of 5 mg/kg once a week for 2 consecutive weeks was well tolerated without evidence of significant drug-related nephrotoxicity (development of proteinuria or serum creatinine elevation), continued weekly dosing at 5 mg/kg was associated with increasing potential for proteinuria. Nephrotoxicity was also demonstrated after single doses of 7.5 mg/kg (with concomitant probenecid) or 10 mg/kg (without concomitant probenecid). Animal data indicate that the nephrotoxicity was due to renal proximal tubular cell injury.

Due to the prolonged anti-CMV effects in both urine and semen specimens in patients receiving doses  $\geq 3$  mg/kg, the applicant investigated longer dosing intervals in order to increase patient convenience and decrease the potential for nephrotoxicity. In animal studies the development of nephrotoxicity was schedule dependent; increased incidences of nephrotoxicity were seen when the same total dose was subdivided versus when administered as a single dose. In humans, persistent anti-CMV effects were observed when cidofovir was administered on an every other week schedule (maintenance). Extension of the dosing interval to three weeks or greater was associated with evidence of resumption of CMV replication.

The concomitant administration of probenecid was associated with a reduction in cidofovir-related nephrotoxicity. Pharmacokinetic results indicate probenecid reduces tubular secretion of cidofovir. The applicant states that, in animals, nephrotoxicity is due to slow (saturable) export of cidofovir at the luminal membrane which results in high concentrations of cidofovir accumulating in the proximal tubule. Thus, reduced secretion into the proximal tubules should result in decreased nephrotoxicity.

Patients were also hydrated prior to cidofovir administration in an effort to further reduce nephrotoxicity. Within each dose level cohort of four to five patients receiving cidofovir without concomitant probenecid in the Phase I/II studies, the first two patients received saline pre-hydration (one liter of normal saline infused over approximately 60 minutes immediately prior to cidofovir infusion) during the first four weeks of study drug administration. After examination of the hydration status of patients developing a serum creatinine  $\geq 2$  mg/dL the applicant determined there was a potential relationship between hydration status and development of cidofovir-associated nephrotoxicity.

Based on Phase I/II results, the applicant chose the dosing regimens for investigation in Phase II/III studies: 5 mg/kg cidofovir once a week for two weeks, followed by either 3 mg/kg or 5 mg/kg every other week. All cidofovir infusions in Phase II/III studies were administered with prehydration and concomitant oral probenecid.

**III. CHEMISTRY:**

Chemical name: 1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine dihydrate

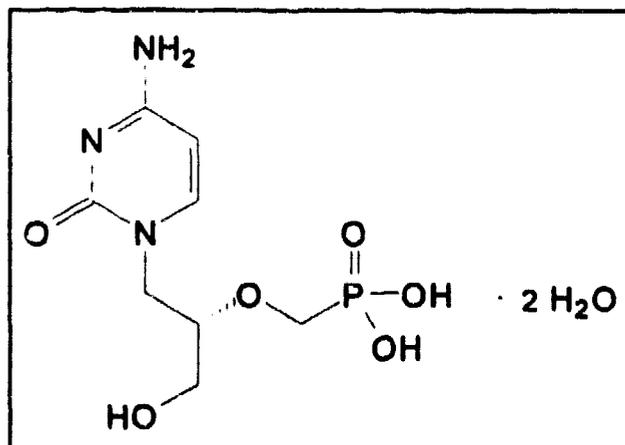
Molecular formula:  $C_9H_{14}N_3O_6P \cdot 2H_2O$

Molecular weight: 315.22, as dihydrate

Partition coefficient (octanol/aqueous buffer):  $\log P = -3.3$  at pH 7.1

Aqueous solubility  $\geq 170$  mg/mL at pH 6-8

Structural formula:

**IV. FORMULATION:**

Cidofovir intravenous is a clear solution packaged in single-use 5 mL USP Type I glass vials. The formulation does not contain a preservative and is suitable for single use only. The commercial batch size is 130 L (9.75 kg cidofovir). The formulation of cidofovir intravenous is shown in the following table.

Ingredient	Composition per mL (5 mL per vial)
Cidofovir (Based on Anhydrous Material) <sup>1</sup>	0.075 g/mL
5.0 N Sodium Hydroxide, NF	For pH Adjustment (Target to pH 7.4)
1.0 N Hydrochloric Acid, NF	For pH Adjustment (Target to pH 7.4)

<sup>1</sup> Cidofovir intravenous is compounded at 101% label strength (75.75 mg/mL) to account for degradation losses on terminal sterilization (-0.0% label strength loss).

Five lots of cidofovir 75 mg/mL and two lots of cidofovir 25 mg/mL were used as supplies for all toxicology and clinical studies. All lots of the 75 mg/mL solution were manufactured using the proposed commercial formulation. The 25 mg/mL formulation differs only in strength from the 75 mg/mL formulation.

**V. INDICATION:**

Cidofovir intravenous is indicated for the treatment of CMV retinitis in patients with acquired immunodeficiency syndrome (AIDS).

## VI. DOSAGE AND ADMINISTRATION: (From proposed label)

THE RECOMMENDED DOSAGE, FREQUENCY, OR INFUSION RATE MUST NOT BE EXCEEDED. CIDOFOVIR MUST BE DILUTED IN 100 MILLILITERS OF 0.9% (NORMAL) SALINE PRIOR TO ADMINISTRATION. TO MINIMIZE POTENTIAL NEPHROTOXICITY, PROBENECID AND INTRAVENOUS SALINE PREHYDRATION MUST BE ADMINISTERED WITH EACH CIDOFOVIR INFUSION.

**Induction Treatment:** The recommended dose of cidofovir is 5 mg/kg body weight (given as an intravenous infusion at a constant rate over 1 hr) administered once weekly for two consecutive weeks.

**Maintenance Treatment:** Following completion of induction treatment, the recommended maintenance dose of cidofovir is 5 mg/kg body weight (given as an intravenous infusion at a constant rate over 1 hr) administered once every two weeks.

**Probenecid:** To minimize the potential for nephrotoxicity, a course of probenecid must be administered orally with each cidofovir dose. Two grams must be administered at 3 hours prior to the cidofovir dose and one gram administered at 2 and again at 8 hours after the completion of the 1 hour cidofovir infusion (for a total of 4 grams).

**Hydration:** To minimize the potential for nephrotoxicity, patients should receive a total of one liter of 0.9% saline intravenously with each infusion of cidofovir. The saline solution should be infused over a 1-2 hour period immediately before the cidofovir infusion.

### Dose Adjustment:

**Changes in renal function during VISTIDE therapy:** Because of cidofovir's potential to cause renal impairment, dose adjustment is required for decreases in renal function that occur during treatment. Demonstration of an increase in serum creatinine of 0.3 to 0.4 mg/dL above pre-cidofovir therapy baseline requires that the patient's dose be reduced from 5 mg/kg/dose to 3 mg/kg/dose. Demonstration of an increase in serum creatinine of  $\geq 0.5$  mg/dL or development of  $\geq 3+$  proteinuria requires discontinuation of cidofovir therapy. *Note: These recommendations are not based on pharmacokinetic data, they are based on observations (nephrotoxicity, laboratory data) made during clinical trials.*

**Pre-existing renal impairment:** The most appropriate initial and maintenance doses of VISTIDE for patients with serum creatinine concentrations  $> 1.5$  mg/dL or creatinine clearances  $\leq 55$  mL/min are not known. When the potential benefits of therapy exceed the potential risks, dose adjustments should be made based on the following table.

Calculated Creatinine Clearance (mL/min)	Induction dose (once per week for 2 weeks)	Maintenance dose (every other week)
41 - 55	2.0 mg/kg	2.0 mg/kg
30 - 40	1.5 mg/kg	1.5 mg/kg
20 - 29	1.0 mg/kg	1.0 mg/kg
$\leq 19$	0.5 mg/kg	0.5 mg/kg

Note: the recommended dose adjustments are based on calculations, not actual data.

No data are available concerning VISTIDE dosing for patients undergoing dialysis.

## VII. PHARMACOKINETICS:

Pharmacokinetic data for cidofovir in humans are available from a total of four clinical studies. The pharmacokinetics of cidofovir were examined at five dose levels in three Phase I/II studies in a total of 42 HIV-infected patients (with or without asymptomatic CMV infection). The three Phase I/II studies included evaluation of the pharmacokinetics of intravenous cidofovir, the oral and subcutaneous bioavailability of cidofovir, and the effects of hydration and concomitant probenecid on the pharmacokinetics of cidofovir. In a Phase II/III study in patients with AIDS and relapsing CMV retinitis, pharmacokinetic data were obtained for intravenous cidofovir at two dose levels in a total of 10 patients during the maintenance phase of cidofovir therapy with concomitant probenecid and hydration. The numbers of patients receiving various doses of cidofovir, with different combinations of probenecid and hydration, are indicated in the following table.

CONCOMITANT TREATMENT	DOSE				
	1 mg/kg	3 mg/kg	5 mg/kg	7.5 mg/kg	10 mg/kg
No probenecid	5	10	2	0	10
2 g probenecid/hydration	0	0	2	0	0
4 g probenecid/no hydration	0	0	2	0	0
4 g probenecid/hydration	0	12	6	4	0

### Summary of study designs:

\*\*Data pooled from several studies are presented throughout this review. Summaries of all pharmacokinetic studies are provided in this section.

NOTE: For all cidofovir intravenous infusions, the required volume of cidofovir solution was added to a 100 mL normal saline infusion bag. The entire volume was infused over 1 hour.

Study 101 Cidofovir lot numbers: 504A92-01 (25 mg/mL), 504D92-01 (75 mg/mL), 504G92-01 (25 mg/mL).

5 patients received IV cidofovir 3.0 mg/kg per week. All patients received 4 doses.  
5 patients received IV cidofovir 10.0 mg/kg per week. One patient received 4 doses and four patients received 2 doses.

The first two patients at each dose were hydrated with an IV infusion of 1 liter 0.9% normal saline over 1 hour just prior to the cidofovir infusion.

Serum samples were drawn after the first and fourth infusions at 0, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hours (relative to the beginning of the infusion). In 3 patients administered 10 mg/kg, samples were also drawn at 18 and 29 hours. Urine samples were collected during the first infusion and fourth infusion over the following intervals: 0-4 hr, 4-8 hr, 8-12 hr, and 12-24 hr.

Five patients received IV cidofovir at 3.0 mg/kg together with concomitant hydration and oral probenecid. Hydration was administered as described above. Probenecid tablets were administered as follows (4 grams total):

2 grams at 3 hours prior to cidofovir infusion  
1 gram at 2 and 8 hours post infusion.

Serum samples were drawn at 0, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hours. Urine was not collected from patients administered cidofovir with probenecid.

**Study 102** Cidofovir lot numbers: 504A92-01 (25 mg/mL), 504D92-01 (75 mg/mL).

5 patients received cidofovir at 1.0 mg/kg by IV, oral, and subcutaneous routes, with a 2 week washout period between administrations. Pharmacokinetic results were presented for the IV doses.

5 patients received cidofovir at 3.0 mg/kg by IV, oral, and subcutaneous routes, with a 2 week washout period between administrations. Pharmacokinetic results were presented for the IV and SC doses.

5 patients received cidofovir at 10.0 mg/kg by IV and oral routes, with a 2 week washout period between administrations. Pharmacokinetic results were presented for the IV and oral doses.

For subcutaneous administration, the appropriate volume of cidofovir was directly injected (up to two divided doses). For oral administration, cidofovir was diluted to 30 mL with tap water and administered orally, followed by an additional 100 mL of tap water taken orally.

Serum samples were drawn at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hours. Urine was collected over the following intervals: 0-4 hr, 4-8 hr, 8-12 hr, and 12-24 hr.

**Study 103** Cidofovir lot numbers: 504A92-01 (25 mg/mL), 504G92-01 (25 mg/mL), 504K92-01 (75 mg/mL).

Pharmacokinetic results are available for patients who received the following treatments:

N	DOSE	REGIMEN	1L prehydration	Probenecid regimen
2	5 mg/kg	Twice/week for 1 week	Yes	None
2	5 mg/kg	Twice/week for 1 week	Yes	Low
3	5 mg/kg	Once/week for up to 4 weeks	Yes	High
2	5 mg/kg	Once/week for up to 4 weeks	No	High
2	7.5 mg/kg	Once every 3 weeks for 4 doses	Yes	High
1	7.5 mg/kg	Once every 3 weeks for 4 doses	Yes (+ 1L after 4th dose)	High
1	7.5 mg/kg	Once every 3 weeks for 4 doses	Yes (+ 1L after all doses)	High
1	5 mg/kg	Once/week for 2 weeks, then every other week	Yes	High

**Probenecid Regimens:**

Low- 1 gram 3 hrs prior to cidofovir infusion, 0.5 grams 2 and 8 hrs after the end of the infusion (2 grams total).

High- 2 grams 3 hrs prior to cidofovir infusion, 1 gram 2 and 8 hrs after the end of the infusion (4 grams total).

**Hydration:**

IV infusion of 1L 0.9% (normal) saline over 1 hour just prior to cidofovir administration. Several patients received additional infusions after cidofovir.

Serum and urine samples were collected from all patients during the first infusion of cidofovir (week 1), during the fourth infusion in 3 patients (5 mg/kg cidofovir, high dose probenecid, week 4), and during the third infusion in 2 patients (7.5 mg/kg cidofovir, high dose probenecid, week 7). Serum samples were drawn at 0, 1, 2, 3, 4, 8, 12, 24, and (for a few patients) 72 hours. Urine samples were collected over the following intervals: 0-4 hr, 4-8 hr, 8-12 hr, and 12-24 hours.

**Study 107** Cidofovir lot numbers: 504K92-01 (75 mg/mL), 504J94-01 (75 mg/mL).

The pharmacokinetics of cidofovir were examined as a part of this open-label study in AIDS patients with relapsing CMV retinitis. All patients received an induction phase of cidofovir at 5 mg/kg/week for two weeks with concomitant oral probenecid (4 gram course) and hydration. Patients were then randomized to receive maintenance doses of cidofovir at 3 mg/kg (n = 7) or 5 mg/kg (n = 3) every other week, with probenecid and hydration.

Serum and urine were collected following the second or third dose of the maintenance phase. Serum was collected at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours after infusion initiation. Urine was collected over the following intervals: 0-4 h, 4-8 h, 8-12 h, 12-24 h.

### Cidofovir Intravenous: Single Dose Pharmacokinetics

The following table summarizes (mean  $\pm$  SD) the pharmacokinetic parameters after an IV infusion of cidofovir (no probenecid) over the dose range of 1 to 10 mg/kg.

(Data pooled from studies 101, 102, 103)

Dose	1 mg/kg (n=5)	3 mg/kg (n=10)	5 mg/kg (n=2)	10 mg/kg (n=10)	Overall (n=27)
AUC- ( $\mu\text{g}\cdot\text{hr}/\text{mL}$ )	8.35 $\pm$ 3.10	19.96 $\pm$ 2.70	27.90	76.17 $\pm$ 17.67	N/A
AUC normalized to 1 mg/kg	8.35 $\pm$ 3.10	6.65 $\pm$ 0.77	5.58	7.62 $\pm$ 1.77	7.25 $\pm$ 1.85
C <sub>max</sub> ( $\mu\text{g}/\text{mL}$ )	3.12 $\pm$ 0.67	7.43 $\pm$ 1.39	11.55	24.14 $\pm$ 4.52	N/A
C <sub>max</sub> normalized to 1 mg/kg	3.12 $\pm$ 0.67	2.48 $\pm$ 0.46	2.31	2.41 $\pm$ 0.45	2.56 $\pm$ 0.54
MRT (hr)	2.70 $\pm$ 0.67	3.54 $\pm$ 0.93	3.15	4.16 $\pm$ 1.64	3.59 $\pm$ 1.28
CLT (mL/hr/kg)	130 $\pm$ 37	152 $\pm$ 18	181	137 $\pm$ 28	145 $\pm$ 28
V <sub>dss</sub> (mL/kg)	274 $\pm$ 62	457 $\pm$ 134	465	472 $\pm$ 131	429 $\pm$ 138
Terminal T <sub>1/2</sub> (hr)	1.68 $\pm$ 0.52	2.72 $\pm$ 0.82	2.42	4.83 $\pm$ 4.11	3.29 $\pm$ 2.78
CLR (mL/hr/kg)	129 $\pm$ 38	129 $\pm$ 23	149	117 $\pm$ 60	126 $\pm$ 42
U(24) (%)	98.5 $\pm$ 14.1	84 $\pm$ 10	85.9	92.7 $\pm$ 41.3	89.9 $\pm$ 26.0
CLR/CLT	0.99 $\pm$ 0.14	0.85 $\pm$ 0.10	0.84	0.88 $\pm$ 0.44	0.88 $\pm$ 0.28

Note: The applicant did not include data from two patients administered 10.0 mg/kg in their summary. For these two patients, AUC is higher and CLR and CLT are lower than in the other patients administered 10.0 mg/kg. The applicant states that elevated serum creatinine levels observed in these patients indicate impaired renal function as a result of cidofovir exposure causing their renal clearance of cidofovir to be decreased. The increases in serum creatinine were not seen until several days after cidofovir administration. The results from these two patients ARE included throughout this review.

(See figure on page 12)

Following IV administration of cidofovir over the dose range of 1 to 10 mg/kg, the pharmacokinetics appeared dose independent; however, this assessment was based on a small number of subjects at each dose level. The mean V<sub>dss</sub> was lower for patients administered 1 mg/kg than patients administered higher doses. Based on animal data, cidofovir accumulates in the kidney; it is possible that the lower V<sub>dss</sub> at the 1 mg/kg dose was due to less renal accumulation. C<sub>max</sub> and AUC both increased proportionally with dose. Aside from the lower V<sub>dss</sub> at the 1 mg/kg dose, no dose dependent trends in pharmacokinetic parameters were evident.

Following a single IV infusion, cidofovir concentrations declined in a biexponential manner with an overall mean terminal half-life of 3.3  $\pm$  2.8 hours. The total clearance of cidofovir was 145  $\pm$  28 mL/hr/kg (185  $\pm$  35 mL/min). The renal clearance was 126  $\pm$  42 mL/hr/kg

(164 ± 65 mL/min). Renal clearance accounted for 88 ± 28% of total clearance. The urinary recovery of unchanged cidofovir following IV infusion was 90 ± 26% of the administered dose. Incomplete urinary recovery of the dose within 24 hours may explain the difference between renal clearance and total clearance. Renal clearance was 160 ± 56% of creatinine clearance, thus active tubular secretion probably occurs during cidofovir elimination. In 6 patients, renal clearance was less than creatinine clearance; incomplete urinary recovery and/or reabsorption may have contributed to this observation.

#### Cidofovir Intravenous: Multiple Dose Pharmacokinetics

In study 101, six patients had cidofovir concentrations determined after both the first and the fourth dose (five patients received 3 mg/kg once each week, and one patient received 10 mg/kg once each week). The table below contains the mean ± SD (range) parameter values from patients receiving 3 mg/kg.

PARAMETER	WEEK 1		WEEK 4	
	AUC <sub>0-∞</sub> (μg·hr/mL)	20.1 ± 2.1	(17.2-22.6)	21.3 ± 4.7
MRT (hr)	3.9 ± 0.8	(3.3-5.3)	5.4 ± 3.6	(2.8-11.6)
CLT (mL/hr/kg)	150 ± 16	(133-175)	146 ± 31	(108-186)
V <sub>dss</sub> (mL/kg)	498 ± 101	(436-675)	698 ± 545	(410-1665*)
k <sub>e</sub> (hr <sup>-1</sup> )	0.216 ± 0.018	(0.185-0.229)	0.230 ± 0.088	(0.130-0.363)
terminal T <sub>1/2</sub> (hr)	3.2 ± 0.3	(3.0-3.7)	3.4 ± 1.3	(1.9-5.3)
C <sub>max</sub> (μg/mL)	7.7 ± 0.5	(7.05-8.16)	7.9 ± 1.7	(5.38-9.52)

\*The increase in V<sub>dss</sub> observed at week 4 is due to one patient with a quantifiable serum concentration at 48 hours. No other profiles had quantifiable concentrations past 24 hours.

As expected based on single dose pharmacokinetics and the one week dosing interval, there was no accumulation of cidofovir. The time 0 concentration of week 4 was 0 ng/mL for all patients. The pharmacokinetics of cidofovir did not appear to change with multiple doses when administered on a once a week schedule.

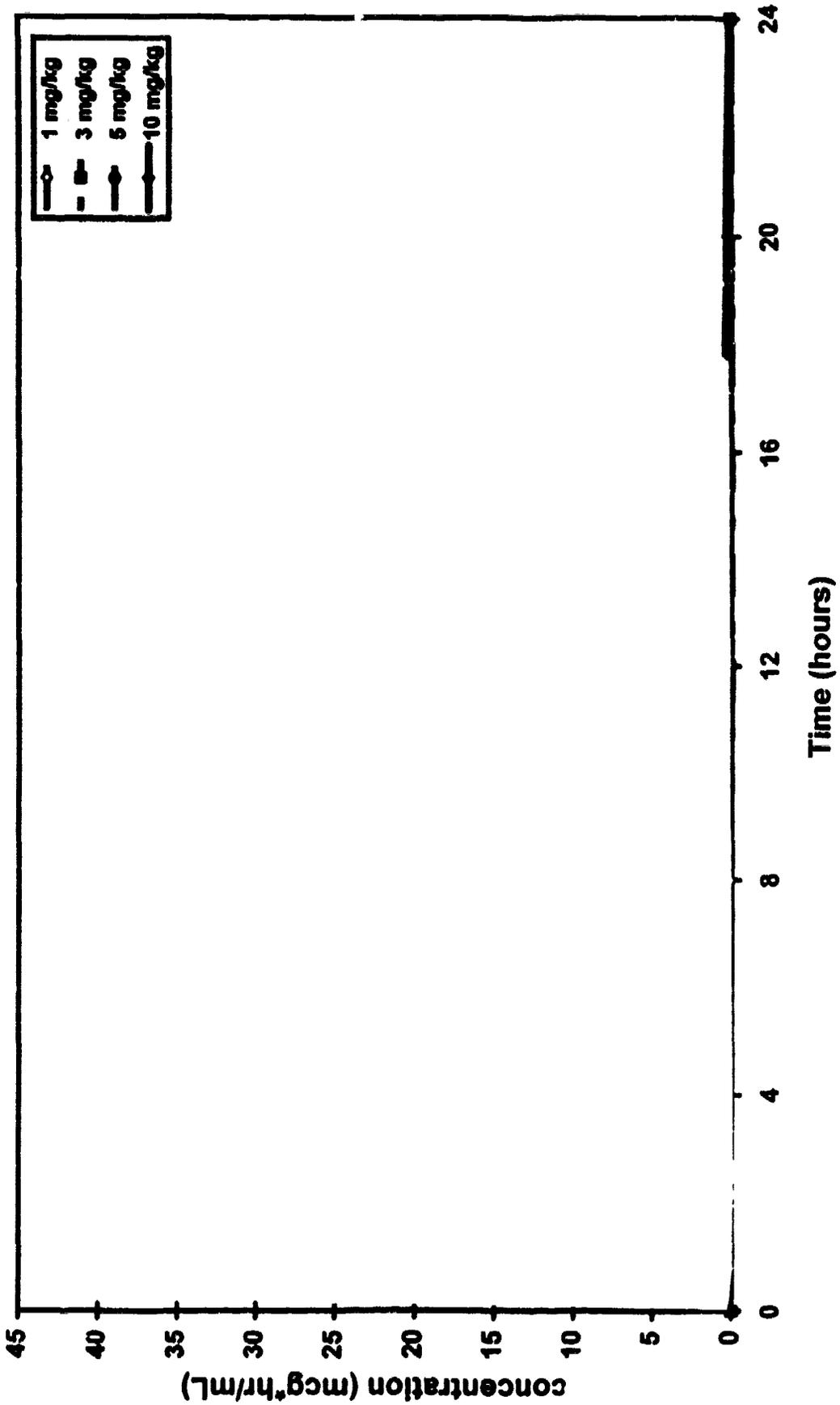
#### Bioavailability

##### Subcutaneous

The pharmacokinetic results (mean ± SD) from the IV and subcutaneous administrations of cidofovir 3 mg/kg in study 102 are summarized in the following table.

PARAMETER	ROUTE	
	SC	IV
AUC <sub>0-∞</sub> (μg·hr/mL)	18.71 ± 1.44	19.78 ± 2.75
MRT (hr)	3.4 ± 0.8	3.2 ± 1.0
k <sub>e</sub> (hr <sup>-1</sup> )	0.344 ± 0.114	0.354 ± 0.132
terminal T <sub>1/2</sub> (hr)	2.21 ± 0.73	2.23 ± 0.90
C <sub>max</sub> (μg/mL)	5.02 ± 0.99	7.20 ± 2.00
T <sub>max</sub> (hr)	1.4 ± 0.8	1.2 ± 0.4
% dose excreted unchanged in urine (24 hrs)	82.2 ± 9.9	80 ± 11
Bioavailability (% based on serum data)	95.3 ± 7.9	NA
Bioavailability (% based on urinary recovery)	104.6 ± 16.0	NA

# Concentration vs. Time Cidofivir administered without probenecid



Cidofovir was well absorbed after subcutaneous administration; bioavailability was  $95.3 \pm 7.9\%$  based on serum data.  $C_{max}$  was reduced by  $28 \pm 12\%$  when cidofovir was administered SC. For individual patients, the serum concentration vs. time curves (following  $T_{max}$ ) were similar when cidofovir was administered IV or SC. Although cidofovir displayed high bioavailability when administered SC, this route of administration was not pursued due to poor patient tolerability.

### Oral

Cidofovir concentrations reached quantifiable levels in the serum of only two of five patients following oral administration at 10 mg/kg; one patient had a maximum serum concentration of 1000 ng/mL, while the other had a maximum concentration of 400 ng/mL. In one patient the bioavailability of oral cidofovir was 20.6%; bioavailability could not be determined for the other four patients.

### **Protein Binding**

The protein binding of cidofovir (Lot #1966-C-9P) in pooled human serum and pooled human plasma was determined by spiking individual samples with [2- $^{14}$ C]-cidofovir (Lot #114-204-056, 3.0 mCi/mL, 0.443 mg/mL) and using centrifugal ultrafiltration to separate free and protein-bound drug. The final concentrations of cidofovir in the samples were 0.6, 2.6, 5.6, 10.6 and 25.6  $\mu$ g/mL at 0.125  $\mu$ Ci/mL. Total and free drug concentrations were determined by mixing equal aliquots of the corresponding unfiltered and filtered samples with scintillation cocktail and analyzing the resulting mixtures on a 2500 TR Liquid Scintillation Analyzer (Packard). The mean  $\pm$  SD (range) percent unbound was  $94.8 \pm 3.8\%$  (89.5-99.1) in plasma and  $99.9 \pm 3.3\%$  (97.3-104.1) in serum. No trend with respect to unbound vs. total concentrations was evident across the concentration range tested.

Cidofovir demonstrated very low protein binding ( $\leq 10\%$ ) in human serum and plasma across the concentration range 0.6 to 25.6  $\mu$ g/mL.

### **Metabolites**

One minor metabolite (<3% administered dose) was detected in the urine of rats, rabbits and monkeys. This metabolite was isolated and identified as the choline adduct of cidofovir monophosphate. Because the applicant attributed all cidofovir elimination in humans to renal excretion, metabolite studies were not performed on serum and urine collected during clinical studies. For completeness, the performance of metabolite studies in humans would be interesting.

### **Effect of Probenecid on Pharmacokinetics**

The effects of probenecid on the pharmacokinetics of cidofovir were evaluated in two Phase I/II studies (101 and 103) and during the maintenance phase of the randomized controlled trial (107). The labeling for cidofovir will indicate that cidofovir should always be administered with the oral probenecid 4 gram regimen and hydration.

The following table contains the mean  $\pm$  SD pharmacokinetic parameters following cidofovir infusions of 3 mg/kg to 7.5 mg/kg with concomitant probenecid (4 gram regimen). (Data pooled from studies 101, 103, 107)

PARAMETER	3 mg/kg (n=12)	5 mg/kg (n=8)	7.5 mg/kg (n=4)	Overall (n=24)
AUC- ( $\mu\text{g}\cdot\text{hr}/\text{mL}$ )	25.7 $\pm$ 8.5	43.9 $\pm$ 9.8	79.7 $\pm$ 32.2	N/A
AUC normalized to 1 mg/kg	8.6 $\pm$ 2.8	8.8 $\pm$ 2.0	10.6 $\pm$ 4.3	9.0 $\pm$ 2.8
C <sub>max</sub> ( $\mu\text{g}/\text{mL}$ )	9.79 $\pm$ 3.74	18.75 $\pm$ 6.80	43.0 $\pm$ 17.1	N/A
C <sub>max</sub> normalized to 1 mg/kg	3.26 $\pm$ 1.25	3.75 $\pm$ 1.36	5.7 $\pm$ 2.3	3.8 $\pm$ 1.7
MRT (hr)	3.4 $\pm$ 0.7	2.9 $\pm$ 0.5	2.8 $\pm$ 0.4	3.1 $\pm$ 0.7
CLT (mL/hr/kg)	126 $\pm$ 34	120 $\pm$ 32	107 $\pm$ 45	121 $\pm$ 34
V <sub>dss</sub> (mL/kg)	358 $\pm$ 79	285 $\pm$ 82	247 $\pm$ 129	315 $\pm$ 96
k <sub>e</sub> (hr <sup>-1</sup> )	0.28 $\pm$ 0.07	0.32 $\pm$ 0.08	0.27 $\pm$ 0.05	0.29 $\pm$ 0.07
Terminal T <sub>1/2</sub> (hr)	2.6 $\pm$ 0.6	2.2 $\pm$ 0.4	2.6 $\pm$ 0.4	2.5 $\pm$ 0.3
CLR (mL/hr/kg)	80 $\pm$ 18 (n=7)	100 $\pm$ 27	90 $\pm$ 55 (n=3)	90 $\pm$ 29 (n=17)
U(24) (%)	74 $\pm$ 3 (n=7)	80 $\pm$ 15	72 $\pm$ 29 (n=3)	76 $\pm$ 15 (n=17)
*CLR/CLT	0.76 $\pm$ 0.04 (n=7)	0.81 $\pm$ 0.16	0.72 $\pm$ 0.29 (n=3)	0.77 $\pm$ 0.15 (n=17)

\*Contribution of CLR to CLT may be underestimated because (1) there may have been incomplete sample collection for some patients and (2) urine was only collected for 24 hours, some elimination in urine may have occurred after 24 hours.

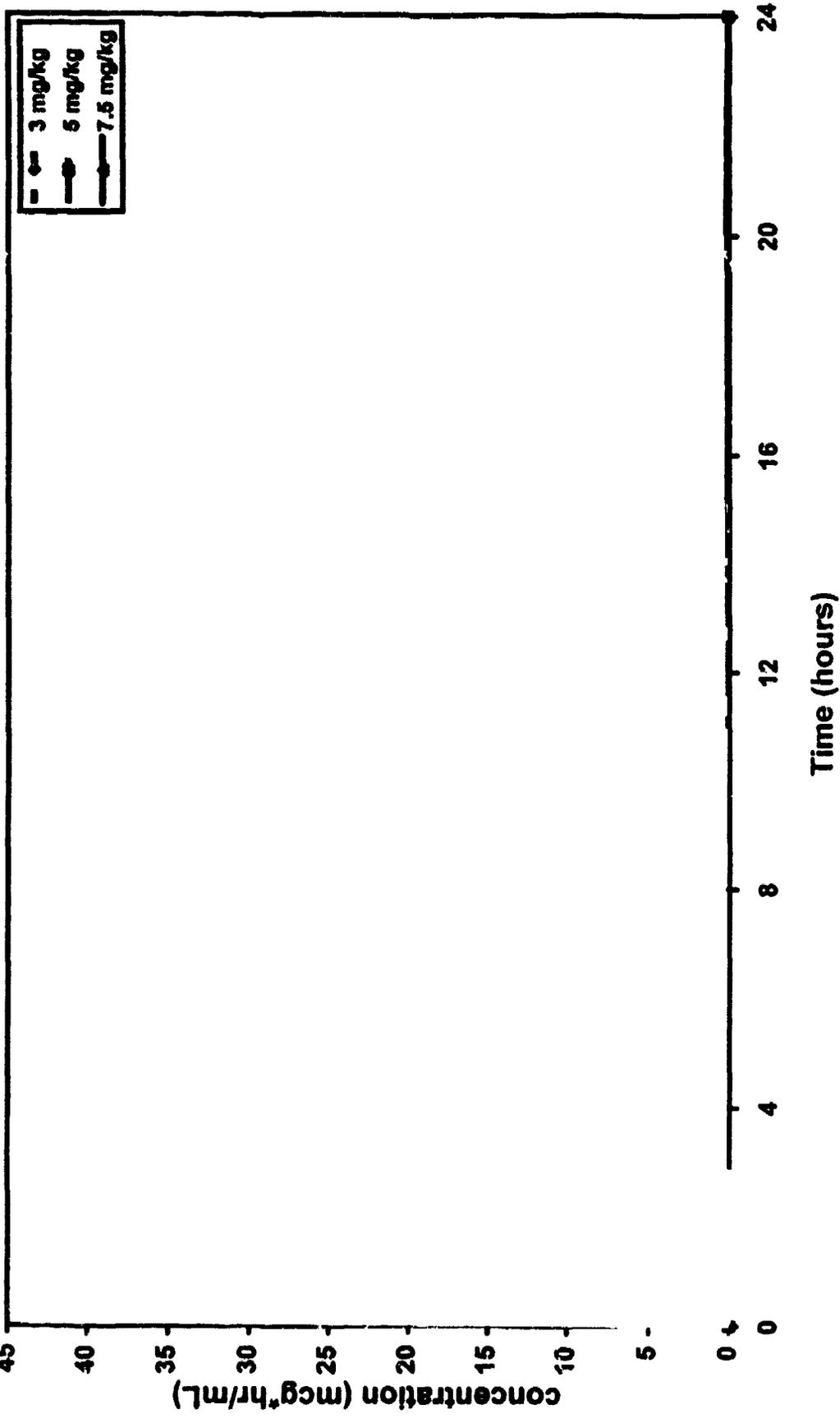
(See figure on page 15)

In study 103, 5 mg/kg cidofovir was administered with various combinations of hydration and probenecid to a small number of patients. The following table contains the mean (range) parameter estimates for the various treatments.

PARAMETER	TREATMENT			
	No probenecid Hydration (n=2)	2 g Probenecid Hydration (n=2)	4 g Probenecid Hydration (n=3)	4 g Probenecid No hydration (n=2)
AUC- ( $\mu\text{g}\cdot\text{hr}/\text{mL}$ )	27.9 (26.7 - 30.1)	33.8 (32.8 - 34.9)	50.6 (45.4 - 58.1)	45.6 (43.7 - 47.5)
C <sub>max</sub> ( $\mu\text{g}/\text{mL}$ )	11.55 (10.5 - 12.6)	12.5 (10.7 - 14.2)	26.1 (24.0 - 29.8)	15.0 (12.8 - 17.1)
MRT (hr)	3.2 (2.5 - 3.8)	3.6 (3.1 - 4.0)	2.6 (2.5 - 2.8)	3.4 (3.1 - 3.7)
CLT (mL/hr/kg)	181 (166 - 195)	148 (143 - 152)	100 (86 - 110)	110 (105 - 115)
CLR (mL/hr/kg)	149 (128 - 170)	175 (n=1)	72 (67 - 76) (n=2)	110 (102 - 118)
V <sub>dss</sub> (mL/kg)	465 (382 - 549)	448 (393 - 602)	211 (198 - 223)	316 (287 - 334)
Terminal T <sub>1/2</sub> (hr)	2.4 (1.8 - 3.0)	3.1 (2.7 - 3.5)	2.2 (1.9 - 2.8)	2.5 (2.4 - 2.6)

Due to the very small numbers of patients in each group, it is not possible to compare the pharmacokinetics between groups statistically. The 2 gram probenecid regimen does not appear to affect the pharmacokinetics of cidofovir. The 4 gram regimen appears to increase AUC and C<sub>max</sub> and decrease CLT, CLR, and V<sub>dss</sub>. The effect of 4 grams of probenecid on cidofovir pharmacokinetics appears to be greater when patients are hydrated prior to cidofovir administration. However, these statements regarding the effects of the different probenecid/hydration regimens on the pharmacokinetics of cidofovir must be viewed cautiously, due to the small number of patients.

### Concentration vs. Time Cidofovir administered with 4 grams of probenecid



The following table contains the mean  $\pm$  SD parameter estimates for all patients administered cidofovir without probenecid or with the 4 gram regimen of probenecid. (Data pooled from studies 101, 102, 103, 107)

PARAMETER	Cidofovir without probenecid (n = 27)	Cidofovir + 4 g probenecid (n = 24)*	P-value unpaired t-test
AUC normalized to 1 mg/kg dose ( $\mu\text{g}\cdot\text{hr}/\text{mL}$ )	7.25 $\pm$ 1.85	8.98 $\pm$ 2.82	0.012
C <sub>max</sub> normalized to 1 mg/kg dose ( $\mu\text{g}/\text{mL}$ )	2.58 $\pm$ 0.54	3.84 $\pm$ 1.67	0.0005
MRT (hr)	3.59 $\pm$ 1.28	3.14 $\pm$ 0.65	0.134
CL <sub>t</sub> (mL/hr/kg)	145 $\pm$ 28	121 $\pm$ 34	0.009
V <sub>dss</sub> (mL/kg)	429 $\pm$ 138	315 $\pm$ 96	0.001

\*2 cidofovir/probenecid patients did not receive hydration

Patients administered 4 grams of probenecid with their IV cidofovir regimen had significantly higher AUC and C<sub>max</sub> values, significantly slower total clearance and significantly smaller volumes of distribution than patients administered cidofovir without probenecid. As indicated by the applicant, probenecid appears to inhibit the renal tubular secretion of cidofovir. The applicant suggests that probenecid also decreases the accumulation of cidofovir in the kidneys. Both the decreased V<sub>dss</sub> observed when probenecid was added to the cidofovir regimen and the results of a <sup>14</sup>C study in animals support this suggestion.

It should be noted that the individual effects of probenecid and prehydration on the pharmacokinetics of cidofovir were not investigated thoroughly. Also, the rationale for choosing the 4 gram regimen of probenecid was not addressed. However, the current combination of probenecid and prehydration was investigated clinically and resulted in less cidofovir related nephrotoxicity than early studies using cidofovir alone or cidofovir plus prehydration.

#### Maintenance Treatment: Cidofovir plus Probenecid

Pharmacokinetic results are available from 10 patients who participated in study 107, a Phase II/III open-label, randomized, multicenter, dose ranging study designed to determine the safety and efficacy of intravenous cidofovir for the treatment of relapsing CMV retinitis in AIDS patients. All patients received an induction phase of cidofovir at 5 mg/kg/week for two weeks with concomitant oral probenecid (4 gram course) and hydration. Patients were then randomized to receive maintenance doses of cidofovir at 3 or 5 mg/kg every other week, with probenecid and hydration. The endpoint in this study was time to CMV retinitis progression. Clinical data presented to the FDA at this time are from an interim intent-to-treat analysis on the first 100 patients enrolled in the trial. The applicant states that the median time to CMV retinitis progression, as documented by retinal photographs, was 115 days in the 5 mg/kg group and 49 days in the 3 mg/kg group. The applicant's assessment of time to progression excludes a high percentage of the patients who entered the study; uninformative censoring was used. The analyses were repeated by an FDA statistical review, Dr. Alan Muhly, using informative censoring. Using the alternative analyses, the median time to CMV retinitis progression was 49 days in the 5 mg/kg group and 35 in the 3 mg/kg group. Pharmacokinetic parameters (mean  $\pm$  SD, range) for patients receiving maintenance doses of 3 mg/kg (n = 7) or 5 mg/kg (n = 3) are summarized in the following table.

PARAMETER	DOSE = 3 mg/kg (n = 7)	DOSE = 5 mg/kg (n = 3)
AUC ( $\mu\text{g}\cdot\text{hr}/\text{mL}$ )	29.9 $\pm$ 8.8 (21.7 - 46.8)	36.1 $\pm$ 11.2 (28.5 - 49.0)
C <sub>max</sub> ( $\mu\text{g}/\text{mL}$ )	11.0 $\pm$ 4.5 (7.6 - 20.8)	14.0 $\pm$ 4.2 (11.2 - 18.8)
MRT (hr)	3.76 $\pm$ 0.78 (2.41 - 4.70)	2.86 $\pm$ 0.51 (2.28 - 3.24)
CLT (mL/hr/kg)	107 $\pm$ 26 (64 - 138)	147 $\pm$ 39 (102 - 175)
CL <sub>R</sub> (mL/hr/kg)	80 $\pm$ 18 (52.9 - 98.3)	112 $\pm$ 32 (78 - 139)
V <sub>dss</sub> (mL/kg)	342 $\pm$ 90 (175 - 439)	340 $\pm$ 96 (261 - 447)
24 hr recovery in urine (% Dose)	74.3 $\pm$ 2.9 (70.0 - 77.2)	76.4 $\pm$ 2.8 (73.8 - 79.4)

Pharmacokinetics were determined for too few patients to allow investigation of pharmacokinetic/pharmacodynamic relationships.

### Special Populations

Cidofovir has not been administered to pediatric or elderly patients. Pharmacokinetic data are available for patients between the ages of 28 and 55 years.

The effects of gender on pharmacokinetics were not investigated. Pharmacokinetic data are available for 3 female patients. Results for the 3 female patients were within the range of results from the male patients.

The effects of race on cidofovir pharmacokinetics were not investigated. The majority of patients for whom pharmacokinetic data were available were Caucasian.

No data are currently available on the pharmacokinetics of cidofovir in patients with creatinine clearance values below 60 mL/min. A protocol describing a study designed to determine the pharmacokinetic profile of cidofovir in subjects with varying degrees of renal function has been submitted to the FDA. Subjects will be assigned to one of six treatment groups based on measured creatinine clearance.

GROUP	N	MEASURED CrCl
1	5	$\geq 91$ mL/min
2	5	61-90 mL/min
3	5	36-60 mL/min
4	5	11-35 mL/min
5	5	$\leq 10$ mL/min (on CAPD)
6	5	$\leq 10$ mL/min (on high-flux hemodialysis)

All subjects will receive cidofovir IV 0.5 mg/kg. Non-dialysis subjects will receive the 4 gram probenecid regimen and hydration.

The applicant plans to use the results of this study to develop a dosing nomogram for patients with renal impairment.

In the absence of clinical data, the applicant has proposed a dosing nomogram based on the index of renal function (ratio of calculated creatinine clearance in the impaired subject to that in a normal subject) and predicted AUC values. This nomogram assumes that cidofovir is cleared completely by renal elimination and that tubular secretion and glomerular filtration are impaired to the same extent.

Calculated Creatinine Clearance (mL/min)	Induction dose (once per week for 2 weeks)	Maintenance dose (every other week)	Predicted AUC ( $\mu\text{g}\cdot\text{hr}/\text{mL}$ )
41 - 55	2.0 mg/kg	2.0 mg/kg	30 - 40
30 - 40	1.5 mg/kg	1.5 mg/kg	31 - 41
20 - 29	1.0 mg/kg	1.0 mg/kg	28 - 40
$\leq 19$	0.5 mg/kg	0.5 mg/kg	$>20$

The applicant designed this nomogram to provide doses that produce similar AUC values in patients with varying degrees of renal function. Assuming that the volume of distribution does not change,  $C_{\text{max}}$  is anticipated to increase only slightly with impaired renal function. The predicted AUC values for patients with renal dysfunction were calculated from the observed AUC in patients with normal renal function at the proposed clinical dose of 5 mg/kg with 4 g probenecid and hydration. The following equation was used:

$$\text{Predicted AUC} = (\text{AUC}_{\text{normal}}/\text{Dose}_{\text{normal}}) \times \text{Dose}_{\text{modified}} \times (\text{CrCl}_{\text{normal}}/\text{CrCl}_{\text{impaired}})$$

where  $\text{AUC}_{\text{normal}}$  is the AUC in patients with normal renal function.  $\text{Dose}_{\text{normal}}$  is the normal dose (5 mg/kg),  $\text{CrCl}_{\text{normal}}$  is the CrCl in patients with normal renal function (100 mL/min),  $\text{Dose}_{\text{modified}}$  is the proposed dose in patients with impaired renal function, and  $\text{CrCl}_{\text{impaired}}$  is the CrCl in patients with impaired renal function.

#### Drug Interactions

Formal drug interaction studies have not been performed. The applicant states that in light of the intermittent administration of cidofovir (every other week during maintenance therapy), potential interactions will be minimized.

Probenecid increases serum zidovudine concentrations by inhibiting glucuronidation and also decreasing renal excretion. Patients receiving probenecid with cidofovir had their daily zidovudine dose decreased by 50% on the day of cidofovir/probenecid administration. This dose adjustment is recommended in the cidofovir label.

The applicant evaluated the effect of cidofovir on zidovudine pharmacokinetics in study 102. The protocol for study 102 permitted coadministration of zidovudine and cidofovir in patients already on zidovudine therapy. For each of these patients, zidovudine was administered once 24 hours prior to cidofovir/placebo administration; serum samples for zidovudine were collected over this 24 hour period. Zidovudine was then coadministered with the cidofovir or placebo dose and serum samples were collected for 24 hours. Data are available for patients receiving cidofovir at different doses (0, 1, 3, or 10 mg/kg), by different routes of administration (IV, SC, PO), and during different periods (weeks 1, 3, and 5). Data are available for three patients receiving placebo, 2 patients at 1 mg/kg, 2 at 3 mg/kg, and 3 at 10 mg/kg. For most patients, data are available after at least 2 different routes of cidofovir administration. Relative zidovudine total clearance (with cidofovir compared to without cidofovir) was  $1.34 \pm 0.93$  for all administrations combined (including placebo). The relative zidovudine clearance was  $1.21 \pm 0.52$  when only placebo administration are considered and was  $1.41 \pm 1.09$  when only patients receiving active cidofovir are considered. There was no evidence that cidofovir altered the pharmacokinetics of zidovudine. There was also no relationship between cidofovir AUC and the change in zidovudine clearance. Due to the small number of patients, the effects of dose, route of cidofovir administration, and week of study were not considered.

In the proposed package labeling, the applicant states that concomitant administration of cidofovir and agents with nephrotoxic potential (e.g., amphotericin B, aminoglycosides, foscarnet, IV pentamidine) has not been evaluated in a clinical study and should be avoided.

As discussed with respect to zidovudine, it is important to also consider potential interactions with probenecid. In the cidofovir label, the applicant states that probenecid is known to interact with the metabolism and/or renal tubular secretion of many drugs, listing the following: acetaminophen, acyclovir, ACE inhibitors, aminosalicic acid, barbiturates, benzodiazepines, bumetanide, clofibrate, methotrexate, famotidine, furosemide, non-steroidal anti-inflammatory agents, theophylline, and zidovudine. The label does not contain any instructions or information concerning the clinical relevance of these interactions.

The applicant states that a clinical study designed to investigate potential interactions between cidofovir and probenecid and agents commonly used by patients with advanced AIDS and CMV retinitis is expected to begin in the next several months. The applicant currently plans to study patients with AIDS receiving fluconazole and trimethoprim-sulfamethoxazole and the cidofovir/probenecid treatment regimen in a formal pharmacokinetic study. The protocol has not yet been submitted to the FDA for review.

Based on the intermittent dose schedule for cidofovir plus probenecid, no other drug interaction studies have been recommended to the applicant.

#### **VIII. Pharmacokinetic/Pharmacodynamic Evaluations:**

The applicant did not perform any pharmacokinetic/pharmacodynamic evaluations. Due to the very small number of patients for whom both pharmacokinetic data and efficacy data were collected (n = 10, Study 107), the lack of pharmacokinetic/pharmacodynamic evaluation is reasonable.

#### **IX. ASSAY:**

Serum and urine samples were analyzed at  
Some serum samples from study 101 were analyzed by the Drug Delivery/Metabolism Section at Gilead Sciences.

Method: For both urine and serum samples, the concentrations of cidofovir were determined using

*Kellie Schoolar Reynolds 3/27/96*  
Kellie Schoolar Reynolds, Pharm.D.  
Reviewer, Antiviral Drugs Section, DPEIII  
Office of Clinical Pharmacology and Biopharmaceutics

*Janice B. Jenkins 3/27/96*  
Concurrence: Janice B. Jenkins, Ph.D.  
Acting Team Leader, Antiviral Drugs Section, DPEIII  
Office of Clinical Pharmacology and Biopharmaceutics

cc: HFD-530 NDA 20638  
/MO/DPratt  
/CSO/KStruble  
/Biopharm/KReynolds (2 copies)  
/SBiopharm/JJenkins  
HFD-205 FOI

**MICROBIOLOGY REVIEW  
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)**

**NDA#:** 20-638

**REVIEWER** : Dempsey  
**CORRESPONDENCE DATE** : 09/29/95  
**CDER RECEIPT DATE** : 10/04/95  
**REVIEW ASSIGN DATE** : 10/05/95  
**REVIEW COMPLETE DATE** : 03/20/96

**SPONSOR:** Gilead Sciences, Inc.  
353 Lakeside Drive  
Foster City, CA 94404  
(415) 574-3000

**SUBMISSION REVIEWED:** N-000- original NDA and amendment B1

**DRUG CATEGORY:** Antiviral

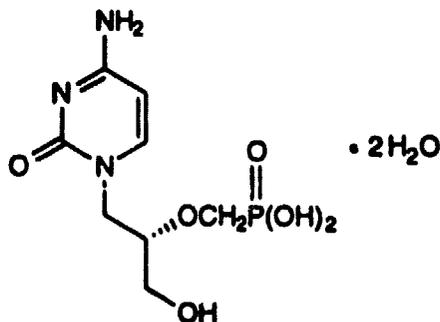
**INDICATION:** Treatment of Cytomegalovirus (CMV) retinitis in patients with AIDS

**DOSAGE FORM:** Intravenous Injection

**PRODUCT NAMES:**

- a. **PROPRIETARY:** Vistide
- b. **NONPROPRIETARY:** Cidofovir
- c. **CHEMICAL:** 1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine dihydrate

**STRUCTURAL FORMULA:**



**SUPPORTING DOCUMENTS:**

## **BACKGROUND**

### Introduction

Cidofovir (CDV or HPMP) is an acyclic nucleoside phosphonate that has antiviral activity *in vitro* and *in vivo* against several herpesviruses, including human cytomegalovirus (HCMV). The active metabolite of CDV is CDVpp, which inhibits HCMV DNA synthesis by acting as a competitive inhibitor of dCTP and as an alternative substrate for HCMV DNA polymerase. The sponsor seeks marketing approval for cidofovir (Vistide<sup>®</sup>) as a treatment for CMV retinitis in patients with acquired immunodeficiency syndrome (AIDS). This review of the microbiology data submitted to NDA 20-638 primarily focuses on those studies which assess activity against HCMV.

### Human CMV Biology

Cytomegaloviruses are a ubiquitous subgroup of herpesviruses (generally classified as  $\beta$  herpesviruses) that commonly infect many species of animals. Although widespread in nature, cytomegaloviruses are species specific, exhibiting a highly restricted host range. In addition to this marked species specificity, characteristics common to all cytomegaloviruses include salivary gland tropism, slow growth in cultured cells and the induction of an identifiable pathology of nuclear and cytoplasmic inclusions in infected cells. Following infection, most cytomegaloviruses persist within the host and may serve as a major source for reinfections.

Human CMV is a double strand DNA virus with a linear 230-240 kb genome. The complete virion is composed of a DNA containing core, surrounded by a 100 nm icosahedral capsid. A protein rich tegument or matrix encloses the capsid. A lipid bilayer envelope containing many viral glycoproteins encloses the complete virion resulting in a mature virion of approximately 150 to 200 nm. Three types of virus particles are released from infected cells: infectious virions, nucleic acid deficient dense bodies (which lack a nucleocapsid and are predominately composed of an enveloped major tegument protein), and noninfectious enveloped particles which have a capsid but no DNA core.

The HCMV genome consists of two segments L (long) and S (short) that are flanked by a series of inverted repeats. This structure allows for the existence of 4 genomic isomers. Viral replication is dependent upon the coordinated expression of viral genes. These genes can be classified according to temporal expression into  $\alpha$  (immediate early);  $\beta 1$  and  $\beta 2$  (delayed early); and  $\gamma 1$  and  $\gamma 2$  (late) genes. Immediate early gene expression does not require *de novo* protein synthesis; whereas delayed early and delayed gene expression require the synthesis of functional  $\alpha$  gene products.  $\beta 1$  gene transcription begins 4-8 hours post infection and is not affected by inhibitors of viral DNA replication.  $\beta 2$  gene transcription occurs 8-24 hours post infection and also is not dependent upon DNA replication.  $\beta$  gene products are likely to be involved in DNA replication and metabolism.  $\gamma 1$  gene products first appear 12-36 hours post infection, and  $\gamma 2$  products are first detected 24-48 hours post infection. Most  $\gamma$  genes code for viral structural proteins.

Human CMV replication is slow relative to other herpesviruses. Accumulation of

replication proteins such as the DNA polymerase and single-stranded DNA binding proteins occurs over a protracted period. The HCMV DNA polymerase, the primary target for CDV activity, is also the ultimate target for the antiviral agents, ganciclovir (GCV), and foscarnet. The DNA polymerase has physicochemical characteristics similar to polymerases of other herpesviruses. Similarities in size, enzymatic characteristics, functional domains, and sensitivity to deoxyribonucleoside analogues have been observed. The two major DNA binding proteins are DB140 (or ICP8) and p52 (or ICP36). ICP8 is a double strand DNA binding protein that associates with the viral DNA polymerase. ICP36 prevents the dissociation of the viral DNA polymerase from the DNA template. Two peaks of viral replication can be observed in cells and occur 18-24 hours and 60-80 hours post infection.

### Human CMV Infection

Human CMV productively infects only humans and human cells. Thus, no animal models of HCMV infection use HCMV as the infecting agent. Human CMV DNA is not significantly homologous to the DNA from other CMV species; however, HCMV strains share about 95% homology in their DNA sequences.

Human CMV infections are endemic, but most are subclinical except in immunocompromised hosts or neonates. Active infection can result from a new primary infection or from reactivation of latent infection. Data from infections in immunocompromised hosts suggest both occur. Many infections of immunosuppressed or immunodeficient individuals occur from reactivation of latent virus. However, superinfection with new strains is supported by the identification of multiple strains of HCMV within one host. Following infection, virus shedding continues for a prolonged period of time and virus can be detected in oropharyngeal secretions, urine, cervical/vaginal secretions, semen, breast milk, tears, feces, and blood. Spread of infection is primarily by close or intimate contact. Sexual transmission represents a major route of virus transmission in adults.

Tissue distribution of HCMV following infection varies with the immune status of the host. Following acute infection in vivo, the virus generally infects and replicates in differentiated epithelial and endothelial cells in a variety of tissues. In situ hybridization studies show that fibroblasts, in addition to epithelial and endothelial cells, are infected. Studies in SCID mice transplanted with human tissue also supports the idea that replication occurs primarily in epithelial tissues; however, the ability to replicate is highly HCMV strain dependent. In immunocompromised patients, viremia is associated with infections of polymorphonuclear cells, monocytes and endothelial cells. In CNS infections, both neuronal and glial cells may be productively infected. In vitro, however, productive infection has been established only in primary cell cultures of differentiated human fibroblasts.

Similar to other herpesviruses, HCMV infections are believed to persist for life. Whether the virus becomes latent or whether low level replication occurs for life is not clear. However, recent studies using polymerase chain reaction assays and in situ hybridization methods have identified that undifferentiated monocytes rather than T lymphocytes in peripheral blood of healthy carriers are sites of viral DNA persistence.

Monocytes and unstimulated (non-activated tissue macrophages) are not permissive for viral replication; whereas, differentiated or stimulated macrophages are permissive. Thus, activation or differentiation of monocytes may serve as the source of reactivation of HCMV infections. Stromal cells in bone marrow have been identified as supporting HCMV replication, but myeloid precursors do not. Experiments demonstrating viral DNA persistence in cultured primary myeloid precursor cells support a role for these cells in latency. In addition, following further cellular differentiation, virus may be reactivated from progeny cells.

Productive HCMV infections are related to the functional immune status of the host. Human CMV infection is the most common congenital infection (0.4-2.3% of live births), however clinical disease occurs only in a small percentage of infected neonates. In general, symptomatic disease occurs in those babies whose mothers had contracted primary HCMV infections during gestation.

Human CMV infection is the most common cause of posttransplant infections in allograft recipients. Human CMV infections are associated with decreased graft survival in solid organ allograft recipients. Major complications include severe CMV infections of the GI tract and interstitial pneumonia. Human CMV infection of bone marrow allograft recipients is most often manifested as pneumonia and has been a leading cause of death of these patients if not treated.

Human CMV seropositivity in HIV-infected patients is associated with an increased risk for development of AIDS. Indeed, transactivation of HIV genes has been widely reported in a variety of in vitro systems. Invasive, clinically significant HCMV disease in AIDS patients primarily occurs as pneumonia, GI disease, or CNS disease (most commonly retinitis).

### Diagnostic Microbiology

Isolation of HCMV from clinical specimens is the primary laboratory diagnostic method for assessing new or reactivated infections. Cell culture methods for isolation, however, are laborious generally requiring culture periods of up to 28 days and observation of cultures for virus induced cytopathic effects. A shell vial method which detects the presence of immediate early viral gene products by immunologic techniques is faster, highly specific and only slightly less sensitive than cell culture for most clinical samples. However, the shell vial assay is markedly less sensitive than cultures for detection of virus from blood.

Following infection, secretion of virus can persist for years and may play a role in transmission. However, the incidence of isolation of CMV from clinical specimens increases with immunologic deficiency. Prognostically, the occurrence of viruria and viremia in immunosuppressed subjects correlates with the likelihood of developing end organ disease. In transplant patients, viremia, even though the viral titers often are small, is highly predictive of invasive disease. In AIDS patients, viruria and viremia are less predictive, and the prognostic value increases with low (<200) CD4 counts. For example, viruria has been reported in up to 50% of AIDS patients without CMV disease symptoms. With the development of CMV disease, however, the frequency of viruria increases. In

most subjects, viremia occurs less frequently than viruria. The majority of AIDS patients with a positive blood culture will have a positive urine culture, thus viremia is strongly associated with viruria. However, the reverse is not necessarily true. Not all patients who are viruric will be viremic. Human CMV also can be isolated with high frequency from semen, but the incidence of recovery of virus from semen has not been associated prognostically with the development of end organ disease.

### Treatment of HCMV Infections and the Development of Resistance

Ganciclovir (GCV) and foscarnet are antiviral agents frequently used for the treatment of CMV retinitis. The development of resistant HCMV isolates to both drugs has been observed clinically as well as experimentally, and the clinical significance of resistance to GCV and foscarnet is being increasingly appreciated in patients with CMV disease. Clinical progression has been observed following isolation of drug-resistant isolates. Moreover, clinical improvement has been reported in patients who have failed one therapy and have drug-resistant isolates following a change in therapy.

Recent estimates suggest that the incidence of development of GCV resistant virus is 5-10% after only 3 months on therapy. The majority of GCV resistant isolates have mutations in the UL97 phosphotransferase gene. Although the role of this gene in HCMV replication or disease pathogenesis is not known, it is believed to be required for initial phosphorylation of GCV. Other GCV resistant isolates have been described with mutations in the DNA polymerase (pUL) gene.

The development of resistance to foscarnet occurs much less frequently than resistance to GCV. However, foscarnet-resistant HCMV strains have been isolated from clinical samples and following in vitro selection under drug pressure. Resistance to foscarnet is mediated by mutations in the DNA polymerase gene.

### **SUMMARY:**

#### In Vitro Anti-CMV Activity

The activity of CDV has been evaluated in several studies utilizing both laboratory strains and clinical isolates of HCMV (summarized in Table 1). In vitro activity was determined by plaque reduction assays in three cell lines (MRC-5, Human embryonic lung - HEL, and Human foreskin fibroblasts-HFF). IC50 concentrations ranged from 0.05 to 1.59  $\mu$ M for laboratory isolates and 0.2-2.8  $\mu$ M for clinical isolates.

**TABLE 1. Reported IC50s for HPMPG against various strains of HCMV.**

HCMV Strain	Cell Line	IC50 (uM)	Reference
AD 169	MRC-5	0.08	1
	HEL	0.05	
18 Clinical Isolates (average)	MRC-5	0.26 +/- 0.23	
	HEL	0.22 +/- 0.23	
AD 169	MRC-5	0.7	2
Clinical Isolates	HFF	0.5-2.0	6
Davis AD 169 (average)	HEL	0.25	8
AD 169	HFF	0.22	11
Davis		0.48	
Towne		0.63	
EC		1.09	
LA		0.95	
CH		2.80	
Mann		0.95	
C8708/17-1-1		1.50	
C8704/9-1-4		0.41	
C8805-37		2.38	
AD 169	HFF	1.0	15
AD 169	HFF	0.5	16
Davis AD 169	HEL	0.32	14
		0.22	
AD 169	HEL	0.25	21
		0.36	
AD 169	HEL	0.20-1.59	3
Davis		0.45-1.27	
Clin. Isol. CM15		0.32-0.72	
CM-F		0.23-1.59	
CM-6		0.32	
CM-7		0.44	
CM-16		1.27	
CM-21		0.13	
CM-24	1.97		

HEL - Human Embryonic Lung Fibroblasts

HFF - Human Foreskin Fibroblasts

uM reported or calculated based on 315 g/Mole.

## In Vitro Drug Interaction Studies

The effect of CDV on the antiviral activity of several antiviral agents was evaluated. Drug:drug interactions studies for antiviral activity were tested for CDV in combination with AZT or ddI with the laboratory HIV strain (HIV-IIIb) in MT-4 cells. Using the "MacSynergyII" program, no synergy or antagonism was observed. In contrast, combination studies of GCV and CDV in normal human dermal fibroblast cells and the Towne strain of HCMV revealed a modest synergistic activity against HCMV infections for these two drugs.

## In Vivo Anti-CMV Activity

The anti-cytomegalovirus activity of CDV (HPMPC) has been evaluated in several animal models of CMV infection. HCMV does not infect other species, thus, animal models for drug activity have utilized animal cytomegalovirus strains, including guinea pig CMV (GPCMV), rat CMV (RCMV), and murine CMV (MCMV).

1. Guinea pig CMV. In a guinea pig infection model using GPCMV, Li et. al (14) reported that HPMPC treatment (5.0 mg/kg/day for 5 days) resulted in lower viral titers in the blood, spleen, and salivary gland. Lower doses of HPMPC were not effective in reducing viral burden.

2. Rat CMV. An immunosuppressed rat model developed by Stahl, et. al (23,24) was used to evaluate the effectiveness of HPMPC as a treatment for RCMV. In this model, Brown Norway rats were immunosuppressed by total body irradiation and were infected with RCMV ( $10^8$  PFUs). Treatment with a single dose  $\geq$  2.0 mg/kg of HPMPC (0.5-10mg/kg) increased the survival time of rats following a lethal infection with RCMV. Treatment with a single dose of 20 mg/kg, but not 5 mg/kg, decreased virus titers isolated from all organs assayed (salivary gland, spleen, liver, and lungs).

3. Murine CMV. Two general models of MCMV infection have been utilized for drug activity testing: immunocompetent mice and severe combined immunodeficiency (SCID) mice. Kern (11) and Kern et al. (12) examined the effect of treatment with HPMPC in immunocompetent mice (first study - mouse strain not identified; second study - Balb/C mice). In both reports, MCMV infection resulted in 70-100% mortality with a mean day of death of 5-8 days post infection. HPMPC treatment (first study- 2X/ day for 5 days; second study- 1X/ day for 7 days) reduced mortality but not the mean day of death at most doses tested. Treatment was effective if initiated at 6 hours or 24 hours post infection at all effective doses. Higher doses (5-10 mg/kg) reduced mortality when administered as late as 48 hours after infection.

Neyts et al. (17), Smeets et al. (20), Kern et al. (12), and Smeets, Sugiyama and Reist (19) evaluated the activity of HPMPC in SCID mice infected with MCMV. Each study differed slightly in the experimental methodology utilized including the concentration of viral inoculum and the doses and schedule of treatment. In all studies, however, 100% mortality was observed following infection in both treated and untreated mice, and treatment with HPMPC significantly delayed the time of death in a dose response effect (Figures 1,2,3). Infrequent dosing (1x-2x/week) was also effective in delaying mortality,

but higher doses generally were required. HPMP treatment also delayed the development of virus titers in most of the tissues assayed.

Figure 1. Neyts et al. (17)

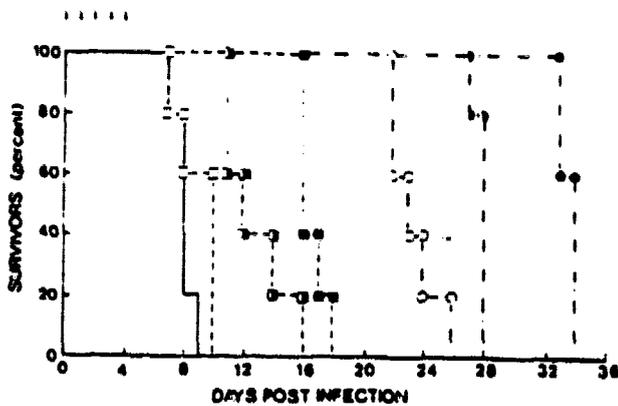


Fig. 1. Survival of SCID mice infected i.p. with MCMV and treated s.c. with DHPG or HPMP. Treatment was initiated 2 hours after infection and was continued for the next 4 days (as indicated by the arrows). Symbols: (—), untreated controls (n = 6); (---), DHPG-treated mice; (---), HPMP-treated mice; (□) DHPG at 1 mg/kg/day (n = 5); (○) DHPG at 5 mg/kg/day (n = 5); (◐) DHPG at 25 mg/kg/day (n = 5); (◑) HPMP at 1 mg/kg/day (n = 5); (◒) HPMP at 5 mg/kg/day (n = 5); (◓) HPMP at 25 mg/kg/day (n = 5).

Figure 2. Smees et al (20)

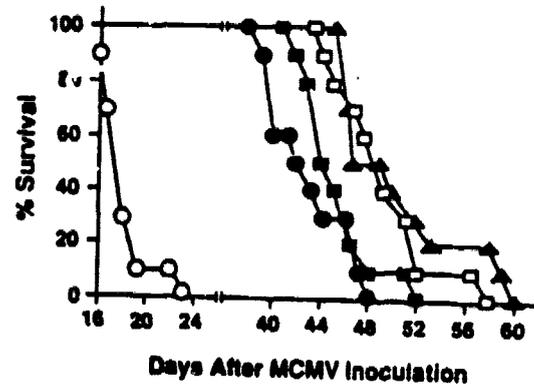


FIG. 3. Effects of 20-day and once-weekly HPMP treatment regimens on survival of SCID mice infected with  $10^{4.3}$  PFU of MCMV. Treatments started 5 days after virus inoculation. Symbols: for once-daily treatments, O, placebo; ●, HPMP at 2.5 mg/kg/day; ■, HPMP at 5 mg/kg/day; ▲, HPMP at 10 mg/kg/day. For once-weekly treatment, □, HPMP at 50 mg/kg/week.

Figure 3. Kern et al. (12)

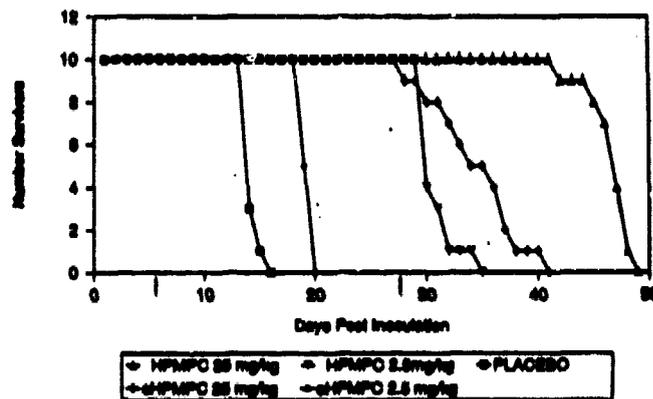


Figure 3. Effect of Twice Weekly Treatment with HPMP or dHPMP on Mortality of SCID Mice Inoculated i.p. with MCMV.

**Reviewer Comment:** In these animal studies, no systematic determination of the dose in conjunction with the optimal schedule of treatment for effects on survival time or viral load was completed. In general, however, higher doses of infrequently administered CDV were required to achieve comparable increases in survival times than were required with more frequent dosing. This observation suggests one reason for the lack of dramatic effects on systemic virus observed in Vistide-treated CMV infected patients.

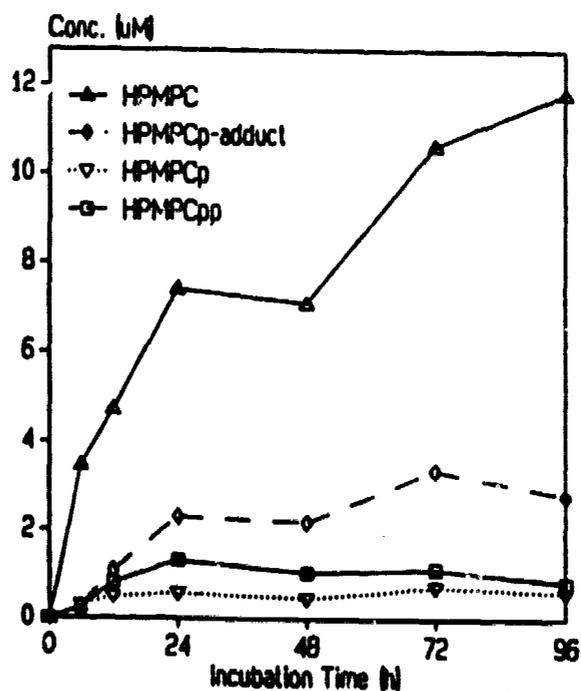
## Mechanism of Action

CDV has been demonstrated in several systems to inhibit HCMV DNA synthesis at concentrations 100-500 -fold lower than that which is required to inhibit cellular DNA synthesis. In these studies, effects on DNA synthesis were measured by  $^3\text{H}$ -thymidine incorporation assays and by CsCl gradient isolation of viral DNA and cellular DNA following exposure of cells to CDV.

Studies of cellular uptake of CDV (using Vero cells and radioactive CDV) suggest that CDV enters these cells by a fluid-phase endocytosis mechanism and may accumulate in the lysosome. Kinetic studies indicate that intracellular CDV concentration is dependent upon its extracellular concentration but approaches only a small percentage (estimated 6%) of the extracellular concentration. Once inside the cell, CDV is phosphorylated by cellular enzymes to the monophosphate and diphosphate derivatives (CDVp and CDVpp). No initial phosphorylation by virus-encoded enzymes is required, and the putative metabolite responsible for the antiviral activity of CDV is CDVpp. After high intracellular levels of CDVpp are achieved, CDVpp is further metabolized to CDVp-choline. The role of CDVp-choline on antiviral activity is not known.

Intracellular concentrations of CDV and its metabolites have been evaluated in MRC-5 cells following exposure to 200  $\mu\text{M}$  CDV for various times (up to 96 hours) (10). CDV (HPMPC) levels increase over time with exposure (Figure 4). Levels of CDVp and CDVpp were 1  $\mu\text{M}$  or less. Levels of the CDVp-choline adduct increased with time to approximately 3  $\mu\text{M}$ . Studies to assess the persistence of intracellular pools of CDV indicate that CDV loss from cells is slow (1/2 removed by 6 hours). Half-lives of the metabolites have been reported as follows: CDVp-6 hours; CDVpp-17 hours; and CDVp-choline adduct-> 48 hours in MRC-5 cells and CDVp-24 hours, CDVpp-65 hours; and CDVp-choline adduct-87 hours in Vero cells.

Figure 4. Ho et al. (10)



Uptake of CDV, and ultimately the concentrations of the CDV metabolites, are cell type dependent. In a report by the sponsor (Mendel and Chen, Vistide™ NDA, Gilead Sciences, Inc.), uptake and concentrations of CDV metabolites were assessed in four cell types: MRC-5 cells (embryonic human lung fibroblast cell line), MDCK cells (canine kidney cell line), A3.01 (human T cell line), and Jurkat cells (human T cell line). Cells were exposed to 10uM [<sup>3</sup>H]CDV for 24 hours at which time the cells were washed, and concentrations of CDV and its metabolites were assessed. Direct measurements of intracellular concentrations of CDV and its metabolites are summarized in Table 2. Intracellular pool concentrations as calculated using mean cellular volume are summarized in Table 3. Intracellular concentrations of CDV and its metabolites are significantly less in T cell lines than in MRC-5 cells, with MDCK cell levels falling in between.

Table 2. (Vistide™ NDA, Gilead Sciences, Inc.)

Cell Line	HPMPC	Metabolite (pmoles / million cells)		
		HPMPCp	HPMPCpp	Choline Adduct
MRC-5	1.71	0.89	1.78	2.52
MDCK	0.41	0.44	1.11	0.76
A3.01	0.16	0.03	0.11	0.26
Jurkat	0.52	0.05	0.21	0.70

Table 3. (Vistide™ NDA, Gilead Sciences, Inc.)

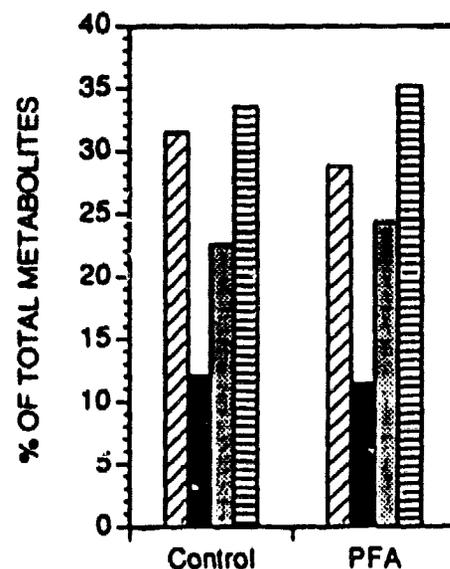
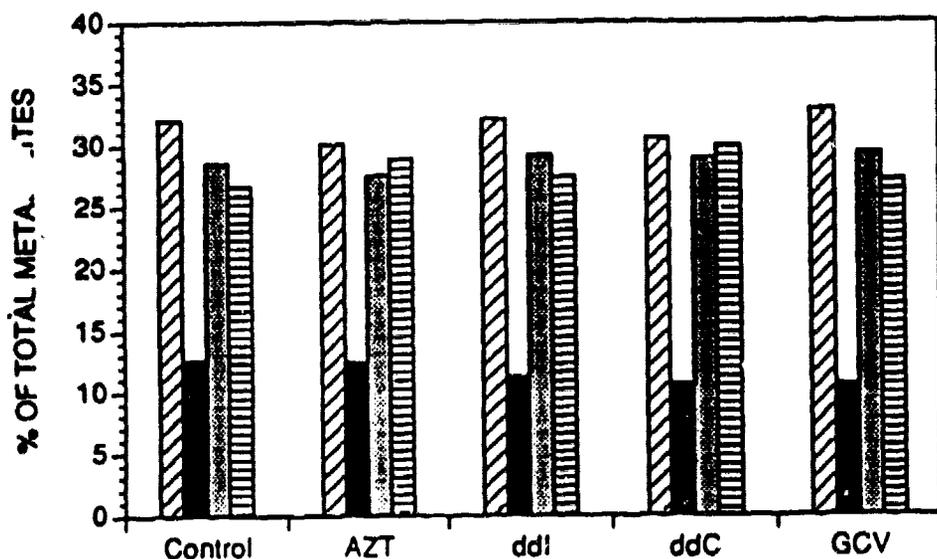
	<u>MRC-5</u> (μM)	<u>A.301</u> (μM)
HPMPC	1.25 (± 0.41)	0.14 (± 0.03)
HPMPCp	0.40 (± 0.14)	0.05 (± 0.02)
HPMPCpp	0.70 (± 0.27)	0.05 (± 0.02)
HPMPCp-choline	1.05 (± 0.38)	0.19 (± 0.03)

Based on reported C<sub>max</sub> serum concentrations (19.6 ug/ml) of the expected clinical dose (5 mg/kg) administered in conjunction with probenecid, the maximum extracellular concentration of CDV is calculated to be 62 uM. Ho et al. (10) reported that intracellular concentrations of CDV approached only 8% of extracellular CDV concentration in MRC-5 cells. Thus, an estimate of the intracellular concentration following a single clinical dose would be 3.7 uM. Based on reported intracellular concentrations of CDVpp from studies outlined above, the estimated intracellular concentration would be of the active metabolite CDVpp would be between 1 and 2 uM.

Experiments which examined the effect of other common anti-viral agents on the metabolism of CDV demonstrated that neither AZT, ddi, ddC, GCV, ACV, nor foscarnet altered the relative concentrations of CDV metabolites at 24 hours (Figures 5,6). Infection of cells with HSV-1, HSV-2 or HCMV also did not significantly alter the metabolism of CDV.

Figure 5. (Vistide™ NDA, Gilead Sciences, Inc.)

Figure 6. (Vistide™ NDA, Gilead Sciences, Inc.)



HPMPC  
 HPMPCp  
 HPMPCpp  
 Choline Adduct

The purported mechanism of action for antiviral activity is inhibition of viral DNA polymerase. CDVpp acts as both an inhibitor and alternate substrate for HCMV DNA polymerase. CDVpp inhibits HCMV DNA polymerase with a  $K_i$  of 6.6  $\mu\text{M}$  (Table 4). The  $K_i$  values of CDVpp for human DNA polymerases  $\alpha$ ,  $\beta$  and  $\gamma$  are 8-600 fold higher than for viral DNA polymerases. Considering the  $K_i$  values for the human DNA polymerases and the previously calculated estimates of intracellular CDVpp concentration (1-2  $\mu\text{M}$ ; previous paragraph), it is unlikely that the human DNA polymerases will be dramatically affected. The  $K_i$  value and the  $K_i/K_m$  ratio of CDVpp for DNA polymerase  $\alpha$  suggest that although utilization of CDVpp by DNA polymerase  $\alpha$  is relatively weak, it can and does occur. In fact, the  $K_i/K_m$  ratio of CDVpp for DNA polymerase  $\alpha$  is not dramatically different (1.33 x) than the  $K_i/K_m$  ratio of 9 for HCMV DNA polymerase. The  $K_i/K_m$  value of 120 for DNA polymerase  $\beta$  suggests that DNA repair is not likely to be affected,  $K_i/K_m$  value of 1430 for DNA polymerase  $\gamma$  indicates that effects on mitochondrial DNA synthesis should be negligible.

Table 4. (Vistide™ NDA - Gilead Sciences, Inc.)

Inhibition Constants of CDVpp Against DNA Polymerases from HCMV, HSV, and Human Cells.

Enzyme	$K_m$ (dCTP) $\mu\text{M}$	$K_i$ (CDVpp) $\mu\text{M}$	$K_i/K_m$
HCMV DNA polymerase <sup>a</sup>	0.72	6.6	9
HSV-1 DNA polymerase <sup>b</sup>	0.31	0.86	3
HSV-2 DNA polymerase <sup>b</sup>	0.37	1.4	4
DNA polymerase $\alpha^c$	4.4	51	12
DNA polymerase $\beta^c$	4.3	520	120
DNA polymerase $\gamma^c$	0.21	300	1430

<sup>a</sup>Xion et al. (29)

<sup>b</sup>Ho et al. (10)

<sup>c</sup>Cherrington et al. (4)

CDVpp is incorporated into the growing viral DNA chain. Human CMV DNA polymerase was purified from normal human dermal fibroblasts following infection with HCMV (Towne strain). Using synthetic DNA primer/template hybrids, the sponsor determined that CDVpp competes with dCTP for incorporation opposite a G unpaired residue on the template. However, the kinetics of incorporation indicate that the competition is less efficient for CDVpp. Nonetheless, incorporation of a single molecule of CDVpp reduces the rate of DNA synthesis by 31%, but does not act as a DNA chain terminator. Incorporation of two consecutive CDVpp by HCMV polymerases effectively stops DNA elongation; whereas incorporation of two CDVpp separated by one or two

nucleotides slows the rate of synthesis by up to 90%. CDV cannot be excised from the 3' end of the DNA chain by the HCMV DNA polymerase associated exonuclease activity.

## Resistance

### 1. In Vitro Selection Studies

The sponsor performed in vitro selection studies with CDV or GCV drug pressure. HCMV laboratory strain AD169 was propagated under increasing concentrations of CDV or GCV for a period of 10 months. At that time, variants which could replicate in the presence of 8 or 16  $\mu\text{M}$  CDV or 300  $\mu\text{M}$  GCV were selected. Ten plaque purified CDV resistant strains and 2 plaque purified GCV resistant strains were assayed for susceptibility to CDV, GCV, or foscarnet. All of the CDV resistant isolates demonstrated decreased susceptibility to CDV ( $\text{IC}_{50}\text{s} = 7\text{-}15 \mu\text{M}$ ). Six of the ten CDV selected strains were also screened for susceptibility to GCV and foscarnet. The 6 isolates were 5-14X more resistant to GCV, but none exhibited significant resistance to foscarnet ( $\text{IC}_{50} = 80\text{-}150 \mu\text{M}$ ). The two isolates selected under GCV pressure were highly resistant to both CDV ( $\text{IC}_{50} = 11\text{-}13 \mu\text{M}$ ) and GCV ( $\text{IC}_{50} = 267\text{-}300 \mu\text{M}$ ), but remained susceptible to foscarnet ( $\text{IC}_{50} = 100$ ). The parent strain  $\text{IC}_{50}$  values for CDV, GCV and foscarnet were 0.5  $\mu\text{M}$ , 7  $\mu\text{M}$ ; and 80  $\mu\text{M}$ , respectively. Genotypic analysis of the resistant strains was not reported.

The sponsor also has summarized data personally communicated from Snoeck et al. (22) for two strains of HCMV (parent strain AD165) selected following in vitro pressure with CDV which are resistant to CDV ( $\text{IC}_{50} = 7.6\text{-}16.2 \mu\text{M}$ ) and are cross resistant to GCV ( $\text{IC}_{50} = 23\text{-}55 \mu\text{M}$ ) but not to foscarnet ( $\text{IC}_{50} = 120\text{-}190 \mu\text{M}$ ). In addition, two GCV resistant ( $\text{IC}_{50} = 47\text{-}51 \mu\text{M}$ ) strains isolated following selective pressure with GCV were cross resistant to CDV ( $\text{IC}_{50} = 7.3\text{-}7.9$ ) but not to foscarnet ( $\text{IC}_{50} 19\text{-}220 \mu\text{M}$ ).

*Reviewer Comment:* In both studies, all of the CDV-resistant isolates selected following in vitro pressure with CDV were cross resistant to GCV.

Although the majority of GCV resistant isolates contain mutations in gene UL97 which confers resistance to GCV but not to CDV, DNA polymerase mutations have been described in some GCV resistant isolates. The exact frequency of occurrence of DNA polymerase mutants in HCMV in patients with or without drug pressure is not known. To date, the only reports of DNA polymerase mutations which result in GCV resistance also result in resistance to CDV. Lurain et al. (15) isolated three GCV-resistant mutants following in vitro exposure of strain AD169 to increasing concentrations of GCV. The plaque-purified strains D6/3/1, D1/3/4 and D10/3/2 were resistant to GCV and CDV, but remained susceptible to foscarnet. Genotypic analysis of the DNA polymerase gene for each of the mutants as compared to wild-type revealed point mutations which resulted in amino acid changes L501I for D1/3/4 and D6/3/1 and F412V for D10/3/2. Sullivan et al. (27) reported a mutation in the DNA polymerase for strain 750'D100 which resulted in amino acid change A987G and decreased susceptibility to GCV and CDV (note: the body of the paper reported the substitution as A to G; whereas, the abstract reported G to A). No significant change was noted in the susceptibility to foscarnet.

To date, two published studies have assessed the development of foscarnet-resistant isolates following selection under foscarnet pressure. A total of 5 isolates were examined in the two studies. Sullivan and Coen (26) reported isolation of two foscarnet resistant strains following in vitro selection, both of which were resistant to foscarnet but remained susceptible to GCV and CDV. Snoeck et al. (22) reported (in abstract form) that serial passage of HCMV strain AD169 in the presence of increasing concentrations of foscarnet resulted in the isolation of foscarnet resistant strains, but that these strains remained susceptible to both GCV and CDV. The IC50 values were not reported in the abstract. The sponsor, however, has provided a table (data obtained from the Snoeck, et al.) which outlines IC50 values for three HCMV strains isolated under selective drug pressure with either foscarnet. The three reported foscarnet resistant isolates (IC50 = 770-930 uM) remained sensitive to both GCV (IC50 = 1.4-5.9 uM) and CDV (IC50 = 0.06-0.31 uM).

To summarize the in vitro drug selection studies with HCMV, isolates which are cross resistant to CDV and GCV have been detected. No CDV or GCV resistant isolates which are cross resistant to foscarnet have been reported. All CDV resistant isolates examined have been cross resistant to GCV, but not to foscarnet. In addition, many GCV resistant isolates that have been examined (except UL97 mutants) have been cross resistant to CDV, but not to foscarnet. Of the five reported, foscarnet-resistant isolates selected under foscarnet drug pressure in vitro, none were cross resistant to GCV or CDV.

Cross resistant murine CMV isolates also have been generated in vitro with selective drug pressure. Smeets et al. (20) characterized three MCMV isolates developed following pressure with foscarnet, CDV, or GCV. The foscarnet resistant isolate was sensitive to GCV and CDV. The CDV resistant isolate was sensitive to GCV and foscarnet, but the GCV resistant isolate was cross resistant to both foscarnet and CDV.

## 2. Resistance of clinical strains

Several studies have reported the isolation of HCMV strains which are cross resistant to CDV, GCV and/or PFA from patients. The clinical significance of the isolation of resistant viruses is not completely known, although several reports suggest that the development of resistant isolates is associated with failure of drug therapy.

Table 5 (from Vistide™ NDA, Gilead Sciences, Inc.) summarizes cross resistance detected in several studies. Cherrington, et al. (5) isolated a strain of HCMV (RDP-1) from the retina of a patient who had been treated with GCV and PFA. The isolate was cross resistant to GCV, PFA and CDV. DNA sequence analysis of the DNA polymerase gene revealed 6 amino acid substitutions and one amino acid insertion in the gene. The changes had not been reported previously to result in resistance to GCV, PFA or CDV. Strain C9209 was isolated from a patient with CMV retinitis who had received GCV therapy only and was resistant to GCV, PFA and CDV (7,9). A patient who had received oral GCV followed by IV GCV and PFA developed an isolate (MR) which was resistant to PFA, GCV and CDV. Another isolate obtained from an AIDS patient with CMV retinitis who had received PFA treatment was resistant to PFA but not to GCV and CDV.

**Table 5. Susceptibility of PFA Resistant Clinical Isolates to CDV and GCV  
(Vistide™ NDA, Gilead Sciences, Inc.)**

IC<sub>50</sub> (uM)

Strain	CDV [IC <sub>50</sub> (uM)]	GCV [IC <sub>50</sub> (uM)]	PFA [IC <sub>50</sub> (uM)]	Polymerase Genotype
AD-169	0.5	5	80	
RDP-1	15	200	600	A885T, D588N, K513E, N685S, P628L, S655L, Insertion 884S
C9209	1	100	> 400	A885T, G874R, L501F, L890F, N685S, N898D, S655L, Y751H
MR- 1/93	0.4	5.2	78	
MR- 10/93	8.0	160	80	
MR-8/94	10	250	500	
DH (pre)	1	8	88	
DH (post)	1	10	> 400	

<sup>a</sup> Data on file, Gilead Sciences

<sup>b</sup> Genotype includes differences relative to the AD169 strain.

<sup>c</sup> From Vistide™ NDA, Gilead Sciences, Inc.

Tatarowicz, et al. (28) demonstrated that plaque purification of a clinical isolate from a patient prior to GCV therapy resulted in the isolation of two virus populations, one which was sensitive to GCV and one which was resistant to GCV. The resistant isolate was markedly resistant to CDV and somewhat resistant to PFA.

Stanat et al. (25) evaluated clinical isolates from 8 patients for susceptibility to CDV, PFA and GCV. Although the isolates remained relatively susceptible to PFA and CDV, a set of isolates obtained from one patient early and late on GCV were borderline resistant to PFA and HPMPC, and the late isolate only was GCV-resistant.

The development of resistance to cidofovir was assessed in study GS-92-102, a phase I/II dose escalating study in HIV- infected patients with asymptomatic CMV infection. GS-92-101 was designed as dose ranging study to assess the safety, tolerance and pharmacokinetics of single doses of CDV. The study was subdivided into two dose escalation studies: 0.5, 1.0, 3.0 or 10.0 mg/kg/week of intravenous CDV alone and 3.0, 5.0 or 7.5 mg/kg/week of intravenous CDV plus probenecid. The second series included extended dosing intervals and drug interruption for proteinuria. Semen was collected weekly for assessment of anti-viral activity. From these samples, twenty-nine pairs of isolates were obtained from 22 patients. Fifteen of these patients received a single concentration of Cidofovir and 7 patients received two different concentrations of Cidofovir (separated by a 6 month washout period). Table 6 describes the source of the paired samples including the amount of time between collections of samples for isolates and the drug exposure of subjects from whom isolates were obtained. In general, the longer the time between isolates, the more intermittent the therapy.

Table 6. Characteristics of sampling for paired Isolates

Dose (mg/Kg)	Time Between Isolates (weeks)	Total # of CDV Doses Administered in the Time Between Isolates
0.5	3-7	4-8
1.0	3-10	3-4
3.0*	3-34	3-19
5.0*	4-20	4-10
10	4-7	2-4

Figure 7 (Vistide™ NDA, Gilead Sciences, Inc.) outlines the time on therapy for each patient and the IC50 for the paired isolates. Figure 8 (Vistide™ NDA, Gilead Sciences, Inc.) represents the change in IC50 values between the paired isolated isolates. Follow-up isolates (denoted as post exposure on the chart) from 4 subjects showed a 2-4-fold increase in IC50 values. Most follow-up isolates showed no change or a 2-3 fold decrease in values.

Figure 7. (Vistide™ NDA, Gilead Sciences, Inc.)

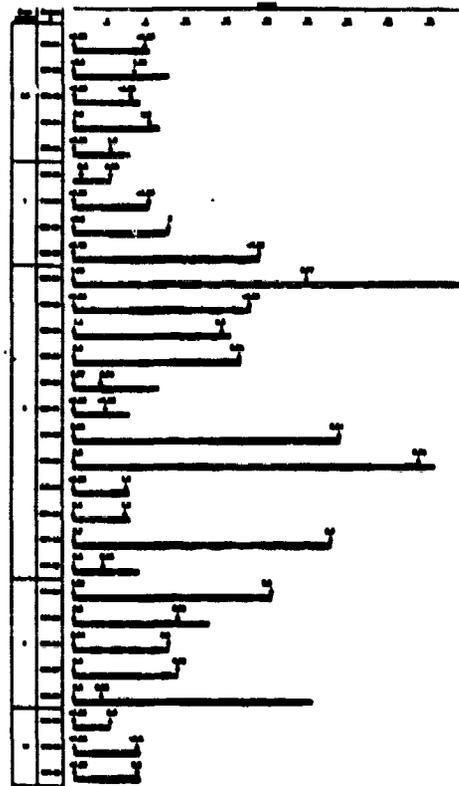
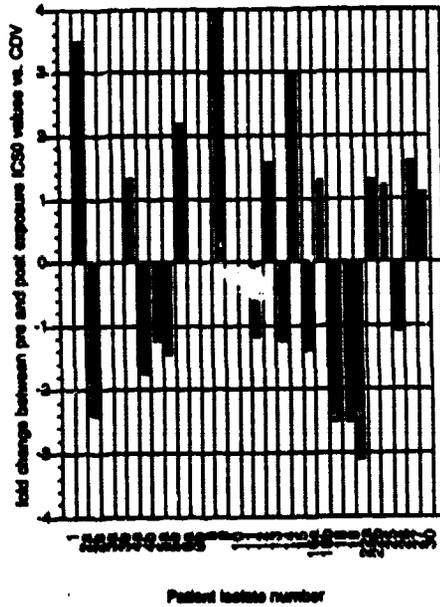


Figure 8. (Vistide™ NDA, Gilead Sciences, Inc.)

Change in Reproducibility of Follow-Up CMV Isolates Compared to Initial Isolates in Study GS-92-101



NDA-020638

FIRM: GILEAD

3 OF 4

TRADE NAME: VISTIDE (CIDOFOVIR)

GENERIC NAME: CIDOFOVIR

**Reviewer comment:** Current estimates for the development of ganciclovir resistance following treatment are that only 8-10% of subjects will develop resistance within several months of initiating treatment. Most of those isolates will have mutations in the UL97 gene, not in the DNA polymerase. While the frequency of mutations in the DNA polymerase which are responsible for resistance to any of the CMV therapies is not known, the frequency is widely believed to be less than that observed for the development of mutations in UL97 gene in response to GCV therapy. This clinical study (GS-92-102) was not an ideal one for assessment of the development of resistance to CDV unless resistance develops rapidly in response to CDV pressure. The duration of treatment was insufficient for the development of resistant isolates. The sponsor indicates that the median duration of therapy between isolates was 8 weeks with a range of 3 to 36 weeks. Most follow-up isolates, however, were obtained from specimens taken after only 3 to 10 weeks of treatment. For samples collected at longer durations (greater time between isolates), most therapy had been intermittent. Finally, several subjects received subtherapeutic levels of CDV which would decrease the likelihood of isolation of resistant virus following a short period of treatment. Thus, no reliable conclusions can be drawn with respect to the lack of development of resistance to CDV in this phase I/II trial, except that it does not occur rapidly or with outstanding frequency.

#### In Vitro Studies from Clinical Trials with CDV.

Three completed clinical studies have incorporated measurement of anti-HCMV activity in vitro. Urine, semen and/or blood specimens were cultured for the presence of virus. Assessments included qualitative culture with assessment of level of cytopathic effects, quantitative culture with an estimate of the plaque forming units of virus in a milliliter of test fluid, or culture with an immunologic assessment of HCMV viral antigen expression (shell vial assay). In one study, HCMV was experimentally assessed by branched chain DNA hybridization assays.

GS-92-101. This study was a phase I/II dose escalating trial to evaluate safety, pharmacokinetics and anti-CMV activity of CDV in HIV-1 positive patients with asymptomatic HCMV infection. Enrolled subjects were to have had positive urine and/or semen cultures on entry and no previous anti-CMV therapy. Thirty-five patients were enrolled. Subjects received 0.5, 1.0, 3.0 or 10 mg/kg/week of CDV without probenecid or 3.0, or 5.0 mg/kg/week with probenecid, or 5.0 mg/kg q 2 weeks with probenecid (first two doses weekly x2), or 7.5 mg/kg q3 weeks with probenecid. Results of this study have been published (13).

Semen HCMV titers were quantitated by serial dilution onto fibroblast monolayers. The sponsor reported that an antiviral positive response was defined as  $\geq 100$  fold reduction in HCMV titer from baseline. The sponsor indicated that 90% (3/5) of patients responded from the 3.0 mg/kg group (both with probenecid (3/5) and without (3/5)), 80% (4/5) responded in the 5.0 mg/kg group with probenecid, 100% (1/1) in the 7.5 mg/kg group; and 100% (4/4) in the 10 mg/kg group. Actual titers which were supplied to the NDA included, for the most part, only two time points; one pre therapy and one either at the end of therapy or at a followup point post therapy. As a result, the actual number of patients demonstrating a 2 log reduction in each group was lower than reported by the

sponsor. However, this difference may be due to sampling at different times, especially as some of the post exposure isolates were obtained when subjects were not receiving CDV.

Semen titers were assessed in a selected number of patients by branched chain DNA assay. The virologic trends assessed by DNA and by PFUs tracked in some patients and diverged in others.

Urine cultures were performed by inoculating 1.0 ml of urine into a tube of fibroblasts. Antiviral activity was qualitatively determined by visual assessment of cytopathic effects read at 8 weeks of culture. Attempts to quantitate the urine cultures based on titer were unsuccessful. A positive response was defined as conversion to culture negativity.

*Reviewer Comment:* The major problem with the definition of a response in this study is the ambiguity in the persistence and magnitude of the effect. For the semen responses, presumably the definition of a  $2 \log_{10}$  reduction in viral titers referred to any single time point as compared to baseline titers. Thus, the titer could fall below 100 fold reduction only at one timepoint and return to baseline later, yet a positive response to therapy would have been scored. For urine cultures, the definition is even more ambiguous. In addition to the issue of timing of a response (occurring at any timepoint, but not necessarily persisting beyond one time point), the definition of conversion to culture negativity was based predominantly on visual examination and subjective scoring of positive or negative responses. Moreover, for those cultures in which quantitative titers have been assessed (based on dilution - Study GS-92-103), baseline PFU values from urine were dramatically low (1-10 PFU/ml). Thus, positive cultures may not have been "strongly positive" at baseline, and conversion to negative culture could have fallen within the experimental error of the assay.

GS-92-103. This was a phase I/II study of the safety, tolerance, pharmacokinetics and anti-viral activity. Twenty-one subjects were enrolled. The planned dosing regimen was to compare the effects (antiviral) when CDV was administered 1x/week for four weeks, 1x/week for three weeks over a twelve week period (four doses), or once every other week (following two consecutive weekly doses) over a six week period (four doses), with probenecid.

Qualitative blood cultures were performed in duplicate by collecting the buffy coat from 10 ml of heparinized bloods, resuspending the cells in 5.0 ml of phosphate buffered saline, and inoculating 1.0 ml of the resuspension onto human embryonic lung cells (MRC-5). Qualitative urine cultures were performed by inoculating 0.2 ml of urine onto MRC-5 cells. Conversion of blood cultures from positive to negative did not occur, and 3 of 6 patients who were negative upon study entry developed positive blood cultures while on study or during follow-up. Several urine cultures from the patients who received higher doses of CDV did convert to negative over time and most remained negative (Table 7) (18)

Table 7. Polis et al. (18) .

Qualitative CMV urine culture results for patients receiving cidofovir

Patient*	Result prestudy	Result at wk																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	18	20	22	24					

\* Patients 13 through 16 received 5 mg of cidofovir per kg weekly for 4 weeks starting at week 1. Patients 17 through 20 received 7.5 mg of cidofovir per kg every 3 weeks for four doses starting at week 1.

**GS-93-106.** This is a phase II/III study of safety and efficacy of CDV for the treatment of peripheral CMV retinitis in AIDS patients. Virologic endpoints included measuring the effect of treatment of viral shedding in the blood, urine and/or semen. The study compared the effect of immediate treatment with deferred treatment. Patients received one dose of CDV (5.0 mg/kg/week X2, then 5.0 mg/kg q 2 weeks plus probenecid). CMV culture data were reported for blood and urine using both the standard culture methods and the shell vial antigen expression system. The shell vial assay was less sensitive in picking up positive cultures at baseline for urine. In contrast, although the shell vial assay has been reported to be less sensitive than standard culture for assessing viremia, in this study, a higher percentage of samples were positive at baseline by the shell vial method. By the culture method, there was no difference over time in the percentage of blood cultures which were positive in the immediate treatment groups. In contrast, with the shell vial method, a marginal response overtime was noted. Urine cultures (assayed by both methods) from subjects enrolled to immediate therapy showed a trend towards a positive (although not complete) response. The same criticisms as outlined for the previous studies would also hold for this study.

Two additional studies, which are either not complete or not completely analyzed,

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## **PACKAGE INSERT**

### Microbiology Section of the Label Proposed by the Sponsor

#### **VIROLOGY**

##### General

*Cidofovir is a cytidine nucleotide analog with potent in vitro and in vivo activity against a broad spectrum of herpesviruses, including human pathogens such as cytomegalovirus (CMV), herpes simplex virus types 1 and 2 (HSV-1, HSV-2), varicella zoster virus (VZV), Epstein-Barr virus (EBV), and human herpesvirus-6 (HHV-6). Cidofovir is also active against adenovirus and human papillomavirus. Many strains of HSV resistant to acyclovir,*

and CMV strains resistant to ganciclovir and foscarnet are sensitive to cidofovir.

**Mechanism of Action**

Cidofovir suppresses CMV replication by selective inhibition of viral DNA synthesis. Biochemical data support selective inhibition of HSV-1, HSV-2 and CMV DNA polymerases by cidofovir diphosphate, the active intracellular metabolite of cidofovir. Cidofovir diphosphate inhibits these viral polymerases at concentrations that are 8- to 600-fold lower than those needed to inhibit human cellular DNA polymerases alpha, beta, and gamma. Incorporation of cidofovir into viral DNA results in reductions in the rate of viral DNA synthesis.

Cidofovir enters cells by fluid-phase endocytosis and is phosphorylated to cidofovir monophosphate and subsequently to cidofovir diphosphate. In addition, a cidofovir phosphate-choline adduct is formed. In contrast to ganciclovir, the metabolism of cidofovir is neither dependent on, nor facilitated by, viral infections. Prolonged antiviral effects of cidofovir are related to the half-lives of metabolites; cidofovir diphosphate persists inside cells with a half-life of 17-65 hrs. Additionally, the phosphate-choline species has a half-life of 87 hrs.

**Antiviral Activity**

In vitro, cidofovir is active against all members of the herpesviridae family tested (HSV-1 and 2, CMV, VZV, EBV, and HHV-6). Antiviral activity is seen at concentrations significantly below those which cause death in cell monolayers. The in vitro sensitivities of a number of viruses to cidofovir are shown in Table 1.

Table 1\*

<b>Cidofovir Inhibition of Virus Multiplication in Cell Culture</b>	
<b>Virus</b>	<b>IC<sub>50</sub> (µM)</b>
Wild-type CMV isolates	0.7 (± 0.6)
ganciclovir-resistant CMV isolates	7.5 (± 4.3)
foscarnet-resistant CMV isolates	0.59 (± 0.07)
HSV-1, HSV-2	12.7-13.7
VZV **	0.79
EBV	0.03
HHV-6	< 6.3

\* data on file

\*\* mean result for 4 human VZV strains (1)

Cidofovir is active at non-toxic doses in several in vivo virus infection models. Murine CMV (MCMV) infection of immunocompetent and immunocompromised mice can be treated

successfully with *cidofovir*. Treatment with *cidofovir* generally was more effective than equivalent doses of *ganciclovir* at decreasing viral burden and increasing survival time. *Cidofovir* was significantly more active than *ganciclovir* against rat cytomegalovirus (RCMV) infection, as measured by mortality, histopathological changes, and virus titers in immunocompromised rats. The clinical relevance of these findings is not known, since controlled clinical trials comparing *cidofovir* and *ganciclovir* have not been performed.

In the infection models in which efficacy is seen, infrequent dosing (e.g., once weekly, 50 mg/kg) of *cidofovir* in mice is at least as effective as fractionation of the same total dose into daily or twice daily dosing schedules. Even single doses produced efficacy in these animal models. Additionally, in contrast to *ganciclovir* which requires existing viral infection for activation, the dosing of mice or rats prophylactically prior to challenge with virus is effective, and the prophylactic state induced can persist up to seven days.

*In vivo* activity against human CMV was confirmed with controlled clinical studies of *cidofovir* for the treatment of CMV retinitis in patients with AIDS, which demonstrated statistically significant delays in time to CMV retinitis progression for patients on VISTIDE when compared to control patients (see section CLINICAL STUDIES).

#### Clinical Virology

Three clinical trials with VISTIDE have examined treatment-related anti-CMV activity through the use of *in vitro* virology studies. Patient specimens were cultured for viable virus in studies GS-92-101, GS-92-103 and GS-93-106.

Study GS-93-101 demonstrated an antiviral effect of *cidofovir* at doses  $\geq 3$  mg/kg in both semen and urine of HIV-infected patients with asymptomatic CMV shedding and showed that development of resistance did not occur under the drug exposure regimens used in this clinical trial. Study GS-92-103 confirmed the anti-CMV effect in urine from similar patients; however, qualitative blood cultures obtained one to 3 weeks after a VISTIDE dose remained positive for CMV. A quantitative anti-CMV effect may not have been observed in this qualitative (+ or -) system. Data from study GS-93-106 obtained from blood and urine cultures showed that VISTIDE therapy in patients with CMV retinitis was associated with pre-treatment positive cultures turning negative and pre-treatment negative cultures staying negative in more instances than with no therapy (deferred group). Three of 4 (75%) pre-treatment positive blood cultures converted to negative following initiation of VISTIDE therapy compared with none of 3 from patients receiving no treatment. Similar results were obtained for pre-treatment urine cultures; 3 of 5 (60%) pre-treatment positive urine cultures converted to negative following VISTIDE therapy compared with none of 3 from patients receiving no treatment. The small number of observations in study GS-93-106 preclude definitive assessment.

Post-treatment virologic responses in patients receiving *cidofovir* at doses  $\geq 3$  mg/kg (studies GS-92-101 and GS-92-103) defined as conversion of pre-treatment positive urine to culture negativity, or a greater than 100-fold (2 log<sub>10</sub>) decrease in CMV infectious units (semen), are shown in the following table:

<b><u>VIROLOGIC RESPONSE</u></b>		
<b><u>Culture Source</u></b>	<b><u>No. Patients Evaluable</u></b>	<b><u>No. (%) Patients Responding</u></b>
<b><u>Urine</u></b>	30	28 (93)
<b><u>Semen</u></b>	19	14 (74)

Comparison of virologic responses in patients receiving  $\geq 3$  mg/kg vs.  $< 3$  mg/kg of cidofovir demonstrated statistically significant dose-related effects in urine and semen ( $p < 0.005$  for each) (Irazoqui et al). Persistence of anti-CMV effect was observed as well; patients with CMV viremia receiving VISTIDE 5 mg/kg/week for 4 consecutive weeks had negative urine cultures for 3 to 8 weeks post-dosing.

#### Viral Resistance

Isolates from semen of 22 patients in a Phase I/II clinical trial (Study GS-92-101) were assessed for in vitro susceptibility to cidofovir. Fifteen patients received VISTIDE at a single fixed dose level during one exposure period. An additional 7 patients received VISTIDE at different dose levels during two different exposure periods; the second exposure period began between 1 and 32 weeks after the end of the first exposure period. Thus, 29 pairs of initial (pre-exposure) and follow-up (post-exposure) isolates were assayed. Initial isolates were obtained immediately before VISTIDE was administered, and follow-up isolates were obtained at a median of 8 (range, 3 to 36) weeks later. The patients received a median cumulative dose of 30 (range, 3 to 67) mg/kg over a median of 8 (range, 2 to 38) weeks per exposure period. Nine patients (6 at 3 mg/kg and 3 at 5 mg/kg) received VISTIDE during exposure periods of more than 12 weeks. Three patients received VISTIDE intermittently (in 2 exposure periods) for more than one year (ref GS-92-101 report). The overall ranges of  $IC_{50}$  values were  $< 0.5$ - $1.85$  and  $< 0.5$ - $2.0$   $\mu$ M for 29 initial and 29 follow-up isolates, demonstrating no shift to reduced susceptibility after drug exposure. A plot of susceptibility changes from baseline (initial) also showed no particular trend; 21 follow-up isolates were considered unchanged ( $< 2$ -fold) whereas 4 were increased (2- to 4-fold), and 4 were decreased (2- to 4-fold) to a limited extent. Thus, no significant changes in susceptibilities to cidofovir were found for human CMV isolates from patients who received various regimens of VISTIDE in this clinical trial.

Following in vitro selection of ganciclovir-resistant human CMV isolates, cross-resistance between ganciclovir and cidofovir was seen with ganciclovir-selected mutations in the CMV DNA polymerase gene but not with mutations in the UL97 gene. No cross-resistance between foscarnet and cidofovir was seen with foscarnet-selected mutants. Cidofovir-selected mutants were cross-resistant to ganciclovir, but susceptible to foscarnet.

Ganciclovir or foscarnet-resistant viruses from clinical studies showed various cross-resistance patterns. Most ganciclovir-resistant viruses were UL97 gene product (phosphorylation defect) mutants and were susceptible to cidofovir and foscarnet. However,

unlike the in vitro findings, some higher level resistant mutants from patients treated long term with ganciclovir or foscarnet and, in some cases, with both ganciclovir and foscarnet, appeared to have resistance to all three agents (ganciclovir, foscarnet and cidofovir). There are inadequate data at this time to assess frequency of such strains in the population being treated. To date, no clinical isolate has exhibited decreased sensitivity to cidofovir as a direct result of VISTIDE administration. In the few clinical isolates with resistance to cidofovir obtained thus far, the resistance has been due to the existence of polymerase mutations selected by in vivo clinical exposure to ganciclovir or foscarnet, or both.

Microbiology Section of the Label Proposed by FDA.

**VIROLOGY**

Mechanism of Action

Cidofovir suppresses CMV replication by selective inhibition of viral DNA synthesis. Biochemical data support selective inhibition of CMV DNA polymerases by cidofovir diphosphate the active intracellular metabolite of cidofovir. Cidofovir diphosphate inhibits these viral polymerases at concentrations that are 8- to 600-fold lower than those needed to inhibit human cellular DNA polymerases alpha, beta, and gamma. Incorporation of cidofovir into viral DNA results in reductions in the rate of viral DNA synthesis).

In Vitro Susceptibility: Cidofovir is active in vitro against a variety of laboratory and clinical isolates of CMV and other herpesviruses (Table 1). Controlled clinical studies of efficacy have been limited to patients with AIDS and CMV retinitis. IC50 values (50% inhibitory concentration) for laboratory and clinical isolates of CMV ranged from 0.5 to 2.8 uM.

Table 1

<b>Cidofovir Inhibition of Virus Multiplication in Cell Culture</b>	
<b>Virus</b>	<b>IC<sub>50</sub> (uM)</b>
Wild-type CMV isolates	0.7 (± 0.6)
ganciclovir-resistant CMV isolates	7.5 (± 4.3)
foscarnet-resistant CMV isolates	0.59 (± 0.07)
HSV-1, HSV-2	12.7-13.7
VZV *	0.79
EBV	0.03
HHV-6	< 6.3

\* mean result for 4 human VZV strains (1)

**Resistance:** CMV isolates with reduced susceptibility to cidofovir have been selected in the presence of high concentrations of cidofovir. IC50 values for selected resistant isolates ranged from 7-15  $\mu$ M.

There are inadequate data at this time to assess the frequency of the development of resistant isolates following VISTIDE administration. In a small phase I/II, dose-ranging study of short treatment duration, twenty-nine paired isolates from 22 patients who had received one of five doses of VISTIDE have been assessed for in vitro susceptibility to cidofovir. Initial isolates were obtained immediately before VISTIDE was administered and follow-up isolates were obtained at a median of 8 weeks later. The overall IC50 values were <0.5 -1.85  $\mu$ M for the initial isolates and <0.5-2.0  $\mu$ M for the follow-up isolates. A plot of susceptibility changes from baseline showed that while most isolates were unchanged (< 2-fold) or were decreased (2- to 4- fold), values for four pairs of isolates were increased (2 - to 4-fold).

**Cross Resistance:** Eight cidofovir-resistant isolates which had been selected in vitro following exposure to increasing concentrations of cidofovir were assessed for susceptibility to ganciclovir and foscarnet. All were cross resistant to ganciclovir, but remained susceptible to foscarnet. Cidofovir-resistant isolates which are cross resistant to ganciclovir and to both ganciclovir and foscarnet also have been obtained from patients following ganciclovir and/or foscarnet therapy. To date, the majority of ganciclovir resistant isolates are UL97 gene product (phosphotransferase) mutants and remains susceptible to cidofovir. Reduced susceptibility to cidofovir, however, has been reported for DNA polymerase mutants of CMV which are resistant to ganciclovir. Clinical isolates which exhibit high level resistance to ganciclovir, due to mutations in the DNA polymerase gene, have been shown to be cross resistant to cidofovir. Cidofovir is also active against some, but not all, CMV isolates which are resistant to foscarnet. The incidence of foscarnet-resistant isolates which are resistant to cidofovir is not known. A few triple-drug resistant isolates have been described. Genotypic analysis of two of these triple-resistant isolates revealed several point mutations in the CMV DNA polymerase gene. The clinical significance of the development of these cross-resistant isolates is not known.

## **CONCLUSIONS:**

1. CDV is active in vitro and in several animal models against the herpesvirus subgroup, cytomegaloviruses. It is also active in vitro against several other species of herpesviruses.
2. In vitro drug interaction studies have not determined any antagonism between CDV and several antiviral agents which may be administered concomitantly to the patient population for which CDV will be indicated.
3. Mechanistically, CDV inhibits HCMV DNA replication. Data indicate that this is the result of activity against the HCMV DNA polymerase. The putative active metabolite is CDVpp, which competes with dCTP for incorporation into the DNA chain. Incorporation of a single molecule of CDVpp into the growing DNA chain slows DNA synthesis. Incorporation of two consecutive molecules effectively stops DNA elongation. If two CDVpp molecules are separated by one or two natural bases, DNA synthesis is markedly slowed (90%).

4. CDVpp is somewhat selective for the viral DNA polymerases. Enzyme inhibition studies indicated that human DNA polymerase  $\alpha$  is inhibited, but to a lesser degree than is the HCMV DNA polymerase. Human DNA polymerase  $\beta$  is inhibited weakly, and DNA polymerase  $\gamma$ , the mitochondrial DNA polymerase, is essentially unaffected.
5. Development of resistance to CDV following clinical exposure has not been demonstrated to date. Studies designed to assess the development of resistance were insufficient in duration to adequately address whether viral resistance to CDV arises after clinical exposure.
6. In vitro selection studies clearly demonstrate the ability to select for CDV resistant isolates following exposure in vitro to increasing concentrations of CDV. All CDV resistant isolates tested have been cross resistant to GCV, but none have been cross resistant to foscarnet.
7. In vitro selection studies demonstrate that GCV resistant mutants which exhibit a mutation in the DNA polymerase gene are cross resistant to CDV. HCMV isolates which are resistant to GCV as the result of mutations in the UL97 phosphotransferase gene remain sensitive to CDV.

#### **RECOMMENDATIONS:**

1. With respect to microbiology, NDA 20-638 (VISTIDE as a treatment of CMV retinitis in AIDS patients) is approved pending final review of the label.
2. The development of resistance of HCMV to Vistide in patients who have received therapy is a concern and has not been adequately addressed. A phase IV commitment from the sponsor is recommended to evaluate the development of resistance to cidofovir and the development of cross resistance to other anti-CMV drugs. Specifically the sponsor should be asked to:
  - a. Monitor the development of resistance to cidofovir in Vistide-treated patients.
  - b. Analyze ganciclovir and foscarnet resistant clinical isolates for susceptibility to cidofovir.
  - c. Evaluate cidofovir resistant isolates (from in vitro selection studies and from clinical samples) for susceptibility to ganciclovir and foscarnet.

Kella P. Dempsey  
Microbiologist

**CONCURRENCES:**

HFD-530/Deputy Dir. [Signature] Signature 3/25/96 Date  
HFD-530/SMicro [Signature] Signature 3/21/96 Date

- cc:  
HFD-530/Original IND  
HFD-530/Division File  
HFD-530/Div Dir Reading file  
HFD-530/Pre-Clin Dep  
HFD-530/MO  
HFD-530/Pharm  
HFD-530/Chem  
HFD-530/SMicro  
HFD-530/Review Micro  
HFD-530/CSO Struble

# Pharmacologist Review

## PHARMACOLOGIST'S REVIEW

NDA 20-638

Date Submitted: October 4, 1995

Date Assigned: October 6, 1995

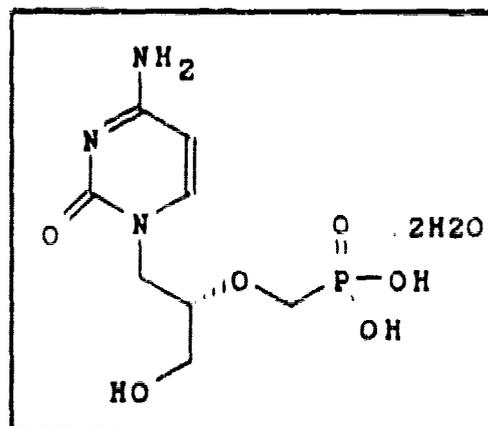
Date Review Completed: October 23, 1995

Assigned Reviewer: Pritam S. Verma, Ph.D.

HFD-530

**SPONSOR:** Gilead Sciences, Inc.  
353 Lakeside Drive  
Foster City, CA 94404

**DRUG:** Trade Name: Vistide™  
Generic Name: Cidofovir  
Other Designations: CDV,  
HPMPC  
Code #: GS-0504  
Chemical Name: (S)-1-(3-  
hydroxy-2-  
(phosphonylmethoxy)propyl]  
cytosine  
Molecular Formula:  
 $C_8H_{14}N_3O_6P \cdot 2H_2O$   
Molecular Weight: 315.21 as  
dihydrate  
Melting Point: 204-212°C  
Physical Appearance: White  
crystalline powder  
Solubility: > 75 mg/ml in water with pH near neutrality



**DOSAGE FORM:** Vistide is supplied as a sterile, clear solution in vials containing 375 mg of anhydrous cidofovir in 5 ml at concentration of 75 mg/ml. The formulation is pH adjusted to 7.4 with sodium hydroxide and/or hydrochloric acid and contains no preservatives. Each single-use vial must be diluted prior to intravenous administration.

**ROUTE OF ADMINISTRATION:** Parenteral administration by intravenous infusion.

**DOSAGE:** Vistide must be diluted in 100 ml of 0.9% saline prior to administration. To minimize potential nephrotoxicity, probenecid and intravenous saline pre-hydration must be

administered with each Vistide infusion.

Induction Treatment: The recommended dose of Vistide is 5 mg/kg body weight (given as an intravenous infusion at a constant rate over 1 hr) administered once weekly for two consecutive weeks.

Maintenance Treatment: Following completion of induction treatment, the recommended maintenance dose of Vistide is 5 mg/kg body weight (given as an intravenous infusion at a constant rate over 1 hr) administered once every two weeks.

**INDICATION:** Treatment of CMV Retinitis in Patients with AIDS.

**RELATED INDS:**

**DMFs:**

**INTRODUCTION:**

Cidofovir (HPMPC) is a phosphonate nucleotide analog of deoxycytidine triphosphate (dCTP). The compound has been shown to

The active intracellular metabolite HPMPC diphosphate (HPMPCpp) is formed by cellular enzymes and has a long intracellular half-life (17-65 hr) in cultured cells. The anti-CMV activity of HPMPC appears to result from the high affinity of HPMPCpp for inhibition of CMV DNA polymerase ( $K_i = 6.6 \mu\text{M}$ ). In so doing, high concentrations of HPMPC and its phosphorylated metabolites may potentially inhibit cellular DNA polymerases thus leading to cytotoxicity. This is one possible mechanism for HPMPC-mediated nephrotoxicity and carcinogenicity. Within kidney, high concentrations of HPMPC may interfere with other cellular functions, possibly by inhibiting the transport of endogenous anions or by depleting the intracellular pool of adenosine triphosphate. Presently, the sponsor has submitted a NDA which describes the development of HPMPC solution for the treatment of CMV retinitis in patients with AIDS.

**BACKGROUND:**

HPMPC is a member of a class of compounds termed phosphonomethylether nucleotide analogues. Unlike acyclovir and ganciclovir which require intracellular activation by the viral enzymatic machinery, phosphorylation of HPMPC in cells to HPMPCpp is independent of virus infection. The two principal systemic toxicities observed in animals are dose-limiting nephrotoxicity and carcinogenicity. Tumors associated with HPMPC administration have been observed in rats and were primarily limited to mammary tissues. Additional tumors were observed on the neck, Zymbal's gland and uterus. Other toxicities included effects on bone marrow (erythroid and myeloid depletion) and testes (hypospermia). In preclinical studies, the tissue distribution, pharmacokinetics and nephrotoxic potential of HPMPC were all affected by the dose and schedule of HPMPC administration and by concomitant administration of probenecid.

**SUMMARY:**

The review of the individual non-clinical toxicity studies can be found in Appendix # 1.

Acute toxicity studies: the minimum lethal single dose of iv HPMPC was shown to be >800 mg/kg in rats and mice. In rabbits, the lethal single dose of iv HPMPC was shown to be >1000 mg/kg. The rabbits showed histomorphologic changes in the kidney (renal tubular nephrosis) at single iv doses of >50 mg/kg. In monkeys, the acute lethal single dose of HPMPC was estimated to be between 40 and 75 mg/kg.

Multiple-dose toxicity studies: among the non-neoplastic changes, nephrotoxicity was the major dose-limiting toxicity observed in most repeat dose iv toxicity studies of HPMPC. These kidney lesions were described as a renal tubular nephrosis characterized by karyomegaly, degeneration, necrosis and/or regeneration of individual proximal convoluted tubule cells. Among the neoplastic changes, HPMPC administration resulted in a dose-related increase in mammary adenocarcinomas in female rats following subscapular subcutaneous injection (at doses of 0.6 mg/kg/weekly and higher) or iv injections (at 15 mg/kg/weekly) and Zymbal's gland carcinomas in both male and female rats (at 15 mg/kg/weekly). In the 6-month sc study, the first palpable tumor occurred after administration of as little as 6 doses of HPMPC. In a 1-month iv toxicity study in rats, clino-pathological changes (high) consisted of reduced RBC, hematocrit, hemoglobin, reticulocyte and lymphocyte counts, decreased total protein and albumin, increased BUN and creatinine, and glucosuria and proteinuria. Histopathologic evaluation identified nephropathy (tubular necrosis) and bone marrow hypoplasia and lymphoid atrophy in mid

and high doses. A dose of 0.3 mg/kg/day was identified as the NOEL. On the basis of a body surface area conversion factor, and equivalent dose in humans would be 0.043 mg/kg/day. Intravenous injections to rats in a 3-month toxicity study resulted in the death or sacrifice in extremis of 21/26 male and 15/20 female rats (high). Histomorphologic findings included a dose-related bone marrow erythroid depletion and renal tubular nephrosis (mid and high). A dose of 3.0 mg/kg/weekly was considered the NOEL in the rat study. On the basis of a body surface area conversion factor, and equivalent dose in humans would be 0.43 mg/kg/weekly. Intravenous injections of HPMPC in a 6-month toxicity study in rats resulted in the formation of subcutaneous masses in high dose female rats beginning at week 12 of study and were located primarily in areas consisting of mammary gland tissue (cervical/axillary and inguinal areas). Additional subcutaneous masses were present on the head near the ear canal in male rats more frequently than in females. Mammary adenocarcinomas were present in 22/44 high dose females. Zymbal's gland carcinomas were present in 3/44 (high) female and 6/44 (high) male rats. Non-neoplastic lesions were present in the kidneys (karyomegaly) in low, mid and high doses male and high dose female rats. A NOEL could not be identified in male rats. A dose of 3.0 mg/kg/weekly may be considered the NOEL in female rats. On the basis of a body surface area conversion factor, and equivalent dose in humans [female] would be 0.43 mg/kg/weekly. In a 1-month iv toxicity study in monkeys, histopathological findings included renal tubular nephrosis in the mid and high doses, lymphoid depletion in the spleen, thymus and lymph nodes (high), and decreased bone marrow density (high). A dose of 0.1 mg/kg/day may be considered the NOEL. On the basis of a body surface area conversion factor, and equivalent dose in humans would be 0.033 mg/kg/day. In a 3-month iv toxicity, 5/5 male and 4/5 female monkeys (high) and 2/3 females (mid) were either found dead or were euthanized in extremis during weeks 3-12. Histomorphologic finding consisted of renal tubular nephrosis and bone marrow erythroid depletion (mid and high) and testicular degeneration, thymic depletion and lymph node depletion (mid and high). Hepatocellular hypertrophy was also present (mid and high). A dose of 1.0 mg/kg/weekly may be considered the NOEL. On the basis of a body surface area conversion factor, and equivalent dose in humans would be 0.33 mg/kg/weekly. In a subsequent 3-month iv toxicity study in combination with probenecid (30 mg/kg orally one hr pre-dose) in monkeys, histomorphologic findings indicated that renal tubular nephrosis was more severe in the group receiving HPMPC alone (5 mg/kg/weekly) than the group co-administered probenecid. Decreased spermatogenesis was noted with or without probenecid (mid and high). A dose of 1.0 mg/kg/weekly may be considered the NOEL. On the basis of a body surface area conversion factor, and equivalent dose in humans would be 0.33 mg/kg/weekly. In an ongoing 1-year iv toxicity study in combination with probenecid in monkeys, an interim 6-month cohort was sacrificed. No HPMPC-

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related effects were reported at dose level of 2.5 mg/kg/weekly (high) in combination with probenecid. A dose of 2.5 mg/kg/weekly may be considered the NOEL. On the basis of a body surface area conversion factor, and equivalent dose in humans would be 0.83 mg/kg/weekly.

Reproductive and developmental toxicity studies: in a Segment I study in male rats, drug-related effects were limited to reduced body weight gains and food consumption (high). The paternal NOEL was 5 mg/kg/weekly. No gross external alterations in the F1 fetuses and no effect on fertility were observed at paternal dose of 15 mg/kg/weekly. On the basis of a body surface area conversion factor, and equivalent dose in humans would be 2.14 mg/kg/weekly. In a Segment I study in female rats, maternal body weights, body weight gains and food consumption were reduced at doses of 1.2 mg/kg/weekly (low) and higher. The number of dead or resorbed fetuses per litter and the number of early resorptions per litter were increased at doses of 1.2 mg/kg/weekly and higher. The maternal and developmental NOELs could not be identified in the study. In a Segment II study in rats, maternal effects included reduced body weight gains (mid and high) and reduced food consumption (high). Fetal effects consisted of reduced fetal body weights (mid and high) and reduced fetal ossification (high). The maternal and fetal NOELs were 0.5 mg/kg/day. On the basis of a body surface area conversion factor, an equivalent dose in humans would be 0.071 mg/kg/day. In a Segment II study in rabbits, maternal effects consisted of reduced body weight gains, body weights and food consumption at the 1 mg/kg/day dose level. Effects on the fetus consisted of increased resorptions, reduced body weights, retarded fetal ossification and increased incidence of external, soft tissue and skeletal malformations and variations at the 1 mg/kg/day dosage (maternal toxic dose). The maternal and fetal NOELs were 0.25 mg/kg/day. On the basis of a body surface area conversion factor, and equivalent dose in humans would be 0.036 mg/kg/day. In a Segment III study in rats, no effects on mating behavior, fertility or embryotoxicity were detected. In this study, the NOEL for maternal, reproductive and developmental effects was 1.0 mg/kg/day. On the basis of a body surface area conversion factor, and equivalent dose in humans would be 0.33 mg/kg/day.

Special toxicity studies: aqueous formulations of HPMPC (up to 1%) were not eye irritants in acute or subchronic studies in rabbits, and failed to elicit a delayed type hypersensitivity response in mice. When applied to the backs of rabbits as a topical gel (0.3-5%), HPMPC produced a concentration -and schedule-dependent dermal irritation following subchronic exposure. HPMPC topical gels (1-5%) were moderate-severe irritants following acute exposure to rabbit penile mucosal skin.

Mutagenicity studies: in the Ames assay (at concentrations: 1.0-

5000 µg/plate), no significant mutagenic effect was observed. In an in vitro human peripheral blood lymphocyte assay system (at concentrations: 12.5-100 µg/plate), a dose-related increase in number of chromosome and chromatid breaks occurred. HPMPC was found to be clastogenic. In a mouse micronucleus assay (at concentrations: 1000-4000 mg/kg), HPMPC produced a significant increase in polychromatic erythrocytes in both male and female mice at doses of >2000 mg/kg.

For the summary of nonclinical pharmacokinetic and ADME studies, please see Appendix 3.

#### CONCLUSIONS:

In the clinic, the test compound is being administered via iv infusion at a dose level of 5 mg/kg per week for the first two consecutive weeks followed by a maintenance dose level of 5 mg/kg every two weeks. The kinetic data from subchronic/chronic toxicity studies in rats and monkeys showed that the proposed therapeutic dose of 5 mg/kg/once weekly produced a mean steady-state C<sub>max</sub> of 7.79 µg/ml. The corresponding mean AUC value at this dose was 27.9 µg\*hr/ml. The clinical exposure was found to be considerably higher than that achieved in the nonclinical toxicity studies at the NOAELs (rat = 4.86-fold; monkey = 3.56-fold). Based on either the body surface area equivalence factors or drug exposure (AUC values), the dosages used in the clinic are higher than the NOELs/NOAELs identified in animal studies.

In a 19-week chronic study in rats, HPMPC was found to be a potent carcinogen. In animal studies, non-neoplastic toxicities were seen at doses that are comparatively much lower than those used in the clinic. Therefore, the sponsor is requested to monitor the patient closely for the toxicities seen in animals. In rabbits, treatment-related embryotoxicity and fetal malformations were observed at HPMPC dosage of 1.0 mg/kg/day [on the basis of the body surface area, an equivalent toxic dose for humans would be 0.25 mg/kg/day]. This dosage also resulted in significant maternal toxicity. Therefore, based on the results of this study, HPMPC can not be categorized unequivocally as a teratogen because maternal toxicity was also seen at the same dose level. HPMPC can be classified as Pregnancy Category C. Vistide should be used during pregnancy only the potential benefit justifies the potential risk to the fetus.

This NDA in its present form has provided adequate nonclinical safety information to support its approval and labeling.

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Six appendices are attached. These are listed below.

1. Non-clinical toxicology, pharmacokinetics and pharmacology.
2. Tabulated summary of animal toxicity studies.
3. Tabulated summary of animal pharmacokinetic studies.
4. Tabulated summary of human pharmacokinetic studies.
5. Comparison of animal doses with the human therapeutic dose.
6. Comparison of human pharmacokinetic parameters with rat, dog and monkey

  
Pritam S. Verma, Ph.D.  
Reviewing Pharmacologist

**Concurrences:**

HFD-530/DFreeman *DF 11/21/95*  
HFD-530/JFarrelly *JDF 11/14/95*  
HFD-530/PVerma *PJV 11/14/95*

**Disk:**

HFD-530/JFarrelly

cc

HFD-530/NDA 20-638  
HFD-340  
HFD-530/PVerma  
HFD-530/DPratt  
HFD-530/KStruble  
HFD-530/AD'Sa  
HFD-530/WDempsey  
HFD-345/GJames



**Appendices**

## Appendix # 1

Non-clinical toxicology, pharmacokinetics and pharmacology.

## NON-CLINICAL TOXICOLOGY

**Toxicity Studies Summary:** The studies marked with an astrict were conducted in accordance with the FDA Good Laboratory Practice Regulations.

Acute Toxicity Studies

1. Acute Intravenous Toxicologic Studies in Mice and Rats, 41671-3 HPMPC, Lot # 27767-28, July 7, 1989, -21061/89019, 89021)\*
2. Single Dose Acute Intravenous Study in Rats Administered HPMPC Alone and in Combination with Cyclosporin and/or Amphotericin B, Lot # 504A92-01, June 18, 1993, 406-GS-001-92)\*
3. Comparative IV Lethality in Rats, BMY-41671 HPMPC, Lot # 27625-50-1 and BMY-43957-3, 98% R-isomer, Lot # 28593-97, June 22, 1990, 21598/90021)\*
4. Acute Oral Dose-Range Toxicity Study in Rats .41671 HPMPC, Lot # 26848-19-2, January, 1991, .21713/90059)
5. 5. Single-Dose Intravenous Nephrotoxicity Screening Study in Male Rabbits, .41671 HPMPC, Lot # 27411-48-, July 25, 1990, (DAVI-TJ-21618/89076)
6. Single dose ocular toxicity study of cHPMPC and HPMPC following intravitreal administration in rabbits, Lot # LY-803-18, Gilead Sciences, Inc., Foster City, CA, August 22, 1995, (94-TOX-0504-013/HWA-6511-105)\*
7. Single Dose Subcutaneous and Intramuscular Study in Rabbits with HPMPC, test articles # NB-316-63-001/002/003, March 27, 1992 450A-GS-001-92)\*
8. Histopathological Examination of Kidneys from HPMPC-treated Monkeys (kidneys obtained from animals in the PK 1 study: A Summary of Single-Dose and Multiple-Dose Pharmacokinetics of <sup>14</sup>C-HPMPC, Lot # 28648-7, in the African Green Monkey,

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- June 22, 1990, ( 25768/904- 41671-  
01/02)
9. Single-Dose Intravenous Toxicity Studies in Monkeys,  
40085 Lot # 27962-013, and 41671 HPMPC, Lot #  
27411-48-1, March 6,  
1990, 21499, 124005)\*
10. Comparative Single-Dose Intravenous Toxicity Study in  
the Cynomolgus Monkey, 41671 HPMPC (97:3 S,R-isomer  
commercial bulk drug formulation) Lot # 27625-50-1 and  
43957-3 (98% R-isomer) Lot # 28593-97,  
July 3, 1990,  
21597/90025)\*
11. Intravenous Single-Dose Pilot Study in the Monkey,  
41671 HPMPC, Lot # 27767-30, and 40085 PMEA, Lot #  
27962-013a, July,  
1989, 21128/89026)

#### Multiple Dose Toxicity Studies

12. Multiple-Dose Intravenous Nephrotoxicity Study in Male  
Mice, 41671 HPMPC, Lot # C90D727,  
April 15, 1991,  
21830/90129)
13. Fourteen day Repeated Dose Toxicity study of HPMPC  
Following Subcutaneous Injections to Mice, Lot #  
504b93-01, Gilead Sciences, Inc. Foster City, CA,  
September 22, 1995, (94-TOX-0504-014)
14. Intravenous Multiple-Dose (x5) Range Study in Rats,  
41671, Batch # 26870-064 and 40085, Batch #  
26870-059, Phosphonates,  
October 3, 1988, 20887/88032)
15. Report in preparation - summary: Two week repeated dose  
toxicity study of HPMPC and cHPMPC administered via iv  
injection to rats, Lot # 0294-T5, Gilead Sciences,  
Inc., Foster City, CA, April 4, 1994, (94-TOX-0504-007)
16. Multiple-Dose Intravenous Pilot Study in Rats,  
41671 HPMPC, Lot # 26870-64 and 40085 PMEA, Lot #  
26870-059, January,  
1989, 20971/88072)
17. Four week repeated dose toxicity study of HPMPC and  
cHPMPC administered via iv or sc to rats, Lot # 0294-  
T5, Gilead Sciences, Inc., Foster City, CA, June 23,  
1994, (94-TOX-0504-008)

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18. One-Month Intravenous Toxicity Study in Rats, 41671  
HPMPC, Identification # 27337-10, ' '  
January 11, 1990, 21461,  
124004)\*
  19. Thirteen week repeated dose toxicity study of HPMPC  
administered iv to rats, Lot # 504B92-01,  
April 1, 1994, (Study No.  
2-P50, 50-93-230)\*
  20. A 6-month chronic toxicity study of HPMPC following  
once weekly iv administration in rats, lot # 504-B93-  
01,  
September 12, 1995, (94-TOX-0504-016)\*
  21. A 6-month chronic toxicity study of HPMPC administered  
sc to rats, Lot # 504K93-01,  
September 15, 1995, (94-TCX-0504-003\2-  
T70)\*
  22. Multiple-Dose Subcutaneous Nephrotoxicity Screening  
Study in Male Guinea Pigs, BMY-41671 HPMPC, Lot #  
27411-48-1 and Probenecid Lot # 026-0293,  
January 21, 1991,  
21634/90047)
  23. Multiple-Dose Subcutaneous Nephrotoxicity Study in Male  
Guinea Pigs, -41671 HPMPC, Lot # 28648-7,  
November 16, 1990,  
21693/90077)\*
  24. Intravenous study in rabbits treated with HPMPC with  
and without probenecid, Lot # 504A92-01,  
November 13,  
1992, 453-GS-001-92/T0504-00015)\*
  25. Final Report: Five day repeated dose toxicity study of  
HPMPC administered iv with and without orally  
administered probenecid in male rabbits, Lot # 504A92-  
01,  
September 21, 1995, 453-GS-001-9: 504-00058)\*
  26. Multiple-Dose Intravenous Nephrotoxicity Screening  
Study with Probenecid, Lot # 267-0293 and -41671  
HPMPC, Lot # 27625-50-1 in Male Rabbits,  
September 21, 1990,  
21596/90001)
  27. Multiple-Dose Intravenous Nephrotoxicity Screening  
Study in Male Rabbits, 41671 HPMPC, Lot # 27625-50-  
1 and 27411-48-1,

- July 23, 1990, -21497/89092)\*
28. Multiple-Dose Intravenous Pilot Study in Monkeys,  
41671 HPMPC, Lot # 26870-64 and -40085 Lot #  
26870-059, January,  
1989, 20954/88064)
29. Repeated Dose Oral Toxicity Study and Pharmacokinetics  
of HPMPC Administered via Gavage to Cynomolgus Monkeys,  
Lot # 504A92-01,  
April 22, 1992 (2-J57 57-92-77)\*
30. Thirty day repeated dose sc toxicity study and  
pharmacokinetics of HPMPC administered to cynomolgus  
monkeys, Lot # 67-049-DK,  
November 9, 1994, (2-N13/) 13-93-  
43)\*
31. One-Month Intravenous Toxicity Study in Cynomolgus  
Monkeys, 41671 HPMPC, Identification # 27337-20,  
January 11, 1990,  
-21444, -124003)\*
32. Thirteen week repeated dose toxicity study of HPMPC  
administered intravenously to cynomolgus monkeys in  
combination with orally administered probenecid. Lot #  
13H0405,  
December 9, 1994, (Study No. 2-T01/93-TOX-0504-002  
Report 01-94-89)\*
33. Thirteen week repeated dose toxicity study of HPMPC  
administered iv to cynomolgus monkeys, Lot # 504B93-01,  
March 25, 1994,  
(Study No. 2-P51, P51-93-232)\*
34. A 12-month chronic toxicity study of HPMPC following  
once weekly iv administration in cynomolgus monkeys  
with an interim sacrifice at 6 months, Lot # 504-B93-  
01,  
September 18, 1995, (94-TOX-0504-015\86540)\*

#### Special Toxicity Studies

35. Evaluation of HPMPC to induce delayed-type  
hypersensitivity in BALB/cByJ mice, Lot # 1966-D-1,  
August 20, 1992, (Report #  
711-GS-001-93)\*
36. Acute toxicity study of HPMPU administered via  
intravenous injection to Sprague-Dawley rats, Lot # NB  
364-75, August

11, 1992, (2- .582-00001 69-92-94)\*

37. Four week repeated dose iv toxicity study of HPMPU in rats, Lot # 883-3-28, Gilead Sciences, Inc, Foster City, CA, August 17, 1995, (95-TOX-1582-001)

#### Reproductive Toxicity Studies

38. Fertility and general reproduction study of HPMPU administered iv to male rats (Segment I), Lot # 504K92-1, November 22, 1994, (707-003/93-TOX-0504-005)\*
39. Intravenous fertility and general reproduction study of HPMPU in female rats, Lot # 504B93-01, September 15, 1995, (707-00494-TOX-0504-019)\*
40. Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of HPMPU administered intravenously to rats, Lot # 504K92-01, April 4, 1994, (Report # 707-001/93-TOX-0504-004)\*
41. A toxicokinetic study of <sup>14</sup>C-labelled HPMPU after multiple iv administrations to presumed-pregnant rats, Lot # JPS-881-32, September 21, 1995, (94-TOX-0504-005/2-W49)\*
42. Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of HPMPU administered intravenously to New Zealand White rabbits, Lot # 504B93-01, November 21, 1994, (707-006/94-TOX-0504-002)\*
43. Perinatal and postnatal reproduction study of HPMPU administered sc to rats (Segment III), Lot # 504K93-01, September 15, 1995, (94-TOX-0504-011/707-005)\*

#### Mutagenicity Studies

44. Ames Microbial Mutagenicity Assay and E. Coli WP2 uvrA Reverse Mutation Assay, BMY-41671 HPMPU, Lot # 27411-47-1, February 21, 1990, (21203/89037)\*
45. Human Peripheral Blood Lymphocyte Clastogenesis Assay, 41671 HPMPU, Lot # B27411-47-1, December 12, 1990, (21539/89052)\*

- .....
46. Mutagenicity test on HPMPC in an in vivo mouse micronucleus assay, Lot # 1966-C-8P, July 25, 1995, (94-TOX-0504-012/16163-0-455)\*
47. Primary skin irritation in rabbits Lot # C90D731, July 31, 1992, (Report # 92-C-12628/12629/12569/12570/12571)

#### Other Toxicity Studies

48. Primary eye irritation in rabbits, Lot # not available, December 14, 1992, (Report # 421-GS-001/002/003-92)\*
49. A penile irritation study in rabbits with HPMPC topical gel, Lot # 504L92-02, May 13, 1994, (93-TOX-0504-003, Study No. 3337.1)\*
50. A single dose penile irritation study in rabbits with HPMPC topical gel, Lot # 504L92-04, Gilead Sciences, Inc., Foster City, CA, September 21, 1995, (94-TOX-0504-010/SLS-3337.2)\*
51. 5-, 10- and 30-Day repeat dose dermal toxicity in rabbit, Lot # 530-65-3, August 27, 1992, (Report # PH 431-GS-002-93)\*
52. 30-Day repeat dose dermal toxicity in rabbit, Lot # 504L92-02, August 30, 1992, (Report # 431-GS-001-93)\*
53. Three day repeated dose ocular irritation study of HPMPC and cHPMPC in rabbits, Lot # LY-803-56D, Gilead Sciences, Inc., Foster City, CA, August 22, 1995, (94-TOX-0504-004/EWA-6511-106)\*

#### Toxicities Studies Review:

#### Acute Toxicity Studies

1. Acute Intravenous Toxicologic Studies in Mice and Rats, 41671-3 HPMPC, Lot # 27767-28, July 7, 1989, (21061/89019, 89021)\*

Two groups of male and female Sprague-Dawley (Cr1:CD (SD) BR) rats (weight: 198 - 225.8 g male and 161.8 - 184 g female; age: 7 weeks, 5 animals/sex/group), and two groups of male and female (CD-1 (Cr1:CD-1(ICR)BR) mice (weight: 16.9 - 29.2 g; age: 5.5 weeks, 5 animals/sex/group) were administered HPMPC a single

intravenous dose of 400 mg or 800 mg/kg. Control groups (5 male and 5 female) of rat and mice received 0.9% sodium chloride under identical experimental conditions. The objective of the study was to investigate the acute intravenous toxicity of HPMPC in mice and rats. All the animals were observed for 14 days for signs of toxicity and changes in general health and behavior. Results: No deaths were observed in the treated rats or the mice. No clinical signs of toxicity were observed in either group of mice. One female rat (400 mg/kg) showed a tail lesion beginning on day 13. Male rats (800 mg/kg) showed rough hair coats on day 6 and one rat exhibited sporadic soiling for the remainder of the period. One female rat (800 mg/kg) showed rough hair coat for 2 days commencing on day 7. The male rats (800 mg/kg) exhibited lower average body weight gains (12%) at the termination of the study as compared to the controls. After intravenous administration of HPMPC, the estimated single minimum lethal dose in male and female rats and mice was > 800 mg/kg.

**2. Single Dose Acute Intravenous Study in Rats Administered HPMPC Alone and in Combination with Cyclosporin and/or Amphotericin B, Lot # 504A92-01, June 18, 1993, 406-GS-001-92)**

Groups of male and female Sprague Dawley rats (weight: 180-243 g; 5 animals/sex/group) received single iv dose of HPMPC (1.0, 3.0 or 10.0 mg/kg) either alone or in combination with cyclosporin (50 mg/kg) and/or amphotericin B (0.5 mg/kg). There was no control group for either drug. Animals were observed daily for 14 days post-dosing for signs of toxicity. The purpose of this study was to evaluate nephrotoxicity in HPMPC (H) treated rats administered cyclosporin (C) and/or amphotericin B (A). Results: animals receiving H and C (with or without A) showed signs of clinical toxicity including decreased activity, dyspnea, abnormal gait and stance, and red urine. No clinical signs were observed after 24 hr post-dosing. No treatment related body weight changes were measured. At necropsy, mottled kidneys were observed in all H + C, H + A, and H treated (3 mg/kg only) rats. Discolored spleens were present in rats receiving C + H (1 and 10 mg/kg). Male rats dosed with C, A and H (10 mg/kg) showed a small BUN increase. Histopathology: rats receiving H alone had minimal severity kidney lesions characterized as multifocal nonsuppurative nephritis and tubular regeneration, occasionally accompanied by tubular dilatation. The severity of nephrotoxicity did not increase with increasing H dose. These kidney lesions were increased in incidence and severity (ie, up to moderate) in H + C rats with or without A. Kidney lesion severity (but not incidence) was slightly increased in H + A group rats. Minimal-to-moderate multifocal splenic capsulitis occurred in H + C treated rats.

**Comments:** These data suggested that the administration of

.....  
cyclosporin (50 mg/kg), and to a lesser extent amphotericin B, could increase both the incidence and/or severity of the kidney lesions in HPMPC-treated rats.

3. Comparative IV Lethality in Rats, .41671 HPMPC (97:3% S,R-isomer) Lot # 27625-50-1 and 43957-3 (98% R-isomer) Lot # 28593-97, June 22, 1990,  
.21598/90021)\*

Four groups of fasted male and female Sprague-Dawley (Cr1:CD (SD) VAF+ BR) rats (weight: 161 - 192 g male and 133 - 155 g female; age: 47 days, 5 animals/sex/group) were administered via the caudal tail vein at a rate of 0.1 ml/sec HPMPC in a single intravenous dose either .41671 or 43957-3 at 400 or 800 mg/kg in 0.9% sodium chloride. The control group (5 male and 5 female) received 0.9% sodium chloride for the injection under identical experimental conditions. The objective of the study was to investigate and directly compare the estimated minimal lethal single doses of the commercial bulk drug formulations of .41671 (97:3% S,R-isomer mixture) and of 43957-3 (98% R-isomer) to the rats. Results: in the present study, no deaths were reported. Male animals given .41671 at either dose gained moderately less weight where as female rats (800 mg/kg) had a slight reduction in weight gain. The estimated minimal lethal intravenous single doses of .41671 and 43957-3 are greater than 800 mg/kg in both the male and female rats.

Comments: The R-isomer of HPMPC is inactive and the animals treated with the isomer appeared clinically normal throughout the study, and they were not different from the control group.

4. Acute Oral Dose-Range Toxicity Study in Rats 41671 HPMPC, Lot # 26848-19-2 January, 1991, 21713/90059)

Three groups of fasted male and female Sprague-Dawley (Cr1:CD (SD) BR VAF/PLUS) rats (weight: 179 - 251.6 g male and 161.8 - 184 g female; age: 6 weeks, 5 animals/sex/group) were administered HPMPC orally by gavage in a single dose (< 2.0 ml/100 g) of 500, 1000 and 2000 mg/kg in saline. A control group (5 male and 5 female) of rats received saline under identical experimental conditions. The objective of the study was to investigate the acute toxicity of HPMPC in rats when given orally. All the animals were observed for 15 days for signs of toxicity and changes in general health and behavior. Results: one female rat (2000 mg/kg) died on day 13. Orally administered HPMPC induces a gastrointestinal irritation which appears to be dose limiting. The single dose of 500 mg/kg may be considered a NOAEL.

Comments: With a conversion based on body surface area, the equivalent oral NOAEL in humans would be 71 mg/kg.

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**5. Single-Dose Intravenous Nephrotoxicity Screening Study in Male Rabbits,** 41671 HPMPC, Lot # 27411-48-1,  
, July 25, 1990, 21518/89076)

Four groups of male New Zealand White rabbits (weight: 2.7-3.0 kg; age: unknown; 2 animals/group) were administered HPMPC by intravenous injections at a rate of 0.1 ml/sec (via the lateral ear vein) at doses of 10, 25, 50 and 100 mg/kg in sterile saline (<2 ml/100 g). A control group received sterile saline under similar experimental conditions. The purpose of this study was to assess the nephrotoxicity of HPMPC in the rabbits and to define a single dose producing moderate to severe nephrosis in this species. Results: a single dose of 50 or 100 mg/kg of HPMPC produced a tubular nephrosis of minimal to slight severity in this species. An IV dose of 25 mg/kg may be considered a NOAEL in rabbits.

**Comments:** In the single dose IV study, with the dose conversion based on body surface area, the equivalent NOAEL for humans (based on renal effects) is 6.25 mg/kg.

**6. Single dose ocular toxicity study of cHPMPC and HPMPC following intravitreal administration in rabbits, Lot # LY-803-18, Gilead Sciences, Inc., Foster City, CA, August 22, 1995, (94-TOX-0504-013/HWA-6511-105)\***

Six groups of male rabbits (3 animals/group) were administered (using a 27-gauge needle) HPMPC at dose levels of 10 µg/eye (G1), 100 µg/eye (G2) and 1000 µg/eye (G3) or cyclic HPMPC 10 µg/eye (G4), 100 µg/eye (G5) and 1000 µg/eye (G6) as a single intravitreal injection into the right eye of each animal. The control material, 0.9% saline, was administered as a single intravitreal injection into the left eye of each animal. The purpose of the study was to assess the ocular inflammation and pathological changes through 28 days in rabbits. Results: are summarized Table 1. Microscopic changes noted in control and treated eyes included vitreal hemorrhage and fibrillar precipitates. These changes were considered to be due to the mechanical aspects of the intravitreal injection procedure.

Table 1

Single dose ocular toxicity of HPMPC and cyclic HPMPC following intravitreal administration in rabbits

Group	Drug	Dose ( $\mu\text{g}/\text{eye}$ )	Major Findings
1	HPMPC	10	None
2	HPMPC	100	Red conjunctivae, squinting, opaque eye, cloudy discharge, swollen eyelid (Days 4-12, 2/3 rabbits)
3	HPMPC	1000	Red conjunctivae, squinting, opaque eye, cloudy discharge, swollen eyelid (Day 4-16, 3/3 rabbits); WBC in aqueous humor (Day 3, 1/3 rabbits); endophthalmitis (3/3), inflammation of the iris and elevated intraocular pressure (Day 15, 2/3 rabbits). Histological findings: corneal edema, vascularization or inflammation; hemorrhage, fibrillar precipitate (3/3 rabbits)
4	cHPMPC	10	None
5	cHPMPC	100	None
6	cHPMPC	1000	None

**Comments:** Based on the results of this study, a single intravitreal injection of HPMPC (10  $\mu\text{g}/\text{eye}$ ) may be considered a NOAEL in rabbits and the NOAEL for a single intravitreal injection of cyclic HPMPC was considered to be 1000  $\mu\text{g}/\text{eye}$ . Based on the body surface conversion factor, a single intravitreal injection of 3.2  $\mu\text{g}/\text{eye}$  (HPMPC) or 322.5  $\mu\text{g}/\text{eye}$  (cyclic HPMPC) would be considered an equivalent dose in humans.

**7. Single Dose Subcutaneous and Intramuscular Study in Rabbits with HPMPC, test articles # NB-316-63-001/002/003,**

March 27, 1992

450A-GS-001-92)\*

Groups of male albino New Zealand White rabbits (age: 8-12 weeks; weight: 2.3-2.75 kg; 8 animal/group) were administered a single dose of HPMPC (25, 50 and 75 mg/kg) via sc or im route in one ml dose volume (2 sites x 0.5 ml). Following the dosing, 2 animals from each group were sacrificed at 24, 48, 72 hr and 7 days after the injection. The purpose of the study was to evaluate the local effects of single sc and im injections. At necropsy, all injection sites and adjacent untreated sites were removed and evaluated for histopathology. The results showed that HPMPC via im and sc routes caused minimal to slight irritation at the injection sites following 24, 48 and 72 hr after the treatment. These changes were completely resolved at 7 days.

8. Histopathological Examination of Kidneys from HPMPC-treated Monkeys (kidneys obtained from animals in the PK 1 study: A Summary of Single-Dose and Multiple-Dose Pharmacokinetics of <sup>14</sup>C-HPMPC, Lot # 28648-7, in the African Green Monkey, June 22, 1990, -25768/904. 41671-01/02)

The kidneys collected from 13 African green monkeys administered a single 50 mg/kg dose of HPMPC (IV, IP or PO) and from 6 African green monkeys administered single or multiple IV 5 mg/kg/day doses of HPMPC were examined histopathologically. Results: when HPMPC (5 mg/kg/day) was administered intravenously for 10 consecutive days to monkeys that were sacrificed after the 10th dose, renal toxicity was observed in 2 of 2 monkeys. The treatment-related nephrotoxicity was characterized by acute, diffuse necrosis, vacuolar degeneration with loss of brush borders and regeneration of tubule epithelial cells, primarily in the proximal convoluted tubules of the renal cortex. No drug-related histopathologic changes were observed in the kidneys of the monkeys treated with a single dose of 50 mg/kg, regardless of route of administration (IV, IP or PO).

9. Single-Dose Intravenous Toxicity Studies in Monkeys, -40085  
PMEA, Lot # 27962-013, and 41671 HPMPC, Lot # 27411-48-1,  
March 6, 1990,  
21499 -124005)\*

Groups of male and female Cynomolgus monkeys (weight: 3.0 - 4.4 kg; age: adult; one animal/sex/group unless noted) were administered a single intravenous injection of either HPMPC or PMEA in a total volume of less than 6.41 ml in 1N NaOH: PMEA [75 mg (one female) and 175 mg/kg] and HPMPC [40 mg (one female) and 75 mg/kg]. Monkeys were observed for toxic effects for a period of time that ranged from 14 - 21 days. There were no control animals for either of the test compounds. The purpose of the study was to evaluate the potential toxic effects of the two test compounds when administered once via intravenous injection to the male and female Cynomolgus monkeys. Results: with HPMPC, one female (75 mg/kg) died on day 21. Significant and progressive elevations in creatinine and BUN were noted in the female after the first week of treatment, and her condition continued to worsen, before her death, with significant loss in body weight. Transient body weight loss and decreased food intake were also noted in the female (40 mg/kg) during the first week after the treatment. The male (75 mg/kg) had decreased defecation on day 4 and 11 and decreased urination on day 11. The female monkey given 40 mg/kg was observed to be hypo-active on day 3 and 7. With PMEA, the male monkey given 150 mg/kg died on day 6 after the treatment. Prior to his death on day 1 after treatment, the animal had emesis, decreased activity, body tremors, hunched body posture and decreased food intake. The single lethal dose of

HPMPC and PMEA are between 40 - 75 mg/kg and 75 - 150 mg/kg, respectively, when administered intravenously to Cynomolgus monkeys.

**Comments:** PMEA and HPMPC were tested for their potential toxic effects when administered once intravenous to Cynomolgus monkeys. HPMPC was found to be approximately two times more toxic than PMEA.

**10. Comparative Single-Dose Intravenous Toxicity Study in the Cynomolgus Monkey, - 41671 HPMPC (97:3 S,R-isomer commercial bulk drug formulation) Lot # 27625-50-1 and -43957-3 (98% R-isomer) Lot # 28593-97, July 3, 1990, -21597/90025)\***

Two groups of fasted male and female Cynomolgus macaque monkeys (weight: 2.2 - 2.6 kg; age: unknown; 1 animal/sex/group) were administered HPMPC, 41671 (97:3% S,R-isomer formulation) or 43957-3 (98% R-isomer) as a single intravenous injection (via femoral vein) of 75 mg/kg in sterile saline (3.0 ml/kg and a dose rate of 0.1 ml/second) during a two week toxicity study. There was no control group in the study. The intent of this study was to investigate and directly compare the single IV dose toxicity of two isomeric formulations of HPMPC. The results of this study were compared with data obtained in a previous toxicity study in monkeys using an isomeric formulation (>99% S-isomer) in the previous toxicological study (# 9). **Results:** No deaths were reported. Signs of toxicity included transient, slight whole-body blue skin color and transient lack of weight gain for the male; weight loss, increased serum creatinine and decreased serum phosphorus for the female; and pale kidney cortices at necropsy for 41671-treated monkeys. These changes were not observed in the 43957-3-treated monkeys. The estimated minimal lethal IV single doses for both the isomers in both the sexes are greater than 75 mg/kg. In comparison, the previously reported minimal lethal IV single dose of 41671-3 (>99% S-isomer) was estimated to be greater than 40 mg/kg and less than 75 mg/kg. In conclusion, 43957-3 (98% R-isomer) appears to be less acutely toxic than 41671 (97:3% S,R-isomer), the present commercial bulk drug formulation; and neither is more acutely toxic than 41671-3 (>99% S-isomer), the formulation used in the toxicological study (# 2), when given as a single IV dose of 75 mg/kg to the male and female monkeys.

**Comments:** The S-isomer (>99%) of HPMPC is more nephrotoxic than the present commercial bulk drug formulation (97:3% S,R-isomer).

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11. Intravenous Single-Dose Pilot Study in the Monkey, 41671  
HPMPC, Lot # 27767-30, and 40085 PMEA, Lot # 27962-013a,  
July, 1989,  
21128/89026)

Groups of male and female Cynomolgus monkeys (weight: 3.8 - 5.5 kg; age: unknown, 1 animal/group) were administered single intravenous dose via a saphenous vein in a total volume of <6.5 ml/kg at a rate of 0.1 ml/sec of either HPMPC (150 or 400 mg/kg) or PMEA (150 or 500 mg/kg) in 0.9% sodium chloride. A control group was not included in this study. The objective of the study was to investigate the acute toxicity of HPMPC and PMEA following a single IV injection. Results: with HPMPC, both single doses were lethal within 5 - 6 days after the treatment. The single lethal dose is, therefore, estimated to be less than 150 mg/kg. With PMEA, single lethal dose is estimated to be greater than 150 mg/kg but less than 500 mg/kg.

#### Multiple Dose Toxicity Studies

12. Multiple-Dose Intravenous Nephrotoxicity Study in Male Mice,  
.41671 HPMPC, Lot # C90D727,  
April 15, 1991, .21830/90129)

Groups of male mice (CD-1 (Cr1:CD(ICR)BR) VAF-PLUS) (weight: 24 - 32.7 g; age: 4 - 5 weeks, 5 animals/group) were administered HPMPC by intravenous injection at a rate of 0.05 ml per second (via the caudal tail vein) as either a single dose of 200 mg or 1000 mg/kg, two doses of 100 or 500 mg/kg, or 5 consecutive daily doses of 40 or 200 mg/kg, so that the total cumulative dose was either 200 or 1000 mg/kg in sterile saline (<2 ml/100 g). Two control groups received sterile saline under similar experimental conditions. The objective of the study was to investigate the nephrotoxic potential of HPMPC in mice following either a single dose of 200 or 1000 mg/kg, 2 doses of 100 or 500 mg/kg, or 5 consecutive daily doses of 40 or 200 mg/kg. Results: this study showed that a cumulative drug exposure/time relationship, i.e., greater effects per body weight were apparent when the HPMPC dose was divided into smaller doses with maximal effects noted with daily doses. There were no distinct or consistent drug-related macroscopic or microscopic alterations noted in the kidneys of the treated groups.

Comments: Based on the morphologic findings, the mouse is not as susceptible to the nephrotoxic effects of HPMPC as other laboratory animal species tested including the rat, guinea pig, dog and monkey. A low bioavailability (3%) of the drug in mouse is observed in other experiments, suggesting that a high rate of metabolism of the compound may be an issue in mouse susceptibility.

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13. Fourteen day Repeated Dose Toxicity study of HPMPC Following Subcutaneous Injections to Mice, Lot # 504b93-01, Gilead Sciences, Inc. Foster City, CA, September 22, 1995, (94-TOX-0504-014)

Groups of male and female mice (5/sex/group) received sc HPMPC either 0 (vehicle control), 3 (low), 10 (mid) or 30 mg/kg/day (high) for 14 consecutive days, or 70 mg/kg/once weekly on study days 1, 7 and 14. Results: all animals survived until scheduled sacrifice. Clinical signs of toxicity observed were limited to injection site lesions (redness, scab formation and roughness) that were present in all mice receiving daily (but not weekly) injections. Histopathology: changes were present only at the injection sites (epidermis, dermis and associated subcutaneous tissues). The changes consisted of chronic inflammation characterized by mild-moderated acanthosis, dermal mononuclear cell infiltration and fibrosis in both the control and treated mice. Epidermal ulceration and subcutaneous accumulation of fibrillar eosinophilic material showed dose-dependent increased severity (mild-severe) in daily treated animals. Conclusion: a NOEL could not be identified in this study.

14. Intravenous Multiple-Dose (x5) Range Study in Rats, 41671, Batch # 26870-064 and 40085, Batch # 26870-059, Phosphonates, 1 October 3, 1988, -20887/88032)

Groups of male Sprague-Dawley (Cr1:CD (SD) BR) rats (weight: 136 - 160 g; age: 6 - 7 weeks, 5 animals/group) were administered intravenously via the tail vein in a total volume of 0.25 ml/100 g at a rate of 0.05 ml/sec either HPMPC or PMEA in 0.9% sodium chloride at a daily doses of either 10 (low), 100 (mid) or 250 mg/kg/day (high) for 5 consecutive days. Following treatment, the rats were observed for 10 days. A control group (5 animals) received 0.9% sodium chloride for injection under identical experimental conditions. The objective of the study was to investigate the toxicity of HPMPC and PMEA in the male rats. Results: 3 of the 5 rats died 4 - 6 days after treatment (high). Body weight losses or significantly decreased weight gains were noted in the intermediate and high dose groups. Dose-related nephrotoxicity (cortical tubular nephrosis) was apparent in the intermediate and high dose groups. Other major findings included a transient leukocyte reduction due to a decrease in lymphocytes and slight lymphoid depletion at the high dose group, and a slight increase in hepatocellular mitoses at the intermediate dose level. At the end of the observation period, elevations in ALT were evident in intermediate and high dose groups. In the low-dose animals, a slight increases in hepatocellular mitoses in one rat were detected. On the other hand in PMEA treated animals, transient and dose dependent decreases in body wight gain occurred during treatment in the intermediate and high dose

groups. Nephrotoxicity was detected at the high dose level and correlated with increased BUN. Other toxicity included slight transient leukocyte reduction, decrease in neutrophils in both the intermediate and high dose groups, slight lymphoid depletion, reticulocytopenia, slight elevations in ALT and increased hepatocellular mitoses in the high dose group. No toxicity was observed at the low dose.

At necropsy, pale kidney cortices were noted for 4 intermediate and 2 high dose HPMPC as well as 2 high dose PMEA-treated rats; evidence of gastro-intestinal hemorrhage or ulceration and/or liquid/watery intestinal contents were noted for 3 high dose HPMPC and 2 high dose PMEA treated rats. Histopathological finding included a dosage related tubular nephrosis in kidney sections from the intermediate and high dose HPMPC and the high dose PMEA-treated rats, lymphoid depletion in thymic and/or splenic sections from 3 high dose HPMPC and 3 high dose-PMEA-treated rats, and increased mitotic figures in liver sections from one or more low and intermediated dose HPMPC and one of the high dose PMEA-treated rats.

**Comments:** The death of high dose HPMPC-treated animals may be attributed to tubular nephrosis. Nephrotoxicity appears to be major dose limiting toxicity. A dose of 10 mg/kg/day for 5 days treatment of R-isomer may considered a NOEL; however, a NOEL could not be determined for the S-isomer. A dose of 10 mg/kg/day of the S-isomer may be considered a NOAEL; an equivalent dose in humans would be 1.4 mg/kg/day.

**15. Two week repeated dose toxicity study of HPMPC and cHPMPC administered via iv injection to rats, Lot # 0294-T5, Gilead Sciences, Inc., Foster City, CA, April 4, 1994, (94-TOX-0504-007)**

Groups of male Sprague-Dawley rats (5 animals/group) received HPMPC via iv injections at dose levels of 0 (vehicle control, saline), 7 (low), 28 (mid) or 98 mg/kg/once weekly (high) or cyclic HPMPC at a dose of 98 mg/kg/once weekly for two weeks. **Results:** no treatment-related clinical signs of toxicity or clinical pathology changes were noted in any treatment group. Histomorphological findings indicated that no test article-associated nephrotoxicity or other systemic histomorphological change occurred in any group administered HPMPC or cyclic HPMPC.

**Comments:** A NOEL of 98 mg/kg/once weekly was identified for this study. Based on the body surface conversion factor, an iv dose of 14 mg/kg/once weekly cyclic HPMPC would be considered an equivalent dose in humans.

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16. Multiple-Dose Intravenous Pilot Study in Rats, 41671  
HPMPC, Lot # 26870-64 and -40085 PMEA, Lot # 26870-059,  
January, 1989,  
20971/88072)

Groups of male Sprague-Dawley (Cr1:CD (SD) BR) rats (weight: 175 - 220 g; age: 43 days, 5 animals/group) were administered drug intravenously for 14 consecutive days via the tail vein in a total volume of 1.0 ml/kg at a rate of 0.05 ml/sec either HPMPC (0.3, 1.0, 3.0, 10.0, or 50 mg/kg/day) or PMEA (1.0, 10.0 or 50 mg/kg/day) in 0.9% sodium chloride. Following treatment, rats were observed for 10 days. Control groups received 0.9% sodium chloride for the injection under identical experimental conditions. The objective of the study was to investigate the toxicity of HPMPC and PMEA treatment for 14 days and to select appropriate dose levels for a one-month toxicity study in this species. Results: with HPMPC, doses of 10 or 50 mg/kg/day resulted in a significant depression of body weight gain. Overt toxicity consisting of hunched body posture, dehydration and emaciation that occurred during the 2nd week of the drug treatment at 50 mg/kg/day. Nephrotoxicity characterized by tubular nephrosis was observed at doses of 3 mg/kg/day or more and severity was dose-dependent. Elevations in BUN, creatinine and AST and a decrease in bone marrow cellularity were apparent at 10 and 50 mg/kg/day. Additional findings noted at 50 mg/kg/day consisted of decreases in hematocrit, total leukocyte count, percent and absolute lymphocyte and reticulocyte value and lymphoid depletion. No evidence of toxicity was detected at 0.3 or 1.0 mg/kg/day. With PMEA, no overt toxicity was observed at doses up to 50 mg/kg/day.

Comments: For both the isomers, a dose of 1 mg/kg/day for 10 days may be considered a NOAEL. With a body conversion based on body surface for humans, an equivalent dose will be 0.14 mg/kg/day.

17. Four week repeated dose toxicity study of HPMPC and cHPMPC administered via iv or sc to rats, Lot # 0294-T5, Gilead Sciences, Inc., Foster City, CA, June 23, 1994, (94-TOX-0504-008)

Groups of male Sprague-Dawley rats (4 animals/group) received once weekly iv injection of HPMPC at dose levels of 0 (vehicle control, saline), 7 (low), 28 (mid) or 98 mg/kg/once weekly (high) or once weekly sc injection at a 28 mg/kg/once weekly for 4 consecutive weeks. An additional group received iv injections of cyclic HPMPC at a dose of 98 mg/kg/once weekly for 4 weeks. Results: no treatment-related clinical signs of toxicity were observed. ALT and AST values were slightly elevated at the HPMPC sc 28 mg/kg/once weekly dosage.

Comments: For cyclic HPMPC, a NOEL of 98 mg/kg/once weekly was identified in this study. Based on the body surface conversion

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factor, an iv dose of 14 mg/kg/once weekly of cyclic HPMPC would be considered an equivalent dose in humans.

18. One-Month Intravenous Toxicity Study in Rats, 41671  
HPMPC, Identification # 27337-10,  
January 11, 1990, ( -21461/1 L-124004)\*

Three groups of male and female rats (CrI:CD BR) (weight: 227 - 279 g males and 159 - 195 g females; age: eight weeks; 10 animals/sex/group) were administered HPMPC by intravenous injection (via lateral tail vein) 0.3, 1.0 and 5.0 mg/kg/day in sterile saline (0.2 ml/100 g) for a period of 29 - 30 consecutive days. A control group received sterile saline under similar experimental conditions. This study was designed to investigate the toxicological potential of HPMPC when administered intravenously to the rats for a period of one month. Results: four of ten high-dose males died or were sacrificed in extremis during the 3rd or 4th week of the treatment. Overt toxicity including tremors, rough hair coat, hypothermia and respiratory distress were noted in the high-dose group males during the 3rd and 4th week of treatment. In both the males and females in high-dose group, mean body weight, body weight gains and food intakes were decreased significantly. Clinicopathologic changes in the high-dose group consisted of reductions in red cell count, hemoglobin, hematocrit, reticulocyte and lymphocyte counts, increase in serum urea nitrogen and creatinine, and decreases in total protein and albumin levels. Glucose and elevated protein in the urine were also observed. Pale kidneys, increased kidney weights and decreased thymic weight were noted at the necropsy in the high-dose group. Nephropathy with significant tubular necrosis of renal tubules was detected microscopically and was moderate at the high-dose in both male and female monkeys, and minimal at the intermediate dose where only the males were affected. In addition, lymphoid atrophy was observed in the spleen, thymus and mesenteric lymph node, and bone marrow hyperplasia was present in the high-dose males. Mean prostate/seminal vesicle weight in the high dose males was lower (absolute weight  $p < 0.01$ ) than the control. Relative brain, heart, liver and adrenal gland weight in the high-dose males and females were significantly higher ( $p < 0.01$ ) than in the control. However, abnormal histopathological findings were not observed in these organs. Conversely, upon microscopic examination, compound-related lesions were found in the kidney, spleen, thymus, bone marrow and mesenteric lymph node in the males of the high-dose group. As to the clinical chemistry parameters, high mean AST and cholesterol levels in the high-dose group males were apparently treatment-related effects. No significant toxicity was observed at a daily dose of 0.3 mg/kg.

Comments: As evident from the macroscopic and microscopic examinations, the kidney is the major organ affected by

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treatment with the drug. A dose level of 0.3 mg/kg may be considered the NOAEL for this study. With a dose conversion based on body surface area, the equivalent intravenous dose for humans would be 0.04 mg/kg/day. Treatment-related minimal tubular necrosis was seen in the males; the equivalent human dose at which these lesions may be seen is 0.14 mg/kg/day for kidney. Atrophy of the seminal vesicle was observed histologically. In addition, lymphoid atrophy was observed in the spleen, thymus and mesenteric lymph nodes; hypoplasia was present in the bone marrow. Although, relative weight increases in brain, heart, liver and adrenal were not accompanied by abnormal histopathological findings; significant increases in AST and cholesterol levels suggest that liver may be a possible site of toxicity for the compound. Therefore, seminal vesicles, thymus, bone marrow, spleen and liver may be possible sites of toxicity in humans.

19. Thirteen week repeated dose toxicity study of HPMPC administered iv to rats, Lot # 504B92-01, April 1, 1994, (Study No. 2-P50/ -P50-93-230)\*

Four groups of male and female rats (strain: Tac:N(SD)fBR; age: 3-5 weeks; 10-26 animals/sex/group) were administered HPMPC by intravenous injection at dose levels of 0 (vehicle control), 3 (low), 15 (mid) or 60 mg/kg once per week (high) for a period of 13 weeks. In control and high dose groups, 10 animals/sex were designated as recovery animals which were scheduled to receive 13 weeks of the test article administration followed by a 4-week non-treatment recovery period. Results: 2/16 females by week 12 (mid), and 21/26 males and 15/20 females by week 9 (high) died or euthanized in extremis (severe tail ulceration). The animals (died or euthanized) revealed histomorphologic changes in the kidney (minimal to mild tubular cell hypertrophy and degeneration of renal tubules in males and minimal, multifocal degeneration of tubular epithelial cells and segmental tubular collapse in females), bone marrow (minimal to mild depletion of hematopoietic cells in the epiphyseal region in both males and females) and administration site (severe, focally diffuse, exudative dermatitis with epidermal effacement and an array of morphologic changes of the epidermal cells in both sexes). Clinical Observations: lesions of the tail ranging from erythema to ulceration to missing anatomy were seen at all dose levels in both sexes. Erythema, swelling, sloughing, necrosis, scabbing and/or ulceration of the skin covering the tail of a number of animals (mid and high) were seen. Body Weight and Food Consumption: there was an obvious depression in weight gain in males (15%) and females (21%) by week 4 and 45% and 27% by week 8 in males and females, respectively (high). The body weight gain (mid) was depressed (8% in the males and 5% in the females) during the first weeks of treatment as compared to respective

control animals. During the recovery period, some compensatory weight gain was noted; the males and females still gained less weight (13% and 11%, respectively) than the controls. Statistical evaluation of mean food consumption values revealed a significant depression in animals (high) as compared to the controls.

Hematology: a significant increase in platelet count on day 31 in males (high); significant decreases in erythrocyte, hematocrit and/or hemoglobin values in male and/or female (high) on days 31, 38, 58, and 86 (recovery); increases in the mean cell volume values in males (high) on day 58; significant increases in the mean cell hemoglobin values in males (low, mid and high) on days 38, 58 and 86; and significant increases in mean cell hemoglobin concentration values in males (low, mid and high) on day 86 were observed. The absolute and percent polymorphonuclear leukocyte values were significantly decreased in the mid and high dose females on day 38 and in the high dose males on day 58. The percent lymphocyte value was increased in the mid and high doses females on day 38. Clinical Chemistry: statistical significant changes were noted in the males included increases in albumin/globin ratio (low and mid); chloride values (mid and high); creatinine, phosphorus and sodium values (high). Significant decreases were noted for the following parameters: triglyceride value in males (high); ALP value in males (mid and high); ALT value in females (low, mid and high); and bilirubin values in both sexes (mid and high). Organ Weights: significant decreases in absolute spleen weight in male (mid) and female (high) were noted. The adrenal, liver and kidney-to-final body weight values in females (high) were significantly increased compared to the controls. Following the recovery period, male mean and absolute and relative testes weights were significantly decreased (high), mean relative kidney and adrenal weights were significantly decreased (male, high), and relative heart weight was significantly increased (female, high). Toxicokinetics: the pharmacokinetic parameters are summarized in Table 2. There were no obvious trends in the pharmacokinetic parameters over the course of the study at any of the dose levels tested. AUC values following the first dose indicated apparent dose proportionality.

Table 2

Non-compartmental Pharmacokinetic Parameters Following Once a Week Administration of Cidofovir at 3, 15 and 60 mg/kg/week

Dose mg/kg/day	PK parameters	Sample Periods (Weeks)			
		1	4	8	12
3	AUC <sub>0-∞</sub> (μg*hr/ml)	3.27	3.69	3.4	4.86
	CL <sub>TOT</sub> (l/hr/kg)	0.92	0.81	0.88	0.62
	Vdss (l/kg)	28.63	5.83	4.43	11.62
	T <sub>1/2</sub> (hr)	31.2	12.1	8.9	27.7
	Cmax (μg/ml)	0.46	0.76	0.79	0.87
	AUC/Dose	1.11	1.23	1.13	1.62
	15	AUC <sub>0-∞</sub> (μg*hr/ml)	12.66	14.62	20.85
CL <sub>TOT</sub> (l/hr/kg)		1.19	1.03	0.72	1.17
Vdss (l/kg)		2.33	2.65	3.18	6.83
T <sub>1/2</sub> (hr)		5.16	11.15	12.21	11.16
Cmax (μg/ml)		2.7	3.2	5.5	2.6
AUC/Dose		0.84	0.97	1.39	0.84
60		AUC <sub>0-∞</sub> (μg*hr/ml)	46.38	40.59	51.69
	CL <sub>TOT</sub> (l/hr/kg)	1.29	1.48	1.16	0.93
	Vdss (l/kg)	1.26	1.97	1.29	0.71
	T <sub>1/2</sub> (hr)	3.6	3.3	3.5	4.1
	Cmax (μg/ml)	12.7	9.22	14.64	na
	AUC/Dose	0.77	0.68	0.86	1.07

**Microscopic Examination:** revealed changes similar to those seen in the interim sacrifice (kidneys, bone marrow and administration sites) with the addition of degeneration of testicular seminiferous tubules and hypospermia in the males. By the end of the recovery period, the lesions (high) were less severe than noted following the treatment; however, the lesions were still present in the bone marrow, administration site, testes/epididymides and kidneys.

**Comments:** As evident from the macroscopic and microscopic examinations, the kidney, hematopoietic system, administration site and testes are the major organs affected by treatment with the drug. A dose level of 3 mg/kg may be considered the NOAEL for this study. With a dose conversion based on body surface area, the equivalent intravenous dose for humans would be 0.43

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mg/kg/day.

Amendment to Study No. 2-P50

P50-93-230, April 1, 1994.

Specific notations were made for the two animals which developed mammary adenocarcinomas during the iv treatment of HPMPC. The results are summarized in Table 3.

Table 3

Development of mammary adenocarcinomas in rats during the 13-week iv treatment of HPMPC

Group	Dose (mg/kg/day)	Animal No.	Number of Masses
Mid	15	115	1
High	60	142	3

Comments: Previously, the sponsor has made an attempt to demonstrate sc route specific tissue distribution and alteration in expected pharmacokinetic profiles of HPMPC in Sprague-Dawley rats. The sponsor contended that specific higher HPMPC concentrations in the axillary/thoracic mammary tissue via the sc route led to the formation of adenocarcinomas in rats (Toxicology study). The data submitted at that time did not conclusively support the hypothesis. The pathology report of the 13-week iv treatment of HPMPC in rats, however, demonstrated that the test compound induced the formation of mammary adenocarcinomas via the iv route also. The test compound, therefore, appeared to be a very potent carcinogen. The pathology report further supported the assessment.

20. A 6-month chronic toxicity study of HPMPC following once weekly iv administration in rats, lot # 504-B93-01

September 12, 1995, (94-TOX-0504-016)\*

Groups of Sprague-Dawley male and female rats (20 animals/sex/group) were administered HPMPC intravenously at dose levels of 0 (vehicle control), 0.6 (low), 3.0 (mid) or 15.0 mg/kg once weekly (high) for a period of 6 months. An additional (10/sex) rats were assigned to the control and high dose groups and were retained untreated for a 4-week recovery period following the termination of treatment. A further cohort of rats (14/sex/group) were included for the toxicokinetic evaluation. Results: during the treatment period, 4 deaths occurred (2 low dose females, 1 mid dose male and 1 high dose female) due to blood collection procedures. One (low) male was found dead during week 23. The cause of death is unknown. Two males (one mid dose with lymphosarcoma during week 23 and one high dose with severe

discharge from a head mass during week 26) were sacrificed in extremis. Clinical Signs: masses were first detected at week 12 and persisted throughout the treatment and recovery phase. Total incidence (palpable masses only) for all animals was (control to high, respectively): 1/30, 1/20, 1/20 and 21/44 females, and 0/30, 0/34, 0/34 and 21/44 males. Multiple masses (2/5) were present in 10/44 high dose females. Histopathologically, degeneration/atrophy of the tubular epithelium, epididymal oligo/aspermia, cardiomyopathy, congestion and/or edema of the lungs, atrophy of the optic nerve and hemorrhage of the thymus and mediastinal lymph node were recorded. In addition, cutaneous lesions not associated with the masses were noticed on the cervical, scapular and thoracic regions in males and females from all groups: 6/60 (control), 2/54 (low), 1/54 (mid) and 2/88 (high) beginning week 3 of treatment. The lesions were treated with sterile water and Proviiodine. The etiology of these lesions remains unknown. Body Weights and Food Consumption: body weight gains were lower ( $p=0.05$ ) in males and females (high) and were considered to be HPMPC-related. Statistically significantly lower weights (high) were recorded in males during the recovery phase. Statistically significant lower food consumption recorded in males (low and high) throughout the study and statistically significant higher food consumption in males (mid) at week 13 when compared to the controls. Hematology: test article-associated changes in the high dose included reduced RBC, hematocrit and hemoglobin; increased WBC, lymphocytes and neutrophils. Segmented neutrophils were higher in females (high) during the recovery period. Examination of the individual values generally showed that leukocytosis correlated with the presence of neoplasms. Clinical Biochemistry: statistically significantly higher levels of creatinine, glucose, calcium, cholesterol, chloride and lower levels of AST, total bilirubin, ALP, LDH and potassium were observed in males and females (high) when compared to controls. Many of these changes were also observed at lower dose levels. Only higher creatinine and chloride levels and lower LDH levels were significant during the recovery phase (high) animals. Organ Weights: testes weights (high) were statistically lower when compared to the controls (absolute, relative to body weight and brain weight) during the main study and recovery phase. Toxicokinetics: repeated administration of HPMPC led to apparent increase in  $AUC_{0-6}$  values from week 1 to 26. These changes were greater at high dose level (2.6-fold increase for males vs 1.9-fold increase at the mid dose level). The data suggested that repeated exposure to HPMPC led to a decrease in renal clearance of HPMPC. The changes were greater for males than females. The half-life of HPMPC (high) was not affected by the repeated exposure. Gross and Histopathological Examinations: subcutaneous masses (Table 4) were found in females (high) beginning week 12 and located primarily in areas containing mammary gland tissue (cervical/axillary and inguinal areas). Additional subcutaneous masses were present on the head near the ear canal and more

frequently in males than females. By the end of the treatment period (week 27), total mass incidence observed at necropsy, in main study, toxicokinetics and recovery animals (control to high), respectively, was 1/30 (3%), 1/20 (5%), 1/20 (5%) and 23/37 (62%) females, and 0/30 (0%), 0/20 (0%), 0/20 (0%) and 10/32 (31%) males. Non-neoplastic lesions attributable to HPMPC were present in the kidneys (karyomegaly), testes (degeneration/atrophy), epididymides (oligo-/aspermia) and uterus (glandular epithelial hyperplasia). Dose-related kidney changes were found in males and females (only high). Compared to the severity of these kidneys lesions in males (high), renal changes were less severe in females (high) or absent in females in the recovery cohort, suggesting lesion reversibility.

**Table 4**  
Incidence of selected neoplastic findings in high dose-animals

Organ	Lesion	Main study		Recovery		Toxicokinetics	
		♂	♀	♂	♀	♂	♀
Mammary gland	adenocarcinoma	0/20	9/20	0/10	7/10	0/2	6/7
Zymbal's gland	carcinoma	2/20	1/20	2/10	0/10	2/2	2/7
Skin	squamous cell papilloma	1/20	0/20	0/10	0/10	0/2	0/7

**Comments:** HPMPC is a potent carcinogen. Non-neoplastic lesions attributable to the test articles were present in kidneys, testes, epididymis and uterus. The kidney and uterine tissue lesions appeared reversible based on the lower severity and/or incidence in high dose recovery cohorts. A NOAEL could not be determined in this study. Based on the results from this study, the NOAEL for kidney lesions was 3 mg/kg/week for females and less than 0.6 mg/kg/week for males. The NOAEL for testicular lesions was 3 mg/kg/week. Finally, a NOAEL for neoplastic lesions was 3 mg/kg/week in male and female rats. With a dose conversion based on body surface area, the equivalent intravenous dose for humans would be 0.43 mg/kg/day.

21. A 6-month chronic toxicity study of HPMPC administered sc to rats, Lot # 504K93-01.  
September 15, 1995, (94-TOX-0504-003\2-T70)\*

Four groups of male and female rats (20-30 animals/sex/group) were administered HPMPC (solution in Sterile Water for Injection, USP, 10 ml/kg) via once weekly subcutaneous injection at dose levels of 0 (vehicle control), 0.6 (low), 3 (mid) or 15 mg/kg (high) for a period of 26 weeks. The injections were being

rotated among four separate administration sites in the scapular region. Due to the presence of tissue masses, all main study animals, toxicokinetic animals and animals designated for the recovery phase were sacrificed after receiving 19 of 26 scheduled weekly injections. Results: one (high) male was found dead on day 29 and one (mid) female was euthanized on day 100 in a moribund condition due to tumor growth. Terminal body weights were reduced in high dose males only. Absolute liver, kidney and testes weights were decreased in high dose males. Treatment-related changes in hematologic parameters were limited to decreased MCHC in the low, mid and high dose groups. Treatment-associated clinical chemistry changes consisted of increased creatinine, calcium and ALT values in high dose males. Toxicokinetics: these data showed that repeated sc administration of HPMPC in rats led to a decreased absorption of the drug (Table 5). The rate of absorption after 5 doses (high) was approximately 4-fold slower than observed previously for a single 3 mg/kg dose.

**Table 5**  
 Non-compartmental Pharmacokinetic Parameters Following Once a Week Administration of Cidofovir at 3, 15 and 60 mg/kg/week

Dose mg/kg/day	PK Parameters	Sample Periods (Weeks)		
		1	5	14
0.6	AUC <sub>0-∞</sub> (µg*hr/ml)	0.14	0.49	0.55
	T <sub>max</sub> (hr)	1	1	1
	C <sub>max</sub> (µg/ml)	0.28	0.30	0.44
3	AUC <sub>0-∞</sub> (µg*hr/ml)	1.31	4.11	3.18
	T <sub>max</sub> (hr)	1	1	1
	C <sub>max</sub> (µg/ml)	1.06	1.72	1.94
15	AUC <sub>0-∞</sub> (µg*hr/ml)	8.83	7.32	8.71
	T <sub>h</sub> (hr)	1	2	2
	C <sub>max</sub> (µg/ml)	6.7	2.38	4.6

Non-neoplastic histopathologic changes were observed in the skin administration sites and bone marrow. A minimal severity zonal bone marrow hypoplasia was present only in high dose males and females. An analysis of the cell types in the bone marrow suggested that it was a stem cell effect since both erythroid and myeloid cell elements were minimally affected. No treatment-related histopathologic changes were observed in kidneys, liver or testes. Neoplastic changes: several animals exhibited masses that were anatomically located on the neck, chest, axillary region, abdomen, administration site and face. Masses were not

observed in the control animals. There was a dose-related increase in the number of masses noted in females. The time of first observation of a mass was day 43 (mid) in a female (Table 6). The incidence of mammary adenocarcinoma was as follows: 0/30 (control), 4/20 (low), 7/20 (mid) and 12/30 (high). Serum prolactin levels did not correlate with tumor incidence. Of the twelve (high) females with adenocarcinomas, 5 were multiple tumors classified histologically as mammary adenocarcinomas. There was no relationship between the dose administered and the size of the masses. A number of the masses located in the axillary and abdominal region became ulcerated. Three males (mid) each exhibited a mass on the chest, abdomen or face. There was no relationship to dose administered or onset of these findings.

Table 6

Following the HPMPC subcutaneous administration, female rats per group with mass(es) in the axilla, chest or abdomen

Week	Control (n=30)	Low (n=20)	Mid (n=20)	High (n=30)
5	0	0	0	0
6	0	0	1	0
7	0	0	1	0
8	0	0	1	0
9	0	0	3	0
10	0	1	3	2
11	0	1	3	2
12	0	1	3	4
13	0	2	4	5
14	0	3	5	6

**PATHOLOGY NARRATIVE:** Mammary glands: the majority of the tissue masses were diagnosed as mammary gland adenocarcinoma associated with an inflammatory component. These occurred only in female rats and the incidence was as follows: control=0; low=4; mid=7; and high=12. The mammary adenocarcinomas diagnosed had neoplastic epithelial cells that were arranged predominantly in an alveolar pattern. Ductal and papillary structures were also present and often in combination. Occasionally, alveoli with a single epithelial cell layer were observed; most often the epithelium was multiple-layered. These proliferating epithelial cells obliterated the original alveolar architecture, yet a glandular pattern was maintained. The neoplastic cells were composed of

scant eosinophilic cytoplasm that was vacuolated to varying degrees. Epithelial cells in single and/or double layers tended to be elongated or rectangular compared to cells proliferating in alveoli-forming nests where cells were more rounded with less cytoplasm. Nucleoli were prominent in some cells but barely visible in others. In some tumors there was a heavy population of large vacuolated cells usually located near the basilar border of proliferating cells. These large vacuolated cells were variable in morphology; the majority were elongated and resembled myoepithelial cells. This myoepithelial component was present in all tumors. There were bands of fibrous connective tissues transversing the tumor and were more prominent in larger tumors. Mononuclear inflammatory cells were prominent in this connective tissue. Polymorphonuclear cells were also present, particularly near the surface when ulcerative skin lesions were present. The tumor masses were generally circumscribed lesions expanding into adjacent fat, but infiltration into underlying muscle was observed in several cases. Axillary lymph nodes: there was no evidence of metastasis in the lymph nodes. There was some variation in size and some lymph nodes were considered more "active" than others. Lymph nodes diagnoses as active appeared to have germinal centers that were slightly more prominent than seen in the majority of controls. Additional gross lesions: the facial masses in a male and female (mid) were diagnosed as Zymbal's gland adenoma and carcinoma, respectively. The facial mass in a male (high) was diagnosed as a chronic inflammatory focus. The mass attached to the salivary gland in a male (high) was diagnosed as anaplastic carcinoma. The skin lesions (mid, 2 females) were diagnosed as acute inflammation in the skin and ulcerate dermatitis.

**Comments:** Based on these data, the sc administration of HPMPC to rats resulted in a dose-related increase in mammary adenocarcinomas in females rats. With regard to the pathology report, only gross lesions have been evaluated histopathologically. Secondly, no information is available concerning pre-neoplastic lesions (hyperplasia, inflammation, etc.) in non-tumor mammary glands. Finally, since the mammary tumors were associated with an inflammatory component, the drug-induced inflammation may have contributed to mammary tumor formation in the study.

was consulted to review the histopathology slides of cervical-thoracic masses and facial masses in rats (2-T-70 Toxicity Study). According to his analysis, the vast majority of tumors were mammary adenocarcinomas with varying degrees of tissue invasion. A few tumors were from Zymbal's glands (auditory sebaceous glands) and possibly preputial/clitoral glands. He commented that the test article should be considered a potent carcinogen. A NOAEL could not be identified in this study.

22. Multiple-Dose Subcutaneous Nephrotoxicity Screening Study in Male Guinea Pigs, -41671 HPMPC, Lot # 27411-48-1 and Probenecid Lot # 026-0293, January 21, 1991, (21634/90047)

Four groups of normal male guinea pigs (Hartley) (weight: 294 - 345 g; age: 5 weeks; 3 animals/group) were administered HPMPC at 2.5, 5, 10 or 25 mg/kg/day. In addition, HPMPC at 5 mg/kg/day with 15 mg/kg/day probenecid injected subcutaneously into the dorsal median posterior lumbar area in sterile saline (<4 ml/kg) for five days. A control group received sterile saline under similar experimental conditions. The purpose of this study was to evaluate the nephrotoxicity of HPMPC in the guinea pigs, and to determine if multiple doses (5 days) would induce moderate to severe nephrotoxicity in this species. Results: HPMPC induced a nephrosis in guinea pigs similar to that observed in other species and this toxicity appeared to be dose-limiting. - Microscopically, nephrosis was moderate at 2.5 mg/kg/day and severe at doses of 5 mg/kg/day or more. Due to the overwhelming nephrotoxicity induced by a 5 mg/kg/day dose, it was not possible to evaluate the potential amelioration of this toxicity by probenecid. Based on the results of this study, the sponsor has chosen the dose of 2.5 mg/kg/day to evaluate with multiple dose levels of probenecid.

Comments: Guinea pigs is the most sensitive species known to the nephrotoxicity of HPMPC. The sponsor has chosen a dose level of 2.5 mg/kg/day to study the nephrotoxicity. With a conversion factor based on the body surface area, an equivalent dose in humans would be 0.42 mg/kg/day.

23. Multiple-Dose Subcutaneous Nephrotoxicity Study in Male Guinea Pigs, 41671 HPMPC, Lot # 28648-7, - , November 16, 1990, (21693/90077)

Four groups of normal male guinea pigs (Hartley) (weight: 302 - 375 g; age: unknown; 5 animals/group) were administered HPMPC subcutaneously in sterile saline (<5 ml/kg) into the dorsal median posterior lumbar area. Dosing regimens were (1) single 25 mg/kg dose administered on day 1 with sacrifices on day 8 and day 15; (2) single doses of 12.5 mg/kg administered on day 1 and day 4 with a sacrifice on day 8; (3) single doses of 6.25 mg/kg on day 1, 4, 8 and 12 with a sacrifice on day 15 and (4) daily doses of 5 mg/kg for 5 consecutive days with a sacrifice on day 8. A fifth and a sixth control groups received sterile saline under similar experimental conditions. The purpose of this study was to investigate different dosing regimens on the nephrotoxicity induced by HPMPC in the guinea pig. Results: the regimen employed had a marked effect on the severity of the nephrotoxicity induced by the subcutaneous administration of a total dose of 25 mg/kg of HPMPC to guinea pigs. For example, 25 mg/kg as a single dose

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induced minimal nephrotoxicity and 25 mg/kg subdivided into equal fractions and given in 2, 4 or 5 doses induced greater nephrotoxicity, the severity of which, in general, was directly proportional to the number of doses.

**Comments:** Clearly, the results suggested that HPMP-*C*-induced nephrotoxicity was regimen dependent. In other words, the toxicity depended on the duration of the drug exposure (AUC) rather than on the drug intensity ( $C_{max}$ ).

**24. Intravenous study in rabbits treated with HPMP-*C* with and without probenecid, Lot # 504A92-01,**  
November 13, 1992, 453-GS-  
001-92/T0504-00015)

Groups of male New Zealand White rabbits (5 animals/group) were administered HPMP-*C* via the iv route at dose levels of 0-(vehicle control, I), 25 mg/kg (II), 25 mg/kg + 3 ml/kg probenecid via oral gavage once daily (III) or 25 mg/kg + 3 ml/kg probenecid via oral gavage tid (IV) for 5 days to evaluate the local effects of iv injections. **Results:** no clinical signs were observed in any animal in any dose group during the study. A statistically significant increase in CK and chloride (IV), and a statistically significant decrease was observed in lymphocytes (III and IV). Terminal necropsy revealed mottled kidneys in one animal (I), four animals (II and III) and in all animals (IV). **Histopathology:** treatment-related changes of the kidneys and liver could not be determined because of a larger number of lesions in the kidneys and livers which were compatible with a concurrent protozoan disease, Encephalitozoon cuniculi.

**Comments:** Because of the histopathological discrepancies, this is not a valid study. The sponsor was requested to repeat the study.

**25. Final Report: Five day repeated dose toxicity study of HPMP-*C* administered iv with and without orally administered probenecid in male rabbits, Lot # 504A92-01,**  
September 21, 1995, ( 453-GS-  
001-92a/T0504-00058)

Groups of male New Zealand White rabbits (5 animals/group) were administered HPMP-*C* via the iv route at dose levels of 0 (vehicle control, I), 25 mg/kg (II), 25 mg/kg + 150 mg/kg probenecid via oral gavage once daily (III) or 25 mg/kg + 50 mg/kg probenecid via oral gavage tid (IV) for 5 days to evaluate the local effects of iv injections. **Results:** no clinical signs were observed in any animal in any dose group during the study. A minor but statistically significant increase in chloride and a decrease in urea nitrogen was observed in II and IV, respectively when compared to the control. Terminal necropsy revealed pale kidneys in all 5 animals (II) and (IV) and 4 animals in (III).

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Histopathology: treatment-related changes were present in the kidneys of 3 rabbits (II), 3 rabbits (III) and in all five rabbits (IV). The kidney changes were characterized by a minimal slight tubular degeneration (nephrosis) and minimal to moderate tubular dilatation. In one rabbit (IV), a focal area of heterophils (polymorphonuclear leukocytes) was present.

Comments: The study data indicated that no significant differences exist between the three groups of HPMPC treated rabbits (with or without probenecid) with respect to the type of kidney lesions or the severity of these lesions.

**26. Multiple-Dose Intravenous Nephrotoxicity Screening Study with Probenecid, Lot # 26F-0293 and -41671 HPMPC, Lot # 27625-50-1 in Male Rabbits, September 21, 1990, -21596/90001)**

Four groups of normal male New Zealand White rabbits (weight: 2.9 - 3.5 kg; age: unknown; 3 animals/group) were administered HPMPC by intravenous injection at a rate of 0.1 ml/sec (via the marginal ear vein) with 25 mg/kg/day in sterile saline (<2 ml/100 g) and total daily probenecid doses of 0, 25, 50 or 150 mg/kg/day for five days. These dose levels provided for probenecid: HPMPC dose ratios of 0:1, 1:1, 2:1 and 6:1, respectively. A fifth group of animals received only probenecid at the highest dose of 150 mg/kg/day and a sixth control group received sterile saline under similar experimental conditions. The intent of this study was to determine whether daily probenecid treatment would ameliorate nephrotoxicity resulting from the five daily doses of HPMPC in rabbits. Results: HPMPC (25 mg/kg/day)-induced nephrotoxicity under a five day regimen in the rabbit was completely prevented by probenecid when co-administered with the test compound at a ratio of 6:1, and partially ameliorated at a 2:1 ratio. But; the percent urinary recovery of HPMPC were decreased with the co-administration. The mean percent HPMPC decreased from 71.5% to 9.8% as the dose of probenecid increased from 25 - 150 mg/kg.

Comments: Percent urinary recovery of HPMPC were decreased with increasing doses of probenecid; thus, this may suggest that probenecid inhibits the renal transport and urinary excretion of HPMPC. Further, an accumulation of HPMPC can occur with the co-administration since HPMPC is primarily excreted unchanged in the urine after an IV injection.

**27. Multiple-Dose Intravenous Nephrotoxicity Screening Study in Male Rabbits, 41671 HPMPC, Lot # 27625-50-1 and 27411-48-1, July 23, 1990, 21497/89092)\***

Four groups of normal male New Zealand White rabbits (weight: 3 - 3.7 kg; age: unknown; 2 animals/group) were administered HPMPC by

intravenous injection at a rate of 0.1 ml/sec (via the marginal ear vein) 10, 25, 50 and 100 mg/kg/day in sterile saline (<2 ml/100 g) for a period of 5 consecutive days. A control group received sterile saline under similar experimental conditions. The intent of this study was to investigate the multiple dose nephrotoxicity to rabbits and to establish a dose regimen for further screening studies. Both the animals receiving HPMPC at 100 mg/kg/day were sacrificed on day 5 of the study due to decreased food intake and laboratory test results indicative of nephrotoxicity. All other animals were sacrificed on study day 8. Results: food consumption was markedly reduced in animals treated with HPMPC at 25, 50 and 100 mg/kg/day. No other drug-related clinical signs of toxicity or changes in body weight occurred. The group mean serum urea nitrogen and creatinine values were slightly increased on day 5 for the group given 100 and on day 8 for group given 50 mg/kg/day of the test compound. In conclusion, rabbits given HPMPC intravenously at dosages of 10 mg/kg/day or more for 5 days developed a dose-related nephrotoxicity characterized microscopically by degenerative, necrotic, atrophic and/or regenerative changes in the proximal convoluted tubules of the kidneys.

Comments: A NOAEL could not be determined in this study.

28. Multiple-Dose Intravenous Pilot Study in Monkeys, -41671  
HPMPC, Lot # 26870-64 and 40085 PMEA, Lot # 26870-059,  
January, 1989,  
20954/88064)

Groups of male and female cynomolgus monkeys (weight: 2.4 - 4.9 kg; age: unknown, 1 animal/group) were administered either HPMPC (0.1, 1.0, 10.0 or 50 mg/kg/day) or PMEA (1.0, 5.0, 10.0, 25.0 or 50 mg/kg/day) in 0.9% sodium chloride intravenously for 14 consecutive days via a saphenous vein in a total volume of <5.0 ml/kg at a rate of 0.1 ml/sec. Control groups received 0.9% sodium chloride for injection under identical experimental conditions. The objective of the study was to investigate the toxicity of HPMPC and PMEA and to select appropriate dose levels for the subacute IV toxicological evaluation to support an initial clinical trial in man. Results: with HPMPC, significant nephrotoxicity (acute tubular necrosis) occurred at doses of 1.0 mg/kg/day or more. Ten or 50 mg/kg/day was lethal within 7 - 9 days and nephrotoxicity was considered to be the major contributing factor. Overt toxicity and death occurred after doses of 10 mg/kg/day or more. At the 10 and 50 mg/kg/day dose levels, decreased activity was apparent in both monkeys on the 7th day of treatment. The 50 mg/kg/day monkey was found dead the following morning while the 10 mg/kg/day monkey died after the 9th dose. Severe nephrotoxicity characterized morphologically by acute tubular necrosis was detected in both the monkeys. Significant elevations in creatinine and BUN were also detected

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prior to death. Other drug-related findings consisted of necrosis of mucosal crypt cells in the colon and/or duodenum at 10 and 50 mg/kg/day, pulmonary congestion along with intra-alveolar hemorrhage and edema, depletion of lymphocytes in lymphoid tissues, lymphopenia, and degeneration and necrosis of gastric mucosa of the stomach at 50 mg/kg/day. With PMEA, no drug-related toxicity at 1 mg/kg/day and slight swelling of hepatocytes at 5 mg/kg/day were observed in animals. At 10 mg/kg/day, no clinicopathologic evidence of toxicity was detected after 14 days of treatment. At 25 mg/kg/day beginning on 10 day of treatment, animals developed significant skin lesions. At 50 mg/kg/day, the animals developed significant epidermal lesions consistent with toxic epidermal necrolysis requiring an early sacrifice after 12 days of dosing. Lymphoid depletion and slight testicular degeneration were observed at this dose level. Marked inappetence and nephrotoxicity (vacuolar degeneration and dilatation of tubules) were also noted.

**Comments:** Based upon the results of these range finding studies, a dose of 1.0 mg/kg/day may be considered the NOEL for PMEA. Based upon the observation of toxicities at 25 and 50 mg/kg/day, a high dose between 10 and 25 mg/kg/day PMEA dose levels should be selected for subchronic nonclinical safety studies in monkeys.

**29. Repeated Dose Oral Toxicity Study and Pharmacokinetics of HPMPC Administered via Gavage to Cynomolgus Monkeys, Lot # 504A92-01, April 22, 1992 (2-J57. -J57-92-77)**

Four groups of male and female cynomolgus monkeys (weight: 3-4 kg; 2 animals/sex/group) were administered HPMPC via oral gavage at 0, 1, 5 and 25 mg/kg twice-a-week for two weeks. The objective of the study was to determine the potential toxicity of the drug. Results: one female (25 mg/kg) was found dead on the morning of day 15. Beginning on day 12, the animal was noted as pale, hypothermic, anorexic, dyspneic and had a watery diarrhea. There was an increased incidence of diarrhea, scant feces, and/or no feces in other animals of the same group. In summary, the oral administration of a 5-25 mg/kg dose of HPMPC over a two-week period to male and female monkeys was responsible for a number of changes. In particular, at 25 mg/kg/day, depressed body weight gains, serum chemistry changes indicative of renal and hepatic alterations as well as changes in serum electrolyte values, enlarged kidney weights, gross and microscopic evidence of tissue alterations involving the large intestines, small intestines and kidney were seen. At the dose level of 5 mg/kg/day, histopathological changes were in the rectum, cecum, colon and jejunum in both males and females.

**Comments:** A dose of 1 mg/kg/day may be considered a NOAEL. Based on the body surface area factor, an equivalent dose in humans

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would be 0.33 mg/kg twice a week for a 60 kg person.

30. Thirty day repeated dose sc toxicity study and pharmacokinetics of HPMPC administered to cynomolgus monkeys, Lot # 67-049-DK, November 9, 1994, (2-N13, 13-93-43)

Groups of male and female cynomolgus monkeys (weight: 2.9-3.4 kg; 3 animals/sex/group) were administered HPMPC via subcutaneous injection at dose levels of 0 (vehicle control), 0.5 (low), 1.5 (mid) or 7.5 mg/kg (high) twice per week (total of 9 injections) for a period of 4 weeks. Clinical Observations: an increased incidence of soft, white or mucoid feces were observed in males (mid). There was an increased incidence of erythema at the injection site (males, high) and one male (high) displayed indication of compromised health such as abnormal feed consumption, pale mucous membranes, dehydration and emaciation. In the females, some animals from each group including the controls displayed erythema at the injection site and a dose-related increase in the incidence of soft, mucoid and/or colored feces (low, mid and high). Body Weight and Food Consumption: mean body weight change values revealed that males and females (high) lost 0.4-0.5 kg mean body weight. The high dose group animals appeared to be less consistent in food consumption as compared to the other groups of animals. Clinical Biochemistry: there was a general depression in the red blood cell mass (erythrocyte count, hemoglobin and hematocrit values) in the males (statistically significant) and females (no statistical significance) on day 30. On day 14, mean clinical chemistry values in the males revealed a significant depression in phosphorus value (high) and significant elevations (high) in creatinine and chloride values. On day 30, there was a significant elevation in creatine and AST values and significant depression in the mean phosphorus value (males, high). In the females, the mean phosphorus value was significantly elevated and the mean chloride value was depressed (high) compared to the controls. Organ Weights: statistical evaluation of mean organ weight data revealed significantly elevated absolute and relative kidney weights and liver to final body weight values (females, high). Gross Pathology: at necropsy, there was a greater incidence of pale kidneys (males and females; mid and high) and a increased incidence of yellow/pale foci in the epidermis (injection site) of males (high). Histopathologic observations - Kidney: drug-related alterations of the renal cortex, in the form of a tubular nephropathy, were observed in males and females (mid and high). The nephropathy was characterized by a number of alterations in proximal convoluted tubules of the cortex. Microscopic changes observed included occasional karyomegaly of tubular cells. A moderate diffuse tubular dilatation affecting nearly all tubules of the renal cortex was observed in one male (high). Glomerular changes were not seen in any of the kidneys affected with the nephropathy.

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Bone Marrow: mild to moderate depletion of erythroid cellular elements was observed in both the femur and the sternum in 2/3 males (high). Myeloid (granulocytic) elements appeared unaffected in number in all of the affected males (high). Skin. Administration Site: changes consisted of hemorrhage, epithelial necrosis, acute inflammation and chronic inflammation. Focal hemorrhage in sc tissue was observed in 1-3 monkeys/sex in all groups, except females (mid). Miscellaneous: other changes included granulomas of the colon and cecum in several monkeys.

**Comments:** The results suggested that the major target organs of HPMPC toxicity were kidney, liver, skin and bone marrow. The low dose level used in the study, 0.5 mg/kg twice per week, may be considered a NOEL. For the low dose, based on the body surface area, an equivalent dose in humans would be 0.16 mg/kg twice a week for a 60 kg person. In the clinic, HPMPC is not being administered to patients via the im route.

31. One-Month Intravenous Toxicity Study in Cynomolgus Monkeys,  
41671 HPMPC, Identification # 27337-20  
January 11, 1990, .21444/ 124003)\*

Three groups of normal male and female Cynomolgus macaque monkeys (weight: 2.9 - 3.6 kg males and 2.7 - 3.4 kg females; age: unknown; 2 animals/sex/group) were administered HPMPC by intravenous injection (via saphenous vein) 0.1, 0.25 and 1.0 mg/kg/day in sterile saline (1.0 ml/kg and a dose rate of 0.1 ml/second) for a period of 30 - 31 consecutive days. A control group received sterile saline under similar experimental conditions. This study was designed to investigate the toxicological potential of HPMPC when administered intravenously to monkeys in a one month sub-chronic toxicity study. Results: one male in the high-dose group was sacrificed in extremis during the treatment period (week 2). This animal exhibited severe signs of toxicity including hypo-activity, tremors, hypothermia, shallow and labored respiration, and decreased food consumption. One female in the high-dose group had decreased food consumption throughout the majority of the treatment period. Animals in the high-dose group lost significant weight as a result of drug treatment. The absolute and relative kidney weights were increased in the males and females of the high-dose group at the 4th week of treatment. In addition, pale kidneys at the necropsy (2 of 4) and renal tubular nephrosis (4 of 4) were observed in the high-dose group. Significant differences in serum chemistry that were treatment-related consisted of decreases in phosphorous levels and increases in creatinine levels at the high-dose. Aside from the kidney, lesions that appeared to be treatment-related in the high-dose group were found in bone marrow, lung, lymphoid tissues in males and in thymus in females. The lung lesion consisted of minimal to mild alveolar edema. Lymphoid atrophy involving the lymph node, spleen and/or thymus was noted in 3 of

4 animals at this dose.

At the week-4 evaluation, the mean glucose levels in the low- and mid-dose groups males were significantly ( $p < 0.05$ ) below the control group mean and the mean chloride level in the mid-dose group female was significantly ( $p < 0.05$ ) below the control group mean. The mean ALT levels appeared to be slightly increased in the high-dose group females. The urine of one male and one female in the high-dose group were positive (+3 and +2) for glucose. No treatment-related differences were apparent in hematologic parameters or ophthalmologic findings between the control and the treated groups. In the mid-dose group, minimal renal tubular necrosis was observed that was treatment-related. At the low-dose, no adverse effects were observed for systemic toxicity in the monkeys.

**Comments:** As evident by renal tubular nephrosis, pale kidneys at necropsy, increased kidney weights, elevated creatinine levels, lower phosphorous levels and glucose in the urine, the primary target organ is the kidney for the systemic toxicity of the test compound in the monkeys. A dose of 0.1 mg/kg/day of HPMPC may be considered the NOAEL in this study. With a dose conversion based on body surface area, the equivalent intravenous dose for humans would be 0.03 mg/kg/day. A treatment-related (0.25 mg/kg/day) minimal tubular necrosis was seen in one monkey; the equivalent human dose at which these lesions may be seen is 0.08 mg/kg/day. This study confirms the results seen in the 4-week study in the rats and suggests that the kidney is a potential site of toxicity in human. Additionally, there is sufficient evidence for lung, liver, thymus, bone marrow and spleen as probable sites of toxicity in humans. The observed toxic effects may be due to renal failure and limited clearance of the drug.

**32. Thirteen week repeated dose toxicity study of HPMPC administered intravenously to cynomolgus monkeys in combination with orally administered probenecid, Lot # 13H0405,**

December 9, 1994, (Study No. 2-

01/93-TOX-0504-002,

'01-94-89)

Six groups of normal male and female cynomolgus monkeys (weight: 3.8-4.2 kg males and 2.4-2.7 kg females; age: unknown) were administered HPMPC via intravenous injection and/or probenecid via the oral route once a week for 13 consecutive weeks at dose levels indicated in the study design (Table 7). Two monkeys per sex per group from Groups 2 and 5 were designated as a 4 week post-dosing recovery group.

Table 7  
Study Design

Group #	# of animals		Treatment	
	♂	♀	HPMPC; iv; (mg/kg/once a week)	Probenecid; po; (mg/kg/once a week)
1	2	2	0	0
2	5	5	0	30
3	3	3	1.0	30
4	3	3	2.0	30
5	5	5	5.0	30
6	2	2	5.0	0

**Deaths:** one male (group 3) was found dead during the first week of study and was replaced. **Clinical Observations:** treatment-related increased incidence of pigmentation of the skin was noted in male and female monkeys (groups 3, 4, 5 and 6). There was an increased incidence of soft feces/diarrhea in animals treated with HPMPC. Other clinical observations noted included abrasions, scabbing, ulcerations, apparent bleeding, apparent dried blood, epistaxis, and/or gaping incision. These lesions were principally mild in nature and posed no threat to the animals. **Body Weights:** the mean body weight gain in the group 6 males and females was considerably less (20%) than that seen in the respective group 1 control animals. **Clinical Pathology:** revealed the absence of apparent significant drug-related changes in any of the treatment groups as compared to the respective control groups. **Organ Weights:** evaluation of mean organ weight data revealed a significant elevation ( $p=0.5$ ) in the group 6 male and female mean absolute kidney weight values as compared to group 1 respective control values. In addition, the groups 2-6 male mean absolute and relative thyroid gland weights were significantly decreased as compared to the control values. **Pathology:** at necropsy, an examination of the animals revealed an increased incidence of pale kidneys in 2/2 group 6 males and females and in 1/3 group 2 females. In the group 6 animals, the grossly observable pale kidneys correlated microscopically with nephropathy; however, the gross finding in the group 2 female had no microscopic correlate. Histomorphologic examination of the tissues revealed apparent drug-related changes in the kidneys and testes. **In the kidneys,** a nephropathy, characterized by epithelial cell hypertrophy and marked karyomegaly of cells lining of the convoluted tubules in the renal cortex was seen in 2/2 group 6 males and females. In the group 5 animals, a minimal degree of karyomegaly was seen in 3/3 males and 2/3 females. In the recovery group 5 animals, minimal karyomegaly was noted in 1/2 males and females. **In the**

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testes, a decrease state of spermatogenesis was seen in 3/3 group 4 males, 2/3 group 5 males and in 1/2 group 6 males. Minimal to moderate decrease in spermatogenesis was also seen in 2/2 group 5 recovery males. The decreased spermatogenesis was characterized by seminiferous tubules which contained fewer numbers of spermatozoa, spermatocytes and spermatogonia. In the groups 4, 5 and 6 males which did not display these changes, it was apparent that these particular animals were somewhat sexually immature. Lesions noted microscopically at the administration site were comprised of fibrosis, hemosiderosis, chronic inflammation (lymphocytes and macrophages), acute inflammation (neutrophils), mineralization and hemorrhage. The lesions were typically seen surrounding the saphenous vein and extended into the subcutaneous tissue of a number of the animals from groups 1, 2, 5 and 6.

Other findings: unique microscopic findings were seen in a group 2 terminal sacrifice female, and included: (1) extramedullary hematopoiesis (primarily erythrocytic) in the liver (mild), spleen (mild), kidneys (cortical interstitium, minimal), pancreatic lymph node (mild) and mandibular lymph node (decreased, mild); (2) marked erythroid hyperplasia and mild myeloid hypoplasia in femoral bone marrow; and (3) bilateral decrease in adrenocortical vacuolation of the zona fasciculata. Additionally, pigment consistent with hemoglobin-derived pigment was moderately increased in the red pulp of the spleen and mildly increased in Kupffer cells of liver sinusoids. No treatment-related microscopic findings were observed in mammary glands of males and females in groups 1-6.

Comments: Based on the data, it may be concluded that the iv administration of HPMPC affected the testes and kidneys of cynomolgus monkeys. At a dose level of 5 mg/kg/once a week of HPMPC, the effects on the kidneys were considered to be mild to marked. When the same dose level of HPMPC was preceded by the oral administration of 30 mg/kg/once a week Probenecid, the effects on the kidneys were significantly reduced and considered to be minimal in severity. Concurrent therapy with Probenecid did not reduce the effects of HPMPC on the spermatogenesis as this effect was seen in males administered 2 mg/kg/once a week of HPMPC preceded by an oral administration of 30 mg/kg/once a week Probenecid. A dose of 1.0 mg/kg/once a week of HPMPC preceded by an oral administration of 30 mg/kg/once a week Probenecid may be considered as a NOEL. On the basis of body surface area conversion, an equivalent dose of HPMPC in humans would be 0.33 mg/kg/once a week.

33. Thirteen week repeated dose toxicity study of HPMPC administered iv to cynomolgus monkeys, Lot # 504B93-01,

March 25, 1994, (Study No. 2-P51

51-93-232)

Four groups of normal male and female cynomolgus monkeys (weight:

2.9-3.6 kg males and 2.7-3.4 kg females; age: unknown; 3-5 animals/sex/group) were administered HPMPC via intravenous injection at dose levels of 0 (vehicle control), 1.0 (low), 5 (mid) or 20 mg/kg once per week (high) for a period of 13 weeks. In low and high dose groups, 3 animals/sex/group were designated as recovery animals which were scheduled to receive 13 weeks of treatment followed by a 4-week recovery period. Deaths: 2/3 females by week 12 (mid), and 5/5 males and 4/5 females by week 12 (high) died or euthanized in extremis. Common necropsy findings included discolored (pale) kidneys, livers and spleens, mottled livers and dark discoloration of various lymph nodes, In some animals, red foci or areas were noted along cardiac surfaces, the external tunics of the gallbladder, the gastric mucosa, within miscellaneous lymph nodes and at the administration site. Due to the high mortality (high), the recovery period was eliminated. Clinical Observations: consisted of pale mucous membranes, abnormal food consumption, dehydration, thin and ruffled coat (low); ruffled coat, vomit, pale mucous membranes, abnormal food consumption, dehydration, thin/emaciated appearance, oily fur, hunched posture, lethargy, prostrate and/or tremors (mid and high). Body Weight and Food Consumption: in the males, mean body weight gains were noted in all groups until day 43 when the mean absolute body weights (mid and high) displayed a decrease and a levelling out thereafter. Average food consumption was generally less (high) in both sexes compared to the controls. Clinical Pathology: a significant depression in the erythrocyte count, hematocrit and hemoglobin values in males and females was seen (high). Statistically significant differences included an increase in the mean cell hemoglobin concentration in males and females (high), an increase in the anisocytosis value in females (high), and an increase in the absolute polymorphonuclear leukocyte values in females (mid). Serum chemistry evaluations displayed biologically relevant increases in urea nitrogen and creatinine values and a significant decrease in mean phosphorus value in males and females (high). Significant decreases were seen in the albumin and albumin/globulin values in females (mid and high). Urine chemistry evaluation revealed significant decreases in the urine sodium values in both sexes (low, mid and high). Organ Weights: significant increases in absolute and relative kidney weights (male, mid) and significant decreases in absolute and relative testes weights (male, low and mid) were noted. Pathology: at termination, there was an increased incidence of discoloration of the lymph nodes, liver, spleen and kidneys in both sexes (mid and high). Microscopic changes were noted in the bone marrow (depletion of hematopoietic cells in mid and high dose males), kidneys (toxic tubular nephrosis in mid and high dose males and females), liver (hepatocellular hypertrophy in mid and high dose males and females), testes (degeneration of the germinal epithelium of the seminiferous tubules (mid and high dose males), and thymus and lymph nodes (lymphoid depletion in mid and high dose males and females)

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**Comments:** The results suggested that the major target organs of toxicity include those tissues which have a cellular component which rapidly proliferates such as the hematopoietic system (thymus, lymph nodes, bone marrow) and germinal epithelium of the seminiferous tubules in the testes, and the organs involved in the metabolism or excretion of the test article (liver and kidney). A NOEL could not be determined in the study. The low dose level used in the study, 1 mg/kg once per week, also displayed slight changes in testes weight and general health status (pale mucous membranes, dehydration, thin, ruffled coat) of the animals. For the low dose, based on the body surface area, an equivalent dose in humans would be 0.33 mg/kg once per week.

34. A 12-month chronic toxicity study of HPMPC following once weekly iv administration in cynomolgus monkeys with an interim sacrifice at 6 months, Lot # 504-B93-01, September 18, 1995, (94-TOX-0504-015\86540)\*

6-month interim sacrifice study: this portion of the study consisted of 1 treated group and 1 control group each consisting of 5 male and 5 female monkeys. The treated animals received probenecid (30 mg/kg/dose) via oral gavage approximately 1 hr prior to being given the iv bolus dose of HPMPC at a dose level of 2.5 mg/kg/once weekly. The control animals received an oral dose of sterile water approximately 1 hr prior to being given the iv bolus dose of the saline vehicle. Results: there were no deaths. Incidental clinical signs seen in the control or treated animals included liquid feces, emesis, skin scabs or lesions, thin fur cover of the hindlimb, forelimb, dorsal thoracic or lumbar regions or tail or swollen hindlimb/hindpaw. There were no clinical signs seen which were considered treatment-related. Hematology: during week 13, statistically significant decreases were seen in eosinophil values of the treated males and in red blood cell counts and hematocrit values of the treated females when compared to the control animals. A statistically significant increase was seen in segmented neutrophils of the treated females. Clinical Biochemistry: during week 13, a statistically significant increase was seen in ALP values of the treated males and in sodium values of the treated females, while a statistically significant decrease was seen in potassium values of treated males when compared to the controls. Histopathology: no treatment-related effects.

**Comments:** Toxicokinetic data were not submitted. Based on results from the present study, the NOEL for HPMPC when treated in combination with orally administered probenecid given to monkeys, was below 2.5 mg/kg/week. On the basis of body surface area conversion, an equivalent dose of HPMPC in humans would be 0.83 mg/kg/once a week.

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Special Toxicity Studies

35. Evaluation of HPMPC to induce delayed-type hypersensitivity in BALB/cByJ mice, Lot # 1966-D-1, August 20, 1992, (Report # 711-GS-001-93)

HPMPC was evaluated for its capacity to induce a delayed-type hypersensitivity (DTH) response in mice. In the induction phase, groups of mice (10/group) received at the base of the tail intradermal injections (0.1 ml/injection) of test or control article administered once a week for three weeks. Test article-treated animals received either 0.2, 0.6 or 3.0 mg/kg of HPMPC in Complete Freund's Adjuvant (CFA; Groups 3, 4 and 5, respectively) or without CFA (Groups 6, 7 and 8, respectively). Control article-treated mice received either sterile water for injection (vehicle negative control; Group 1) or ovalbumin (OVA positive control; Group 2) in CFA. One week after the last injection, mice were challenged with intradermal injections (0.03 ml each) of test and vehicle control articles delivered to the pinna of the right and left ear, respectively. Ear swelling was measured 24 hr following the challenge injection. Results: compared to the vehicle control response, the OVA (0.5) challenge caused a significant positive ear swelling reaction ( $p < 0.001$ ) in OVA-induced mice. In contrast, the test article (0.5%) challenge failed to elicit a positive ear swelling response in any group of HPMPC-induced mice. Conclusion: under the conditions used in this study, HPMPC failed to induce a DTH response in mice.

36. Acute toxicity study of HPMPU administered via intravenous injection to Sprague-Dawley rats, Lot # NB 364-75, August 11, 1992, (69-92-94)

One group of 5 male and 5 female rats were administered a single iv bolus of HPMPU at a dose level of 100 mg/kg to characterize the potential acute toxicity of the drug. Results: following 14 days of observation after the administration, there were no abnormal cageside/clinical observations noted in any of the animals throughout the duration of the study. At necropsy, macroscopic examinations of tissues failed to reveal any apparent test article-related abnormalities. No histopathological examinations were performed. Conclusion: based on the data, it would appear that there is no acute toxicity associated with a single iv administration of 100 mg/kg HPMPU to rats.

37. Four week repeated dose iv toxicity study of HPMPU in rats, Lot # 883-3-28, Gilead Sciences, Inc, Foster City, CA, August 17, 1995, (95-TOX-1582-001)

Four groups of male rats (3/group) received iv HPMPU [GS-1582; the principal degradation product of the test compound] at dose

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levels of 0 (vehicle control), 15 (low), 60 (mid) or 120 mg/kg/once weekly (high) for 4 weeks. Results: no sign of toxicity were noted in any animal throughout the study. Conclusion: based on the results, a dose of 120 mg/kg/once a week may be considered a NOEL. On the basis of body surface area conversion, an equivalent dose of HPMPU in humans would be 17.14 mg/kg/once a week.

### Reproductive Toxicity Studies

**38. Fertility and general reproduction study of HPMPU administered iv to male rats (Segment I), Lot # 504K92-1, November 22, 1994, (707-003/93-TOX-0504-005)**

Groups of male and female rats (strain: Crl:CD BR VAF/Plus; 25/group) were administered HPMPU intravenously at dose levels of 0 (vehicle control), 1.0 (low), 5.0 (mid) or 15.0 (high) mg/kg once per week for 10 weeks. Following the treatment, the male rats were assigned to a 21-day cohabitation period with untreated female rats. Results: two animal were found dead (1 low and 1 mid). Chromorhinorrhea occurred in 4 (controls), 6 (low), 7 (mid) and 8 (high) rats. Body Weights: average body weights were significantly reduced ( $p=0.05$  to  $p=0.01$ ) in the high dose animals for the entire pre-cohabitation injection period (days 1-70). Food Consumption: absolute feed consumption values were significantly reduced (high) for the entire pre-cohabitation period. Mating and Fertility: were unaffected by dosages of the test compound. Terminal Body Weights: were significantly reduced ( $p=0.01$ ) in the high dose rats. Brain weights were comparable among the four dosage groups. The high dose animals had significantly reduced ( $p=0.05$  to  $p=0.01$ ) absolute weight of the left epididymis, individual testes and the left testis minus the tunica albuginea. Mating and Fertility: were unaffected. Sperm Evaluation: the high dose male rats has significantly reduced ( $p=0.05$ ) average for coiled flagellum. Female rats: untreated female rats mated with the treated male rats demonstrated no statistically or biologically important differences among the four dosage groups. Fetal Gross Observations: included a depressed eye bulge and agnathia (1 fetus, mid), a hematoma on the neck (one fetus, mid) and low set ears, open eyelids, ectopic eyes, a split snout, astomia and agnathia (one fetus, high).

Comments: On the basis of these results, an iv dose of 5 mg/kg per week and 15 mg/kg per week may be considered maternal and developmental NOELs, respectively. On the basis of the body surface area, equivalent maternal and developmental NOELs for humans would be 0.71 and 2.1 mg/kg per week, respectively. In the absence of a dose-response curve, the gross external alterations in fetuses might not be related to the test article. The significant reduction in the absolute weights of male sex organs

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(high) might be interrelated with the significant reduction in the high dose group terminal body weights because the ratios of the weights of these organs to the terminal body weights were unaffected.

In a phase 2/3 (GS-93-106) study of the safety and efficacy of HPMP, the test compound is being administered via iv infusion at a dose level of 5 mg/kg per week for the first two consecutive weeks followed by a dose level of 5 mg/kg every two weeks. In the Segment I study in rats, toxicities were seen at doses that are comparatively lower than those used in the clinic. Therefore, the sponsor is requested to monitor the patient closely for the toxicities seen in animals.

**39. Intravenous fertility and general reproduction study of HPMP in female rats, Lot # 504B93-01, September 15, 1995, (707-00494-TOX-0504-019)**

Groups of female rats (25/group) received iv HPMP at dose levels of 0 (vehicle control), 1.2 (low), 6 (mid) or 30 mg/kg (high) for a minimum of 6 and a maximum of 8 weekly injections beginning 15 days before cohabitation with breeder male rats. All rats were sacrificed by carbon dioxide asphyxiation on day 20 of presumed gestation and Caesarean-sectioned. Results: single clinical and necropsy observations occurred in two (high) dams with resorbed litters. One had a perivaginal substance on gestation day (GD) 20, and the other had a green viscous substance present in utero. Body weight gains for the entire gestation period (GD 0-20) tended to be reduced (low and mid) and was significantly reduced ( $p=0.01$ ) in high dose rats. Maternal body weight gains were significantly reduced ( $p=0.01$ ) in mid and high dose animals. High dose animals had essentially no weight gain after GD 13. As a results of these effects on maternal body weight gains, mid and high dose rats has significantly reduced ( $p=0.01$ ) body weights. Absolute (g/day) and relative (g/kg/day) feed consumption values for the entire 14-day prehabitation period were significantly reduced ( $p=0.01$ ) in high dose animals. During gestation, absolute feed consumption values tended to be reduced (low and mid) and was significantly reduced ( $p=0.01$ ) in high dose animals for the entire gestation period. Estrous cycling was unaffected. High dose animals has a slight non-statistically significant reduction in pregnancy (2%). Low through high dose animals had dosage-dependent increases in resorption (embryo deaths) that resulted in reduced live litter sizes, and reduced fetal body weights. This parameters was statistically significant ( $p=0.01$ ) in mid and high dose groups. The high dose group had significantly reduced ( $p=0.01$ ) litter averages for corpora lutea and implantations, observations indicative of impaired ovulation and/or pre-implantation loss and possibly interrelated with the previously described reduced fertility (high). There were statistically significant differences ( $p=0.01$ ) in additional endpoints

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associated with embryo death: a significant increase ( $p=0.01$ ) in the number of dams with all conceptuses resorbed and a significant reduction ( $p=0.01$ ) in the number of dams with viable fetuses. There were only five litters with live fetuses in high dose animals.

**Comments:** Based on these data, maternal and developmental NOELs could not be identified in the study. HPMPC at a dose level of as low as 1.2 mg/kg [equivalent dose in humans = 0.17  $\mu$ /kg] had significant toxic effects on conception rates and early and late stages of gestation in female rats.

**40. Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of HPMPC administered intravenously to rats, Lot # 504K92-01, April 4, 1994, (Report # 707-001/93-TOX-0504-004)**

Four groups of presumed pregnant rats (25/group) were administered HPMPC intravenously at dose levels of 0 (vehicle control), 0.5 (low), 1.5 (mid) or 3 mg/kg/day (high) once daily on days 6-15 of gestation. All rats were sacrificed on day 20 of gestation. Dam Body Weights & Food Consumption: reduced body weight gains were observed late in the injection period (days 12-16) in mid and high dose animals; with the reduction significant at  $P<0.05$ . Body weight gains (mid and high) continued to be reduced after completion of the injection period (day 16-20); with the high dose group reduction significant at  $P<0.01$ . The absolute feed consumption tended to be reduced early in the early injection period (high); after completion of the injection period, the feed consumption values were significantly reduced ( $P<0.05$ ). Live Fetal Body Weights: were reduced in mid ( $P<0.05$ ) and high ( $P<0.01$ ) groups. The number of late resorptions tended to be increased (high) as compared to controls. There was one dead fetus in the litter (high). Delayed fetal ossification was seen (high). These variations were evident as significant increases ( $P<0.05$  to  $P<0.01$ ) in the incidence of litters and fetuses with reversible delays (variations) in ossification of the ribs (wavy ribs and incompletely ossified ribs) and vertebrae (incompletely ossified or not ossified centra or arches in the thoracic or lumbar vertebrae) and reduced litter averages for the number of ossified centers in the caudal vertebrae ( $P<0.01$ ), sternum (sternal centers and xiphoid,  $P<0.01$ ), metacarpals ( $P<0.01$ ), metatarsals ( $P<0.05$ ) and hind limb phalanges ( $P<0.01$ ). Reflecting these events, the number of fetuses with any alteration observed and the mean percentage of fetuses with any alteration were significantly increased ( $P<0.01$ ) in the high dose group.

**Comments:** The maternal and developmental NOEL for HPMPC is 0.5 mg/kg/day. The observed skeletal alterations were not dosage-dependent. No gross external or soft tissue malformations or

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variations were noted.

41. A toxicokinetic study of <sup>14</sup>C-labelled HPMPC after multiple iv administrations to presumed-pregnant rats, Lot # JPS-881-32, September 21, 1995, (94-TOX-0504-005/2-W49)\*

Three groups of presumed-pregnant female rats (14/group) received iv <sup>14</sup>C-HPMPC (3 μCi/kg/day) at dose levels of 0.5 (low), 1.5 (mid) or 3 mg/kg/day (high) during the presumed gestation period corresponding to day 7 through 16 for a total of 10 days. All animals were euthanized on study day 11. There was no control group. Results: there were no abnormal clinical observations or body weight changes during the study. Following 10 consecutive days of treatment, the mean HPMPC recoveries were 0.004, 0.013 and 0.028 μg-equivalents/g of tissue at the low, mid and high dose levels, respectively. The mean litter size, mean litter weight, mean <sup>14</sup>C recovery/litter, and mean percent dose recovered/litter were comparable at all three dose groups. The plasma HPMPC clearance profiles were similar at all dose levels. The Cmax was comparable following the day 1 and day 10 dose administrations and was observed at 2 min following the treatment for each dose group.

42. Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of HPMPC administered intravenously to New Zealand White rabbits, Lot # 504B93-01, November 21, 1994, (707-006/94-TOX-0504-002)

Four groups of presumed pregnant rabbits (20/group) were administered HPMPC intravenously at dose levels of 0 (vehicle control), 0.05 (low), 0.25 (mid) or 1 mg/kg/day (high) on days 6-18 of gestation. Results: two (control) does and one (high) doe aborted on day 23 to 27 of gestation. One (mid) doe and one (high) doe prematurely delivered on day 29 of gestation. Clinical Observations: included abnormal feces, dental problems (missing or broken incisors) and localized alopecia on the limbs (high). Maternal Body Weights: gain (high) for the entire dosage period was reduced (37%) as compared to the control values. Absolute (10%) and relative (12%) feed consumption values (high) for the entire dosage period were reduced as compared to the control values. Litter Observations: the litter averages for late resorptions and percent resorted conceptuses and the number of does with any resorptions were significantly increased (p=0.01) in the high dose group. One doe (high) had a litter consisting of only resorted conceptuses. These effects of drug resulted in significant reductions (p=0.01) in the average for total litter size and live litter size. The high dose also had significantly reduced (p=0.01) live fetal body weights. Fetal Malformations: the high dose group had significantly increased (p=0.01) litter and/or fetal incidence of domed head and associated large

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anterior and posterior fontanelles and holes in the frontal and parietal bones, microphthalmia, opaque area and/or opaque white ring in the eye, angulated hyoid alae, broad, short and/or thickened ribs, bent radius and ulna and bent tibia, fibula and femur. Additional fetal alterations generally classified as malformations and occurring only in the high dose group, but not statistically significant incidence included a meningocele, short snout with associated protrusion of the tongue and short nasal premaxillary and maxillary bones, abdominal distention, barrel chest, edematous hindlimbs, short hyoid alae, and bent humerus. The high dose group also had significantly increased litter and/or fetal incidence ( $p=0.01$ ) of skeletal alterations that were generally identified as reversible retardations in ossification interrelated with the significantly reduced ( $p=0.01$ ) fetal body weights in this group (not ossified pubes) or compression deformations associated with in utero compression (rotated hindlimbs). Incomplete ossified parietal bones and not ossified sternal centra, delays in ossification that also were considered interrelated with significantly reduced ( $p=0.01$ ) fetal body weights ( $p=0.05$ ) occurred in the high dose group. Analysis of fetal ossification sites (high) fetuses revealed additional delays in ossification, interrelated with the significantly reduced ( $p=0.01$ ) fetal body weights. Significant reductions ( $p=0.01$ ) occurred in the litter averages for numbers of ossification sites per fetus in the hyoid, metacarpal, forelimb, phalangeal, tarsal and hindlimb phalangeal bones.

**Comments:** On the basis of these results, an iv dose of 0.25 mg/kg/day may be considered both maternal and developmental NOELs. On the basis of the body surface area, an equivalent maternal and developmental NOEL for humans would be 0.06 mg/kg/day. Treatment-related embryotoxicity and fetal malformations were observed at HPMPD dosage of 1.0 mg/kg/day [on the basis of the body surface area, an equivalent toxic dose for humans would be 0.25 mg/kg/day]. The dosage also resulted in significant maternal toxicity. Therefore, based on the results of this study, HPMPD can not be categorized unequivocally as a teratogen because maternal toxicity was also seen at the same dose level.

**43. Perinatal and postnatal reproduction study of HPMPD administered sc to rats (Segment III), Lot # 504K93-01, September 15, 1995, (94-TOX-0504-011/707-005)\***

Four groups of F0 presumed pregnant female (25/group) rats received sc HPMPD at dose levels of 0 (vehicle control), 0.1 (low), 0.3 (mid) or 1.0 mg/kg/day (high) on days 7-24 of presumed gestation or day 21 postpartum. "Postweaning day" observations were recorded beginning day 22 postpartum. At approximately 90 days of age, the F1 generation male and female rats from the same

dosage group, with the help of a table of random units, were assigned to cohabitation. Mating of the F1 generation rats was confirmed by observation of pregnancy. The F1 generation female rats were Caesarean-sectioned on day 20 of presumed gestation. Results: F0 generation rats and F1 generation litters: one (low) rat died on day 21 of gestation during parturition and one rat (high) was sacrificed in extremis on day 5 of lactation due to a prolapsed uterus. Maternal body weights and body weight gains and feed consumption values were unaffected by the treatment. Dosages of the test article did not affect the duration of gestation, the duration of delivery per pup per litter and the duration of parturition per pup. The average numbers of implantation sites per delivered litter were comparable among the groups. Averages for the numbers of dams with stillborn pups or with all pups dying during lactation, the numbers of liveborn pups, the weaning indices, pup sex ratios, viability, survival and body weights were comparable among the groups and did not differ significantly. F1 generation rats: sexual maturation in the F1 generation male and female rats, learning, short-term retention, long-term retention or response inhibition were unaffected. Mating and fertility of F1 generation were also unaffected by the administration of HPMPC to the F0 dams. The averages for the numbers of days in cohabitation, the numbers of rats mating and the percentages of male rats siring litters and impregnated female rats were comparable among the groups and did not differ significantly. Caesarean-sectioning observations (F1 generation) rats were unaffected. There were no dead fetuses, and no dams resorbed all conceptuses. Conclusion: based on these data, the maternal and reproductive NOELs for HPMPC were 1.0 mg/kg/day. The NOEL for viability and growth in the offspring through day 21 was 1.0 mg/kg/day. The developmental NOEL was 1.0 mg/kg/day. On the basis of body surface area conversion factor, equivalent maternal, reproductive, developmental, and viability and growth NOELs for humans would be 0.14 mg/kg/day.

#### Mutagenicity Studies

44. Ames Microbial Mutagenicity Assay and E. Coli WP2 uvrA Reverse Mutation Assay, 41671 HPMPC, Lot # 27411-47-1, February 21, 1990, 21203/89037)\*

An Ames microbial mutagenicity assay and an E. coli reverse mutation assay were performed to determine the potential of HPMPC to induce base pair substitution of frameshift mutations in Salmonella typhimurium (his-) strains and E. coli strains. The compound was tested with and without exogenous metabolic activation using the S-9 fraction of a rat liver homogenate in the mutation assay at five nominal concentrations (312.5, 625, 1250, 2500 and 5000 µg/plate. In conclusion, the results indicated that HPMPC is not mutagenic in the Ames microbial

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mutagenicity assay or the E. coli reverse mutation assay up to a concentration of 5000  $\mu\text{g}/\text{plate}$ .

**45. Human Peripheral Blood Lymphocyte Clastogenesis Assay,  
41671 HPMPC, Lot # S27411-47-1,  
December 12, 1990, 21539/89052)\***

Based on the results of a preliminary assay with no metabolic activation in which human lymphocyte cultures were HPMPC-treated at levels of 1.56 - 200  $\mu\text{g}/\text{ml}$  for 48 hr, levels of 12.5, 25, 50 and 100  $\mu\text{g}/\text{ml}$  were selected to determine the potential of HPMPC to induce chromosomal aberrations. Appropriate positive and negative controls were included; ganciclovir, a new anti-viral agent, was used as reference compound. In the full assay, human peripheral blood lymphocytes from 2 donors exposed to HPMPC for the last 48 hr of a 72-hr culture period. A total of 400 metaphase cells for each dose group were examined microscopically for aberrations. The results of the chromosome aberration analysis indicate that HPMPC exhibits a statistically significant clastogenic effect in the human peripheral blood lymphocyte chromosome aberration assay at the highest dose tested. Metaphases examined at the three lower doses tested showed elevations above the vehicle control frequency. The reference agent, ganciclovir at 50  $\mu\text{g}/\text{ml}$ , caused significant elevations in the frequency of cells with chromosome aberrations and was slightly more clastogenic than HPMPC at the 100  $\mu\text{g}/\text{ml}$ . The positive control, Mitomycin C at 0.05  $\mu\text{g}/\text{ml}$  was clastogenic causing greater than a seven-fold increase in the frequency of cells with chromosome aberrations. The untreated and solvent (0.1N NaOH) controls were not statistically different from each other and were within the expected ranges for this assay.

**Comments:** HPMPC exhibited a significant clastogenic effect at the highest dose tested. At this dose level the mitotic index was depressed to approximately one-half of the negative control value. At the three lower dose levels, there were increases in the frequencies of chromosome aberrations which appear to be dose-related. Thus, all four dose levels of HPMPC tested increased both the percentage of damaged metaphases and the number of aberrations per cell in a concentration dependent manner.

**46. Mutagenicity test on HPMPC in an in vivo mouse micronucleus assay, Lot # 1966-C-8P, July 25, 1995, (94-TOX-0504-012/16163-0-455)\***

Groups of male and female mice (3 animals/sex/group) received a single ip HPMPC injection at dose levels of 1000 (low), 2000 (mid) or 4000 mg/kg (high) to study the mutagenicity of the test compound in an in vivo mouse micronucleus assay. The animals dosed with the test compound were euthanized approximately 24, 48

and 72 hr after the dosing for extraction of the bone marrow. Due to the excessive mortality observed in the high dose females, a Trial II was conducted testing females at 3500 mg/kg for only the high dose group. Results: HPMPC ( $\geq$  2000 mg/kg) induced a significant increase in micronuclei in bone marrow polychromatic erythrocytes under the conditions of the assay. Conclusion: HPMPC was found to be genotoxic under the conditions of this study.

#### Other Toxicity Studies

47. Primary skin irritation in rabbits, Lot # C90D731,  
July 31, 1992. (Report # -92-C-  
12628/12629/12569/12570/12571)

Four groups of 6 healthy albino New Zealand White rabbits were evaluated for primary skin irritation with four different lots of HPMPC. A 0.5 ml portion of the test article was topically applied to the intact and abraded skin of the rabbits and left in place for 24 hr. Test sites were graded for erythema and edema at 24 and 72 hr after the single sample application. Results: under the conditions of this study, no irritation was observed on the skin of the rabbits. Thus, the test article would not be considered a primary irritant to the skin.

48. Primary eye irritation in rabbits, Lot # not available,  
December 14,  
1992, (Report # 421-GS-001/002/003-92)

Groups of New Zealand White male and female rabbits (age: 8-12 weeks; weight: 2.3-3 kg; 6 animal/sex/group) were administered HPMPC at 0.2, 1 or 5% at a dose level of 0.1 ml/eye (right). The left eye of each animal remained untreated and served as the control. Ocular observations were recorded at 1, 24, 48 and 72 hr after the treatments. No positive ocular responses were observed at 0.2, 1 or 5% in any animal at any observation period. Hence, HPMPC was determined not to be an eye irritant at the doses studied.

49. A penile irritation study in rabbits with HPMPC topical gel.  
Lot # 504L92-02,  
May 13, 1994, (93-TOX-0504-003 Study No. 3337.1)

Groups of male rabbits (3 animals/group) were applied HPMPC topical gel (0.2 ml) to the penis and surrounding prepuce at dose levels of 0 (vehicle control), 0.3% (low), 1.0% (mid) or 5.0% (high) once daily for 3 consecutive days. Test sites were subsequently examined and scored for dermal irritation on study days 1, 2, 3, 4, 7, 10 and 14. Following dermal observations on study day 14, all animals were euthanized and the penis and surrounding prepuce were removed and evaluated. Results: varying degrees of mucosal irritation were observed both macroscopically

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and microscopically in all animals during the study. The response was dose related with low levels of subacute inflammation observed in the control group and increasing levels of mucosal irritation that included cellular infiltrates, hyperplasia, edema and epithelial erosion and/or ulceration noted in the low, mid and high dose groups. The most notable response was observed in the high dose group which included generally higher degrees of the above findings as well as minimal to mild urethritis.

50. A single dose penile irritation study in rabbits with HPMPC topical gel, Lot # 504L92-04, Gilead Sciences, Inc., Foster City, CA, September 21, 1995, (94-TOX-0504-010/SLS-3337.2)\*

Three groups of male rabbits (6/group) were applied HPMPC topical gel on the penile tissue at dose levels of 1% (low), 3% (mid) or 5% (high) to evaluate the potential irritant and/or corrosive effects of the test compound. At approximately 8 hr postdose, the residual HPMPC was removed from 3 animals in each group with dry gauze followed by a distilled water rinse. A similar procedure was performed on the remaining 3 animals per group at approximately 24 hr postdose. Test sites were subsequently examined and scored for dermal irritation up to study day 34. All animals were euthanized and the penis and surrounding prepuce were removed for histopathological evaluation. Results: all treatments resulted in a moderate to severe irritation rating. A slight dose response relationship was evident between the irritation response produced by low, mid and high concentrations. Comparison of the effects of an 8 hr or 24 hr rinse did not reveal any notable differences in the resulting tissue response. In all groups, treatment-related erythema and edema was generally maximal at days 7-14 postdose, and diminished progressively until study termination (day 34). Histopathology: the penile mucosa from all groups had inflammatory changes. The inflammation involved primarily the penile epithelium which was often more severe at the level of the fornix. Varying numbers and distribution of polymorphonuclear infiltrates were present in the epithelial layer. The submucosal vessels were often minimally to mildly congested and mononuclear infiltrates were present in nearly all specimens. The urethra often contained small mineralized concretions. Conclusion: under the conditions of the study, HPMPC was found to be a potent irritant and corrosive agent to the penile tissue of rabbits.

51. 5-, 10- and 30-Day repeat dose dermal toxicity in rabbit,  
Lot # 530-65- August 27, 1992,  
(Report # 431-GS-002-93)

Fourteen groups of male albino New Zealand White rabbits (weight: 2.18-2.75 kg; age: young adult; 4/group) were applied HPMPC Topical Gel to intact skin sites on the clipped backs for periods of 5, 10 or 30 consecutive days to evaluate subchronic dermal irritation and systemic toxicity (Table 8). The test article and control article (lacking HPMPC) formulations were applied at volumes of 0.1 ml/application/site for approximately 8 total hr per day. Each rabbit had one treatment site. Skin irritation: controls - erythema and/or edema were observed in Group 11 (20% PG) on day 1-13, and in control group 12 (0% PG) on day 2-19. The irritation observations included scores as high as moderate to severe erythema and slight edema. HPMPC at 0.03% (20% PG) - erythema and edema were observed on days 2-14 and all animals returned to normal on day 15. HPMPC at 0.1% (20% PG) - erythema and/or edema generally were observed on day 1-2 with necrosis beginning between day 16 and 17. Fissuring of the skin occurred on day 15 (Group 8) and day 20 (Group 3) and sloughing on day 28 (Group 8) and day 25 (Group 3). The incidence in severity of skin irritation was grater in Group 3 (tid) when compared to Group 8 (qd). HPMPC at 0.3% (20% PG) - animals (Group 2) exhibited erythema and/or edema on day 1, necrosis by day 16 and an increase in severity of irritation to maximum scores as the study progressed. Erythema and/or edema occurred on day 2-28 (Group 1) with necrosis beginning on day 15. The incidence and severity of irritation were less in Group 7 with erythema and edema first occurring on day 1 and 20, respectively, and necrosis on day 23. Fissuring of the skin at the application site generally occurred a few days before the onset of necrosis in all three dose groups while sloughing was noted a few days after the onset of necrosis.

**Table 8**  
Dermal and Systemic Toxicity Study of HPMPC Topical Gel in Rabbits

Group	HPMPC			Days of Treatment	Days of Recovery
	Concentration (%)	Cumulative Exposure (mg)	Regimen		
1	0.3	9	tid	10	21
2	0.3	27	tid	30	0
3	0.1	9	tid	30	0
4	0.03	2.7	tid	30	0
5	1.0	10	qd	10	21
6	1.0	30	qd	30	0
7	0.3	9	qd	30	0
8	0.1	3	qd	30	0
9	0.3 (0% PG)	27	tid	30	0
10	0.3 (0% PG)	9	qd	30	0
11	Control (20% PG)	0	tid	30	0
12	Control (0% PG)	0	tid	30	0
13	0.3	1.5	qd	5	14
14	1.0	5	qd	5	14

HPMPC at 0.3% (0% PG) - in Group 9, erythema and/or edema was observed on day 2 with necrosis beginning on day 15 and continuing for the remainder of the study. Fissuring of the skin occurred on day 14 and sloughing on day 28. In Group 10, erythema was observed on day 1-12. HPMPC at 1% (20% PG) - the onset of irritation observed was similar in both Groups 5 and 6 (day 1 for erythema, day 15-16 for necrosis). Fissuring of the skin in Group 5 began on day 8 and sloughing on day 12. In Group 6, the irritation increased in severity to maximum scores by day 17 and continued to study termination. Clinical Observations: one rabbit (Group 4) exhibited clinical signs of decreased activity, abnormal gait, abnormal stance, tremors and/or dyspnea on day 5-7. Mortality: one animal (Group 4) was sacrificed moribund on day 7. Necropsy of this animal revealed hemorrhagic areas on the serosal surface of the colon and cecum, lymphoid depletion in the sacculi rotundas and appendix, small spleen and a gastrointestinal tract with watery contents and distended with gas. Necropsy: skin - lesions at the treated skin sites were

observed in all dose groups except for the Groups 4, 5 and control ( Group 12). The following lesions were noted at the dose sites: crusted red and/or fissured surface in one animal (Groups 2 and 3); moist erythematous and/or erythematous surface in three animals (Groups 6 and 9), two (Group 8) and one (Group 7); crusted surface or slightly crusted surface in two animals (Groups 2, 3 and 9) and one animal (Group 8 and 14); and scaly surface and/or slightly scaly surface in two animals (Groups 7, 10 and 11) and in one animal (Groups 1, 3, 6, 8, 9 and 14). Kidney - lesions observed included the following: bilaterally mottled and/or bilaterally slightly mottled in two animals (Group 5) and one animal (Groups 2 and 12); bilaterally pale or left kidney pale in two animals (Groups 6 and 7), three animals (Group 11) and one animal (Group 4, 10, 13 and 14); left and right scattered subcapsular cysts in one animal (Group 1); and an anterior pale indent in one animal (Group 7). Testes - bilaterally small testes were observed in one animal (Groups 6 and 9) and a slightly small right testis was observed in one animal in Group 13. Other findings included multiple and/or bilateral cysts on the epididymides of one animal (Group 5 and 9). Histopathology: topical administration of 0.03, 0.1, 0.3, or 1.0% HPMPC for up to 30 days, once (qd) or three times a day (tid), resulted in a dose-related increased severity of skin lesions at the treatment site in the rabbits receiving 0.1, 0.3 and 1.0% HPMPC. These treatment related skin changes varied with dose regimen and formulation. When applied at 0.3% and 1.0% (with 20% PG) for 10 days, tid and qd respectively, and evaluated after the 21 day recovery period, the treatment related skin changes were almost completely resolved. When applied once a day at 0.3 and 1.0% HPMPC (with 20% PG) for 5 days and evaluated after the 14 day recovery period, the treatment related skin changes were almost completely resolved in the rabbits receiving 0.3% but persisted in the 1.0% group. No treatment related changes were present in the testes or kidneys of any of the rabbits receiving HPMPC regardless of dose level, treatment regimen or formulation. When compared to the control (Groups 11 and 12), an increased skin irritation was present in the rabbits in Groups 2, 3, 6, 7, 8, and 14. Severe skin lesions with ulcerations of the treatment site were present in the rabbits in Groups 2, 3, 6, 7, 8 and 9. The skin changes in Groups 1, 4, 5, 10 and 13 were comparable to the changes present in the skin of the control rabbits. The skin changes at the treated sites of the rabbits receiving 0.1, 0.3 and 1% HPMPC were characterized by ulceration, dermal fibrosis, vesiculation (epidermis), edema of the dermis, subacute dermatitis and multifocal epidermatitis. When the treated skin site was not completely ulcerated, varying degrees of hyperkeratosis and epidermal hyperplasia (acanthosis) were present at the treated area.

**Comments:** A NOEL could not be determined in the study. Topical administration of 0.03, 0.1, 0.3 or 1.0% HPMPC topical gel

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resulted in moderated to severe skin irritation the rabbits in all test article-treated dose levels with the onset and severity of irritation generally greatest in the higher dose levels of HPMPC administered three times daily for thirty days. Additionally, toxicity appeared to be schedule-dependent; as noted above, 0.3% topical HPMPC administered three times daily appeared to be associated with prolonged toxicity relative to 1.0% topical HPMPC administered once daily.

52. 30-Day repeat dose dermal toxicity in rabbit, Lot # 504L92-  
August 30, 1992, (Repor' #  
431-GS-001-93)

Groups of male and female albino New Zealand White rabbits (weight: 2.04-2.43 kg; age: adult; 4/sex/group) were administered topically HPMPC Gel at concentrations of 0.3, 1.0 or 5% (Groups low, mid and high, respectively). The Gel was applied topically three times daily for 30 consecutive days to the clipped backs of the animals. The test article was applied for approximately 8 hr total per day to the same site and at a volume of 0.1 ml/application/site. Control animals received the control formulation. Half of the animals in each group had intentionally-abraded treatment sites and the other half had intact treatment sites. Skin Irritation: treatment-related erythema was observed in all test article dose groups beginning day 2 (male) and 5 (female). The irritation increased in severity with repeated test article application and in a dose-dependent manner. The time of onset of skin lesions (ie, fissuring of the treatment site) was also dose-related, appearing earliest in the high dose males (day 11) and females (day 10), and latest in the low dose males (day 20) and females (day 13). By day 30, skin sloughing was observed in all test article treatment groups in the majority of animals (both male and female). Treatment site necrosis was present in < 50% of females in all test article dose groups and in one low dose male. The control animals also showed some mild signs of irritation including occasional scores as high as very slight edema and well defined erythema. Necropsy: lesions at the treated skin sites were observed in all dose groups except for the control group. The following lesions were noted at the dose sites: erythematous and crusted area in one mid dose and three high dose males and in all low, mid and high dose females; erythematous and moist area in two low and two high dose males. No significant differences of dermal irritation were observed at intact or abraded sites. Relative to controls, three low dose and all mid and high dose group males had smaller testes. Other findings included pale kidneys in one high dose male, yellow nodules in the mesentery of one control female, cysts adjacent to the ovaries in one low and one mid dose female, and a clear, fluid-filled cyst in the fallopian tubes in one high dose female. Organ weights: compared to the control group values, absolute mean testes weights, relative testes-to body weight ratios and

relative testes to brain weight ratios (in all test groups) were statistically lower and reduced in the mid and high dose groups in a dose-dependent manner. Histopathology: HPMPC Topical Gel (0.3, 1.0 or 5.0%) administration for 30 days resulted in moderate to severe skin irritation at all three dose levels. The skin irritation was characterized by ulceration, dermal fibrosis, epidermal vesiculation, edema of the dermis and subacute dermatitis. These were accompanied in almost all of the rabbits by minimal to slight hyperkeratosis and/or acanthosis (epidermal hyperplasia) of the skin adjacent to the treated area. When abraded and unabraded treated skins were evaluated as a combined group, there was dose-related increased severity of ulceration and dermal fibrosis and a reciprocal decreased severity of epidermal vesiculation and edema of the dermis in the low, mid and high dose groups. Slight to moderate subacute dermatitis was present in all of the treated sites without any dose-related pattern. There were no consistent or remarkable differences between the skin reactions in the males versus females or in the abraded treated skins versus the unabraded treated skins.

Slight to moderately-severe nephrocytomegaly and karyomegaly of the outer cortical tubules of the kidneys were present in all males and females rabbits (high). In the more severely affected kidneys, there was a loss of renal tubules in the outer cortical region of the kidney. These changes were representative of a hypertrophy of cortical renal tubular cells without any signs of hyperplasia or neoplasia. There was no apparent difference between the animals with intact skin and abraded skin.

Spermatogenesis was absent or reduced in the testes of the four males (high) and 3 or 4 males (mid) dose groups. No testicular tissue effects were present in low dose group. Normal follicular activity was present in the treated females.

Comments: A NOEL could not be determined in the study. Studies performed in rabbits treated for 30 days have provided further evidence of a toxicity profile for topical HPMPC that is consistent with the systemic administration; the target organs are skin, kidney and testes. The dermatitis which eventually developed in all animals appeared to be dose-dependent; that is, treatment with 5% topical HPMPC was associated with faster onset of local toxicity than with lower concentrations. Systemic toxicities occurred at exposure concentrations of 1.0 and 5.0% HPMPC topical gel. Decreases in testes weights and microscopic evidence of reduced spermatogenesis were observed in the 1.0 and 5.0% HPMPC treatment groups. Nephrotoxicity, characterized by nephrocytomegaly and karyomegaly of the outer cortical tubules, was observed in the 5% HPMPC treatment group.

53. Three day repeated dose ocular irritation study of HPMPC and cHPMPC in rabbits, Lot # LY-803-56D, Gilead Sciences, Inc., Foster City, CA, August 22, 1995, (94-TOX-0504-004/HWA-6511-106)

Five groups of albino rabbits (3/group) received 0.1 ml of control material (sterile water), HPMPC (0.3% or 1%) or cyclic HPMPC (0.3% or 1%) into the everted lower lid of right eye to determine the relative level of ocular irritation of the test materials. The left eye served as the untreated control. Clinical observation were conducted daily for 36 days. Results: all animals in all groups appeared clinically normal throughout the study. There was no statistically significant difference in mean body weight between control and treated groups. The irritation observed in the eyes of the animals treated with 1% HPMPC and 0.3% cyclic HPMPC was very minimal in nature and generally confined to the conjunctiva. The eyes of the animals treated with the control material appeared normal throughout the study. Histopathology: a few minimal to slight lymphocytic infiltrates occurred in the periductal tissues or conjunctiva of the right nasolacrimal duct of animals given 0.3% or 1% of HPMPC which did not occur in control animals.

Comments: From the results of this study, it appeared that HPMPC (0.3% or 1%) was an irritant to the eyes in rabbits. However, the sponsor believes that the ocular administration of HPMPC was not associated with any macroscopic or microscopic findings in the nasolacrimal duct or lacrimal glands. The sponsor stated that because the occurrence of lymphocytic infiltrates in the conjunctiva of animals was a common background finding due to the exposure of the ocular membrane to environmental and other external influences and because of the overall erratic occurrence of this finding in the animals, the occurrence of lymphocytic infiltrates in and around the nasolacrimal ducts was not considered important.

#### ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION (ADME)

##### Summary of ADME Studies:

1. Pilot Studies of the Urinary Excretion and Pharmacokinetics of HPMPC in the Mouse and Rat, July 2, 1990, (25476)
2. Pharmacokinetics and excretion of <sup>14</sup>C-HPMPC following single dose intravenous or subcutaneous administration to Sprague-Dawley rats (Study No. 94-DDM-0504-002).
3. Tissue distribution of <sup>14</sup>C-HPMPC following single dose intravenous or subcutaneous administration to Sprague-

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- Dawley rats (Study No. 94-DDM-0504-003).
4. Tissue distribution of <sup>14</sup>C-HPMPC at 6 and 24 hr after single dose subcutaneous administration to female Sprague-Dawley rats, Gilead Sciences, Inc., Foster City, CA, September 8, 1994, (94-DDM-0504-004)
  5. Cidofovir concentrations in plasma samples from a thirteen week repeated dose toxicity study of HPMPC administered to rats, Lot # 504B92-01, Gilead Science, Inc, Foster City, CA, December 21, 1994, (GSI #93-TOX-2-P50-BA)
  6. Concentration of HPMPC in plasma samples from a 26-week repeated dose subcutaneous toxicity study in Sprague-Dawley rats, Gilead Sciences, Inc., Foster City, CA, September 8, 1994, (2-T-70/94-TOX-0504-003-BA)
  7. Pharmacokinetics of HPMPC in a six-month chronic iv toxicity study in rats, September 12, 1995, (94-TOX-0504-016/P0504-00052)
  8. Pharmacokinetics, bioavailability, metabolism and tissue distribution of <sup>14</sup>C-cyclic HPMPC in Sprague-Dawley rats (94-DDM-0930-002).
  9. Absolute bioavailability and tissue distribution of three formulations of <sup>14</sup>C-HPMPC following intravenous or topical administration to New Zealand white rabbits, Lot # DB-579-89,  
July 1, 1994,  
Report
  10. Intraocular distribution of radioactivity following single iv or intraocular doses of <sup>3</sup>H-HPMPC in rabbits, October 14, 1993, (93-DDM-0504-001/PH-845GS-001-93/P0504-00007)
  11. Effect of probenecid on the tissue distribution of <sup>14</sup>C-radiolabelled HPMPC following iv administration to rabbits, June, 15, 1995, (93-DDM-0504-002/M -T04-94-36)
  12. Analysis of data from study 2-T04: Effect of probenecid on the tissue distribution of <sup>14</sup>C-HPMPC following iv administration to rabbits, August 2, 1995, (93-DDM-0504-002/P0504-00015)
  13. Oral bioavailability of HPMPC and cyclic HPMPC in beagle dogs, June 7, 1995, (94-DDM-0930-006).

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14. A Summary of Single-Dose and Multiple-Dose Pharmacokinetics of <sup>14</sup>C-HPMPC, Lot # 28648-7 and 28701-18-1 in the African Green Monkey, , July 31, 1991, 257/904  
41671-01/02)
  15. Cidofovir concentrations in serum samples from a 13-week iv toxicity study in cynomolgus monkeys (a report on partial analysis of samples from toxicity study 2-P51), Lot # 504B93-01, Gilead Science, Inc., Foster City, CA, March 8, 1995, (93-TOX-2-P51-BA/PO504-00036)
  16. HPMPC concentrations in serum samples from a 30-day sc toxicity study of HPMPC in monkeys, April 18, 1995, (94-TOX-2-0504-0038)
  17. Effect of probenecid on the urinary excretion of <sup>14</sup>C-HPMPC following iv administration to monkeys, April 22, 1994, (2-T03/PO504-00012)
  18. Effect of probenecid on the urinary excretion of <sup>14</sup>C-HPMPC following iv administration to monkeys, June 27, 1994, (93-DDM-0504-003/PO504-00018)
  19. Cidofovir concentrations in serum samples from a 13-week iv toxicity study in cynomolgus monkeys (a report on partial analysis of samples from toxicity study 2-P51), Lot # 504B93-01, Gilead Science, Inc., Foster City, CA, March 8, 1995, (93-TOX-2-P51-BA/PO504-00036)
  20. Isolation and identification of a metabolite of <sup>14</sup>C-HPMPC from kidney of rat following iv administration, (in progress, not submitted)
  21. Concentration of HPMPC in plasma samples from a 26-week repeated dose subcutaneous toxicity study in Sprague-Dawley rats, Gilead Sciences, Inc., Foster City, CA, September 8, 1994, (2-T-70/94-TOX-0504-003-BA)
  22. Protein Binding of Cidofovir, Cyclic HPMPC, PMEA and PMPA in Human Plasma and Serum, Cidofovir Lot # 1966-C-9P), Gilead Sciences, Inc., Foster City, CA, June 9, 1995 (PO504-00039/95-DDM-XXXX-001)

## ADME Studies Review:

## 1. Pilot Studies of the Urinary Excretion and Pharmacokinetics of HPMPC in the Mouse and Rat,

July 2, 1990, (-25476)

Urinary recovery of HPMPC in the mouse.

Mice were dosed with 25 mg/kg HPMPC either IV, IP or PO and urine collected and analyzed for HPMPC content. The results are shown in Table 1. For both IV and IP routes, the recovery of unchanged drug is extensive (80 and 65%, respectively, by 6 hr), and more than 90% of that recovered is present in the 0 - 4 hr sample. With oral dosing, the urinary recovery is by contrast low (8%). From the limited urine data, an oral bioavailability of about 10% can be calculated in mouse.

**Comments:** The low urinary recovery of the unchanged drug by oral route and the presence of single drug peak in the HPLC profile of the drug suggest that HPMPC is poorly absorbed from the G.I. tract and most of the drug is excreted in to the feces; thus, resulting in the poor oral bioavailability in mouse.

**Table 1**  
Urinary Recovery of HPMPC in the Mouse after the Single Administration (25 mg/kg) via Different Routes

Dose route N=5	Percent Urinary Recovery in Mouse		
	0 - 4 hr	4 - 6 hr	Total (0-6 hr)
IV	76.3	5.5	80
IP	61.6	3.8	65
PO	4.1	3.8	8

Urinary Recovery of <sup>14</sup>C-HPMPC in Rats

Rats were dosed either IV or PO with <sup>14</sup>C-HPMPC at 5 mg/kg, and urine collected for 24 hr. The recovery of the dose estimated to be 60% from the IV dose and 1.8 percent from the oral dose. From the urine data, the oral bioavailability of HPMPC was 3%. The radioactivity in urine from a PO dosed rat eluted from the HPLC as a single peak at the retention time expected for HPMPC

**Comments:** The low oral bioavailability observed in the earlier experiment (23% in African Green monkeys) and 3% in the present study (rat) may be due to an incomplete absorption of the compound rather than due to extensive metabolism of the drug in

liver since HPMPC was eluted as the single peak from the HPLC.

### Single Dose Pharmacokinetics of $^{14}\text{C}$ -HPMPC in the Rat

Male rats were dosed with a single bolus IV injection into the tail vein to provide a dose of 4 mg (84.8  $\mu\text{Ci}$ )/kg of  $^{14}\text{C}$ -HPMPC. Blood samples were obtained at 0, 5, 15, 30 min, 1, 1.5, 2, 4, 8, 12, 16 and 24 hr after dosing. Concentration of the drug decreased biphasically with a terminal half-life estimated to be 8.8 hr and  $C_{\text{max}}$  was 1200 ng-eq/ml. The  $\text{AUC}_{0-\infty}$  was calculated to be 3.5  $\mu\text{g}\cdot\text{eq}\cdot\text{hr}/\text{ml}$ ; total body clearance and  $V_d$  estimated to be 19 ml/min.kg and 1.6 L/kg, respectively.

### Concentration of $^{14}\text{C}$ -HPMPC in Rat Kidney

Two rats were given a single bolus IV injection of a solution of  $^{14}\text{C}$ -HPMPC at 4 mg (84.8  $\mu\text{Ci}$ )/kg. After 24 hr, the kidneys were removed from each rat. The results are shown in Table 2.

**Table 2**  
Concentration of HPMPC in Blood and Kidneys after the Single IV Bolus Injection (4 mg/kg) to Rats.

RAT	$^{14}\text{C}$ -HPMPC (ng-eq/g)	
	Kidney	Blood
# 1	6542	11.5 10.2
# 2	6575 6397	14.9 14

The mean concentration of  $^{14}\text{C}$ -HPMPC in the kidney 24 hr after dosing was 6500 ng-eq/g; whereas, the concentration of the drug in the blood after the dosing was 13 ng-eq/g.

**Comments:** These results suggest that 24 hr after the administration, HPMPC is concentrated in the kidney and only a negligible amount of the drug present in the blood at that time; thus as a result, the clearance of the drug from the kidney may be slow.

**2. Pharmacokinetics and excretion of  $^{14}\text{C}$ -HPMPC following single dose intravenous or subcutaneous administration to Sprague-Dawley rats (Study No. 94-DDM-0504-002).**

Groups of female rats (3/group) received a single dose of  $^{14}\text{C}$ -HPMPC (3 mg/kg, 400  $\mu\text{Ci}/\text{kg}$ ) either via the iv or sc route to study the pharmacokinetics and excretion of total radioactivity.

Animals were placed in metabolic cages; urine, feces and plasma were collected over the next 24 hr. Results: plasma concentrations declined in an apparent multi-exponential manner for both the routes of administration. Mean  $\pm$  S.D.  $C_{max}$  values were  $10.23 \pm 1.59$   $\mu\text{g-equiv/ml}$  and  $2.74 \pm 0.47$   $\mu\text{g-equiv/ml}$  for iv and sc routes, respectively. The corresponding  $T_{max}$  for the sc route was  $0.33 \pm 0.14$  hr. The apparent absorption half-life of the sc dose was approximately 12 min; and the mean  $\pm$  S.D. terminal elimination half-lives were  $6.6 \pm 1.4$  hr and  $4.5 \pm 0.4$  hr for the iv and sc injections, respectively. The sc bioavailability was  $91.5 \pm 15.8\%$ .

Comments: The results of this study suggest that the bioavailability of the sc dose was essentially complete and that absorption from the site of injection was relatively fast.

**3. Comparison of the tissue distribution of  $^{14}\text{C}$ -HPMPC following single dose intravenous and subcutaneous administration to female Sprague-Dawley rats, Gilead Sciences, Inc., Foster City, CA, September 8, 1994, (94-DDM-0504-003)**

Groups of female rats (4/group) received a single dose of  $^{14}\text{C}$ -HPMPC (3 mg/kg, 400  $\mu\text{Ci/kg}$ ) either via the iv or sc route to study the tissue distribution of total radioactivity. Following the dosing, animals were sacrificed at 20 min, 6 hr and 24 hr. Results: following the iv injection, concentrations within mammary tissues were homogeneous, and all were equivalent to the plasma concentrations for the first 6 hr. Thereafter, the tissues levels declined at a slower rate than plasma. Following sc injection (Table 2), rats showed evidence of two distinct distribution patterns: one in which distribution was equivalent to the iv route (2/4 rats) and one in which the axillary/thoracic mammary tissue concentrations were elevated (2/4 rats). The elevated drug concentrations were confined to the left side of the animal (the same side as the injection) and were as much as 14 fold higher than for iv administration. At 20 min post-dose, 26% of the radioactive dose remained at the site of injection in rats with low mammary exposure and 6-10% of the dose in rats with high mammary tissue exposure. Similar elevations of axillary/thoracic levels relative to iv administration was evident at 6 hr post-dose (1/3 rats). At 24 hr post-dose, the mean concentrations of left axillary/thoracic mammary tissue were 0.028 and 0.034  $\mu\text{g-equiv/g}$  for sc and iv routes, respectively.

Table 2

Mean concentrations of total radioactivity in left axillary/thoracic mammary tissue after single dose administration of  $^{14}\text{C}$ -HPMPC to female rats

Route	No of rats	Concentrations at various times after administration ( $\mu\text{g-equiv/g}$ )		
		20 min	6 hr	24 hr
sc	2	0.708	0.050	0.030
	2	8.41	0.795	0.026
iv	4	1.325	0.055	0.034

**Comments:** Following the single sc administration of  $^{14}\text{C}$ -HPMPC, distribution of the radioactivity within the left axillary/thoracic mammary tissue was not uniform (total 4 rats). At 20 min post-dose, 2/4 rats showed radioactivity concentrations in the tissue similar to the values for iv administration, whereas 2/4 rats showed 6-fold higher levels of radioactivity in the tissues than the iv rats. These two sc rats continued to show higher drug concentrations in the tissues for the next 6 hr post-dose. However at 24 hr post-dose, mean HPMPC concentrations of the tissues via either sc or iv route were essentially similar. The results clearly showed that there was no drug accumulation and no reservoir for the drug in the region via the sc route.

4. Tissue distribution of  $^{14}\text{C}$ -HPMPC at 6 and 24 hr after single dose subcutaneous administration to female Sprague-Dawley rats, Gilead Sciences, Inc., Foster City, CA, September 8, 1994, (94-DDM-0504-004)

The distribution of radioactivity into the left and right axillary/thoracic and inguinal mammary tissue was compared at 6 and 24 hr after sc administration of  $^{14}\text{C}$ -HPMPC to the left dorsal region (caudal to the scapular region) of twelve female Sprague-Dawley rats per time point. The results are summarized in Table 3.

Table 3

HPMPC Concentrations in Rat Mammary Tissues Following sc and iv Administrations

Mammary Tissue	Route	Concentration in Tissues ( $\mu\text{g-equiv/g}$ ) Mean $\pm$ SD (range)	
		6 hr	24 hr
Left Axillary/Thoracic	sc	0.161 $\pm$ 0.135 (0.041-0.551) (n=12, 24 samples)	0.615 $\pm$ 0.328 (0.025-1.146) (n=12, 48 samples)
	iv	0.052 $\pm$ 0.024* (n=4, 8 samples)	0.034 $\pm$ 0.003 (n=4, 8 samples)
Left Inguinal	sc	0.046 $\pm$ 0.011** (n=12, 24 samples)	0.025 $\pm$ 0.004 (n=12, 48 samples)
Right Inguinal	sc	0.039 $\pm$ 0.011** (n=12, 24 samples)	0.023 $\pm$ 0.005 (n=12, 48 samples)

\*Significantly different from levels in left/axillary thoracic mammary tissue following sc administration (P=0.032).

\*\*Significantly different from levels in left/axillary thoracic mammary tissue following sc administration (P<0.001).

**Comments:** Following the observation (study # 94-DDM-0504-003) of non-uniform concentrations of HPMPC in axillary/thoracic mammary region of rats (4), the present study was designed to further investigate the phenomenon of variable tissue distribution in greater numbers of rats following sc administration, and to further examine whether or not sc dosing resulted in high local concentrations of drug in the axillary/thoracic mammary tissue. The present study of sc administration of HPMPC to rats showed higher drug concentrations in the left axillary/thoracic mammary tissue than those found in the inguinal mammary tissues of the same animals. Secondly, drug concentrations in the left axillary/thoracic mammary tissue via the sc route, varied greatly between animals, and were as much as 20 to 30 fold higher than those found in the same tissue after iv administration.

5. Cidofovir concentrations in plasma samples from a thirteen week repeated dose toxicity study of HPMPC administered to rats, Lot # 504B92-01, Gilead Science, Inc, Foster City, CA, December 21, 1994, (GSI #93-TOX-2-P50-BA)

Four groups of normal male and female rats (strain: Tac:N(SD)fBR; age: 3-5 weeks; 10-26 animals/sex/group) were administered HPMPC by intravenous injection at dose levels of 0 (vehicle control), 3 (low), 15 (mid) or 60 mg/kg once per week (high) for a period of 13 weeks. Plasma samples were obtained over the course of 12 hrs on weeks 1, 4, 8 and 12. The samples were analyzed using a validated HPLC method. Results: the pharmacokinetic parameters

are summarized in Table 4. There were no obvious trends in the pharmacokinetic parameters over the course of the study at any of the dose levels tested. AUC values following the first dose indicated apparent dose proportionality.

**Table 4**  
Non-compartmental Pharmacokinetic Parameters Following Once a Week Administration of Cidofovir at 3, 15 and 60 mg/kg/week

Dose mg/kg/day	PK Parameters	Sample Periods (Weeks)			
		1	4	8	12
3	AUC <sub>0-∞</sub> (μg <sup>2</sup> hr/ml)	3.27	3.69	3.4	4.86
	CL <sub>TOT</sub> (l/hr/kg)	0.92	0.81	0.88	0.62
	V <sub>dss</sub> (l/kg)	28.63	5.83	4.43	11.02
	T <sub>1/2</sub> (hr)	31.2	12.1	8.9	27.7
	C <sub>max</sub> (μg/ml)	0.46	0.76	0.79	0.87
	AUC/Dose	1.11	1.23	1.13	1.62
15	AUC <sub>0-∞</sub> (μg <sup>2</sup> hr/ml)	12.66	14.62	20.85	12.64
	CL <sub>TOT</sub> (l/hr/kg)	1.19	1.03	0.72	1.17
	V <sub>dss</sub> (l/kg)	2.33	2.65	3.18	6.83
	T <sub>1/2</sub> (hr)	5.16	7.15	12.21	11.16
	C <sub>max</sub> (μg/ml)	2.7	3.2	5.5	2.6
	AUC/Dose	0.84	0.97	1.39	0.84
60	AUC <sub>0-∞</sub> (μg <sup>2</sup> hr/ml)	46.38	40.59	51.69	64.22
	CL <sub>TOT</sub> (l/hr/kg)	1.29	1.48	1.16	0.93
	V <sub>dss</sub> (l/kg)	1.26	1.97	1.29	0.71
	T <sub>1/2</sub> (hr)	3.6	3.3	3.5	4.1
	C <sub>max</sub> (μg/ml)	12.7	9.22	14.64	na
	AUC/Dose	0.77	0.68	0.86	1.07

**Comments:** Clearance was significantly lower at the 3 mg/kg/week dose level than the 60 mg/kg/week dose level. This might be due to underestimation of AUC values at the low dose where plasma levels approached the limit of detection.

6. Concentration of HPMPc in plasma samples from a 26-week repeated dose subcutaneous toxicity study in Sprague-Dawley rats, Gilead Sciences, Inc., Foster City, CA, September 8, 1994, (2-T-70/94-TOX-0504-003-BA)

HPMPc concentrations were determined in plasma samples obtained from a 26-week sc toxicity study in rats. Groups of rats were administered HPMPc via sc route at dose levels of 0 (vehicle control), 0.6 (low), 3.0 (mid) or 15.0 mg/kg/weekly (high). Plasma samples were obtained at 0, 1, 2, 3, 4, 6 and 12 hr post-dose at week 1, 5 and 14 using 4 rats per time point. Samples were analyzed using a validated bioanalytical method for HPMPc in rat plasma. Results are shown in Table 5.

Table 5  
Pharmacokinetic Parameters for HPMPc following Repeated Subcutaneous Administration to Male Rats

Dose (mg/kg/weekly)	Parameter (Plasma)	Week of Study		
		1	5	14
0.6	AUC ( $\mu\text{g}\cdot\text{hr}/\text{ml}$ )	0.14	0.49	0.55
	Cmax ( $\mu\text{g}/\text{ml}$ ), Mean $\pm$ SD	0.28 $\pm$ 0.03	0.30 $\pm$ 0.21	0.44 $\pm$ 0.09
	Tmax (hr)	1	1	1
3.0	AUC ( $\mu\text{g}\cdot\text{hr}/\text{ml}$ )	1.31	4.11	3.18
	Cmax ( $\mu\text{g}/\text{ml}$ ), Mean $\pm$ SD	1.06 $\pm$ 0.10	1.72 $\pm$ 0.27	1.94 $\pm$ 0.11
	Tmax (hr)	1	1	1
15.0	AUC ( $\mu\text{g}\cdot\text{hr}/\text{ml}$ )	8.83	7.32	8.71
	Cmax ( $\mu\text{g}/\text{ml}$ ), Mean $\pm$ SD	6.70 $\pm$ 1.26	2.38 $\pm$ 0.14	4.60 $\pm$ 0.41
	Tmax (hr)	1	2	2

Comments: This study demonstrated that repeated sc administration of HPMPc over the course of 14 weeks to rats, did not result in an accumulation of the test compound at the high dose.

7. Pharmacokinetics of HPMPC in a six-month chronic iv toxicity study in rats, September 12, 1995, (94-TOX-0504-016/P0504-00052)

Groups of Sprague-Dawley male and female rats (20 animals/sex/group) were administered HPMPC intravenously at dose levels of 0 (vehicle control), 0.6 (low), 3.0 (mid) or 15.0 mg/kg once weekly (high) for a period of 6 months. An additional (10/sex) rats were assigned to the control and high dose groups and were retained untreated for a 4-week recovery period following the termination of treatment. A further cohort of rats (14/sex/group) were included for the toxicokinetic evaluation. Blood samples were obtained prior to dosing and at 5, 30 min and at 1, 2, 4 and 6 hr post-dose on weeks 1, 5, 13 and 26. Concentrations of HPMPC were determined by a validated HPLC method. Results: the pharmacokinetic parameters are listed in Table 6. Repeated administration of HPMPC led to apparent increases in AUC values from week 1 to 26. These changes were greater at the high dose level (2.6-fold increase in the AUC for males vs 1.9-fold increase for females at the dose level).

Table 6

Pharmacokinetic parameters of HPMPC in a 26 week iv toxicity study in rats

Parameter	Week	Low dose	Mid dose	High dose	High dose
		♂	♂	♂	♀
C <sub>0</sub> (µg/ml)	1	1.55	6.27	27.4	33.9
	5	1.62	8.71	46.9	47.5
	13	1.73	10.7	63	56.3
	26	2.36	11.4	55.5	59.1
AUC <sub>0-6</sub> (µg*hr/ml)	1	nd	2.62	11.6	13.3
	5	nd	3.35	19.2	19.3
	13	nd	4.41	27.2	22.8
	26	nd	4.89	30.5	24.8
T <sub>1/2</sub> (hr)	1	nd	nd	2.23	1.63
	5	nd	nd	2.79	2
	13	nd	nd	2.08	1.5
	26	nd	nd	2.07	1.59

7. Pharmacokinetics of HPMPC in a six-month chronic iv toxicity study in rats, September 12, 1995, (94-TOX-0504-016/P0504-00052)

Groups of Sprague-Dawley male and female rats (20 animals/sex/group) were administered HPMPC intravenously at dose levels of 0 (vehicle control), 0.6 (low), 3.0 (mid) or 15.0 mg/kg once weekly (high) for a period of 6 months. An additional (10/sex) rats were assigned to the control and high dose groups and were retained untreated for a 4-week recovery period following the termination of treatment. A further cohort of rats (14/sex/group) were included for the toxicokinetic evaluation. Blood samples were obtained prior to dosing and at 5, 30 min and at 1, 2, 4 and 6 hr post-dose on weeks 1, 5, 13 and 26. Concentrations of HPMPC were determined by a validated HPLC method. Results: the pharmacokinetic parameters are listed in Table 6. Repeated administration of HPMPC led to apparent increases in AUC values from week 1 to 26. These changes were greater at the high dose level (2.6-fold increase in the AUC for males vs 1.9-fold increase for females at the dose level).

Table 6  
Pharmacokinetic parameters of HPMPC in a 26 week iv toxicity study in rats

Parameter	Week	Low dose	Mid dose	High dose	High dose
		♂	♂	♂	♀
C <sub>0</sub> (ng/ml)	1	1.55	6.27	27.4	33.9
	5	1.62	8.71	46.9	47.5
	13	1.73	10.7	63	56.3
	26	2.36	11.4	55.5	59.1
AUC <sub>0-6</sub> (ng*hr/ml)	1	nd	2.62	11.6	13.3
	5	nd	3.35	19.2	19.3
	13	nd	4.41	27.2	22.8
	26	nd	4.89	30.5	24.8
T <sub>1/2</sub> (hr)	1	nd	nd	2.23	1.63
	5	nd	nd	2.79	2
	13	nd	nd	2.08	1.5
	26	nd	nd	2.07	1.59

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**Comments:** The data suggested that repeated exposure to HPMPC led to a decrease in renal clearance of HPMPC. The changes were apparently greater for males than for females. The present study demonstrated that males rats displayed greater changes in HPMPC pharmacokinetics than females. Histopathological evaluation of kidney tissue from rats exposed to iv HPMPC once weekly for 26 weeks indicated that a dose-dependent toxicity (characterized by karyomegaly only) was present in males at all dose levels. In females, the dose-dependent toxicity occurred only at the high dose level. Therefore, the greater decrease in renal clearance in male rats compared to female rats reflects the greater nephrotoxicity observed in males.

**8. Pharmacokinetics, bioavailability, metabolism and tissue distribution of <sup>14</sup>C-cyclic HPMPC in Sprague-Dawley rats (94-DDM-0930-002).**

Three groups of 4 male rats received a single dose of <sup>14</sup>C-cyclic HPMPC (5 mg/kg; 330  $\mu$ Ci/kg) by the iv, sc or oral routes and a fourth group of 4 rats received a single iv dose of <sup>14</sup>C-HPMPC (5 mg/kg; 330  $\mu$ Ci/kg) to compare the pharmacokinetics and distribution of <sup>14</sup>C-cyclic HPMPC and <sup>14</sup>C-HPMPC. In a second study, two additional groups of four rats received a single dose of <sup>14</sup>C-cyclic HPMPC (5 mg/kg; 600  $\mu$ Ci/kg) by the iv or oral routes. Serial blood samples were obtained over the course of 24 hr and were processed for the analyses. The animals were euthanized after the last sample; selected tissues were removed from animals in the first study for the analyses. **Results:** total <sup>14</sup>C-activity in plasma following the iv administration showed a multi-exponential decline for both the drugs with similar terminal elimination half-lives of 9-12 hr. The pharmacokinetic and tissue distribution parameters are shown in Tables 7-9.

Table 7

Mean concentration of total radioactivity in tissues (ng-equiv/g) following administration of <sup>14</sup>C-cyclic HPMPC or <sup>14</sup>C-HPMPC via different routes in rats at 5 mg/kg (n=4)

Tissues	<sup>14</sup> C-cyclic HPMPC			<sup>14</sup> C-HPMPC
	iv	sc	oral	iv
Adrenal glands	20	-	-	-
Aqueous humor	-	-	-	-
Brain	2	-	-	9
Cecum	26	-	159	72
Colon	40	-	446	89
Duodenum	19	-	8	19
Heart	13	13	1	33
Ileum	15	-	12	36
Jejunum	15	-	7	26
Kidney	343	351	108	6610
Liver	294	292	19	368
Lung	42	29	4	56
Lymph node	53	-	-	-
Pancreas	28	-	-	40
Rectum	50	-	169	59
Skeletal muscle	13	9	-	24
Skin	26	36	5	45
Spleen	56	45	4	82
Stomach	12	-	18	24
Testes	16	-	-	17
Thymus	27	-	-	-
Vitreous humor	-	-	-	-

Table 8

Mean pharmacokinetic parameters for  $^{14}\text{C}$ -cyclic HPMPc and  $^{14}\text{C}$ -HPMPc at 5 mg/kg (n=4) in rats

Parameters	$^{14}\text{C}$ -cyclic HPMPc			$^{14}\text{C}$ -HPMPc
	iv	sc	oral	iv
AUC ( $\mu\text{g}\cdot\text{eq}/\text{kg}$ )	4.6	3.8	0.16	6.47
C <sub>max</sub> ( $\mu\text{g}\cdot\text{eq}/\text{ml}$ )	-	5.6	0.05	-
T <sub>max</sub> (hr)	-	0.5	0.6	-
MRT (hr)	0.3	0.65	3.02	3.68
Cl (l/kg/hr)	1.1	-	-	0.79
V <sub>dss</sub> (l/kg)	0.32	-	-	2.96
T <sub>1/2</sub> (hr)	0.53	0.43	2.07	9.26
F (%)	-	82.7	3.5	-

Table 9

Relative abundance of radiolabelled metabolites in tissues of a single rat at 24 hr after administration of  $^{14}\text{C}$ -cyclic HPMPc or  $^{14}\text{C}$ -HPMPc at (5 mg/kg; 330  $\mu\text{Ci}/\text{kg}$ )

Tissue	Compound	Percent of total radioactivity attributed to a compound		
		$^{14}\text{C}$ -cyclic HPMPc		$^{14}\text{C}$ -HPMPc
		iv	oral	iv
Kidney	cHPMPc	1.4	-	-
	HPMPc	44.3	-	35.3
	Metabolite I	54.3	-	59.6
	Metabolite II	-	-	5.1
Liver	cHPMPc	4.5	-	-
	HPMPc	44.5	-	64.7
	Metabolite I	51.1	-	28.6
	Metabolite II	-	-	6.7
Lung	cHPMPc	5.8	-	-
	HPMPc	50.7	-	-
	Metabolite I	43.5	-	-
	Metabolite II	-	-	-
Colon	cHPMPc	-	0.7	-
	HPMPc	100	97.4	91.2
	Metabolite I	-	2	1.4
	Metabolite II	-	-	7.4

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**Comments:** The total plasma clearance of cHPMPC (1.1 l/hr/kg) was higher than the published value for the GFR in rats (0.6 l/hr/kg). Total clearance is the sum of renal clearance and other clearance mechanisms, including metabolism. The corrected plasma AUC for cHPMPC accounted for 96.1% of the AUC of total radioactivity in plasma. Therefore, 3.9% of the administered dose of cHPMPC was metabolized to HPMPC following iv injection (i.e., metabolism accounted for 3.9% of the clearance of cHPMPC). Since this value is small, the observed total clearance of cHPMPC must be close to the renal clearance of the drug and reflects significant tubular secretion. The percent of renal clearance attributed to active secretion (calculated as  $100 * (Cl_r - GFR) / Cl_r$ ) was therefore approximately 45% for cHPMPC.

**9. Absolute bioavailability and tissue distribution of three formulations of <sup>14</sup>C-HPMPC following intravenous or topical administration to New Zealand white rabbits, Lot # DB-579-89, July 1, 1994, (2-T39/93-DDM-0504-004, T39-94-69)**

Four groups of male rabbits (4 animals/group) were used to compare the absolute bioavailability and tissue distribution of two topical formulations of HPMPC labelled with <sup>14</sup>C. Group 1 received a single intravenous administration of HPMPC at a dose of 1 mg/kg. Group 2 received 1% w/w HPMPC formulation in a gel prepared from propylene glycol (PG) and hydroxyethylcellulose (HEC) as a single topical application to an abraded skin site (0.7 mg/kg). Group 3 received the same 1% w/w gel formulation of HPMPC in PG/HEC as a single topical application to a normal skin site (0.7 mg/kg). Group 4 received a 1% w/w HPMPC formulation in a gel prepared from HEC alone as a single topical application to a normal skin site (0.7 mg/kg). **Results:** a tabulated summary of significant mean tissue distribution and plasma concentrations data are summarized in Table 10.

Table 10

Summary of significant mean tissue distribution and plasma concentration data of HPMPC in rabbits

Treatment	Intravenous	Topical PG/HEC		Topical HEC
Site	Ear Vein	Normal skin	Abraded skin	Normal skin
Group	1	2	3	4
Dose (mg/kg)	0.96	0.67	0.73	0.72
24 hr feces recovery (%)	<0.07	<0.07	<0.07	<0.07
24 hr urinary recovery (%)	77.5	2.0	21.6	0.6
% dose in kidney at 6 hr	32.0	0.3	21.7	0.2
% dose in kidney at 24 hr	7.7	0.3	3.0	0.1
% dose in testes at 6 hr	0.1	0.0	0.1	0.01
% recovery at administration site rinse at 6 hr	-	80	27	54.7
% recovery at administration site at 6 hr	-	8.6	-	14.8
% recovery at administration site rinse at 24 hr	-	81	52	41.7
% recovery at administration site at 24 hr	-	10.6	-	18.5
C <sub>max</sub> (µg/ml)	3.55	0.02	0.25	-
T <sub>max</sub> (hr)	0.1	6.0	2.0	-
AUC (µg*hr/ml)	3.75	0.05	1.0	-
Bioavailability (%)	100	2.1	41.0	-
T <sub>1/2</sub> (hr)	5.4	-	-	-
V <sub>ss</sub> (ml/kg)	710.5	-	-	-
Cl <sub>T</sub> (ml/hr/kg)	262.7	-	-	-
MRT (hr)	2.7	-	-	-

At 5 min following the iv administration, the mean circulating HPMPC plasma level was 3.55 µg/ml and decreased steadily during the study period. Following the topical administration of PG/HEC gel to abraded skin, the first quantifiable plasma HPMPC level was observed at 30 min post-treatment and peaked at 2 hr post-treatment at 0.25 µg/ml. Following the topical administration of PG/HEC gel to normal skin, the first quantifiable plasma HPMPC level was not observed until 4 hr post-treatment, after which the plasma level peaked at 6 hr post-dose before dropping below the quantifiable level by 8 hr post-dose. The topical administration

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of HEC gel formulation to intact skin produced no quantifiable plasma level of HPMPC at any timepoint during the 24 hr post-dose period.

**Comments:** A comparison of the groups that received a topical administration of the <sup>14</sup>C-HPMPC PG/HEC gel formulation to normal or abraded skin indicated that HPMPC absorption was enhanced by the abrasion process. Secondly, since no measurable plasma HPMPC levels were seen following HEC formulation of HPMPC to normal skin, it appeared that this formulation demonstrated lower absorption than the PG/HEC gel formulation when applied to normal skin of New Zealand white rabbits. Finally, significant percentage of the total dose of HPMPC was recovered in the kidneys and skin sites. This finding is consistent with the observation that these organs have been identified as major target organs in rats and monkeys (nonclinical safety studies)

**10. Intraocular distribution of radioactivity following single iv or intraocular doses of <sup>3</sup>H-HPMPC in rabbits, October 14, 1993, (93-DDM-0504-001/PH-845GS-001-93/P0504-00007)**

Three groups of male rabbits (6/group) received a single dose of <sup>3</sup>H-HPMPC (20 µCi) on one occasion by intraocular dosing at 2 mg (G1), iv dosing at 5 mg/kg (G2) or iv dosing at 5 mg/kg with 90 mg/kg probenecid (G3). Two rabbits per group were sacrificed by carbon dioxide inhalation at 0.25, 1 and 4 hr post-dose. **Results:** all rabbits in G2 and G3 exhibited severe irritability. No additional signs were observed from these rabbits during the remainder of study. Maximum mean plasma radioactivity concentrations of 8.52 and 13.2 µg-eq/ml were observed at 0.25 hr post-dose for G1, and (G2 and G3), respectively. Following both the iv treatments (G2 and G3), mean plasma radioactivity levels were higher than the concurrent concentrations observed in the ocular and brain tissues but lower than the kidney tissues. After the intraocular administration, systemic levels were only quantifiable in one rabbit at 0.25 hr post-dose, and the concentration was less than 1/300th of the concurrent concentrations for either iv treatment. The highest levels of ocular radioactivity following the iv administration were found in the aqueous humor and retina. Following the io administration, cornea radioactivity levels were the highest for all ocular tissues. The lowest level of radioactivity in the ocular tissues for all groups and at most time points was typically found in the vitreous humor. Radioactivity concentrations in the kidneys following the io dosing were relatively constant throughout the 4 hr post-dose sampling period and were less than 1/100th of those following either iv administration.

**Comments:** The route of administration and the presence of co-administered probenecid did not seem to alter the ocular distribution of radioactivity. However, probenecid may contribute

to lower levels of HPMPC in the kidneys.

11. Effect of probenecid on the tissue distribution of  $^{14}\text{C}$ -radiolabelled HPMPC following iv administration to rabbits, June, 15, 1995, (93-DDM-0504-002, '04-94-36)

Six groups of male rabbits (2/group) were utilized to compare the tissue distribution of  $^{14}\text{C}$ -HPMPC (100  $\mu\text{Ci}/\text{kg}$ ) when administered as a single dose of 15 mg/kg iv injection to rabbits. Table 11 summarizes the study design.

Table 11  
Study design

Group #	# of males	Treatment	Route	Blood sample	Urine/feces sample	Tissue sample
1	2	15 mg/kg $^{14}\text{C}$ -HPMPC	iv	30 min	0-30 min	30 min
2	2	15 mg/kg $^{14}\text{C}$ -HPMPC	iv	6 hr	0-6 hr	6 hr
3	2	15 mg/kg $^{14}\text{C}$ -HPMPC	iv	24 hr	0-24 hr	24 hr
4	2	90 mg/kg probenecid	oral	30 min	0-30 min	30 min
		15 mg/kg $^{14}\text{C}$ -HPMPC	iv			
5	2	90 mg/kg probenecid	oral	6 hr	0-6 hr	6 hr
		15 mg/kg $^{14}\text{C}$ -HPMPC	iv			
6	2	90 mg/kg probenecid	oral	24 hr	0-24 hr	24 hr
		15 mg/kg $^{14}\text{C}$ -HPMPC	iv			

**Results:** at 30 min post-dose, rabbits (G1) had the greatest percentage of the radioactivity present in the kidneys, skeleton muscle, skin and blood/plasma. Rabbits (G4) had the greatest percentage of recovered radioactivity present in the skeletal muscle, kidney and blood/plasma. At 6 hr post-dose, the mean recovery of radioactivity in the urine was 83.9% in animals given HPMPC alone and 111.2% in animals given oral probenecid prior to HPMPC administration. The mean concentration of the radioactivity in the kidney was 499  $\mu\text{g-eq}/\text{g}$  in animals given HPMPC alone and 599  $\mu\text{g-eq}/\text{g}$  in animals given oral probenecid prior to HPMPC administration. At 24 hr post-dose, the greatest percentage of the radioactivity was present in the urine, kidney and skeletal muscle of all animals. The mean recovery of radioactivity in the urine was 98.3% in animals given HPMPC alone and 109.4% in animals given oral probenecid prior to HPMPC administration. The mean concentration of HPMPC in the kidney was 268  $\mu\text{g-eq}/\text{g}$  in animals given HPMPC alone and 125  $\mu\text{g-eq}/\text{g}$  in animals given oral probenecid prior to HPMPC administration. These data suggested that clearance of HPMPC from the kidney might have been faster in

the presence of probenecid.

**Comments:** The above data indicated that a single oral dose of probenecid had no discernible effect on the aggregate distribution of radioactivity at the initial time point (30 min). However, clearance of radioactivity from the cortex of the kidney following the iv administration of radioactive HPMPC appeared to have been accelerated by pretreatment with oral probenecid at a 90 mg/kg dose.

**12. Analysis of data from study 2-T04: Effect of probenecid on the tissue distribution of <sup>14</sup>C-HPMPC following iv administration to rabbits, August 2, 1995, (93-DDM-0504-002/P0504-00015)**

Three groups (2/group) of male rabbits were dosed iv with <sup>14</sup>C-HPMPC (15 mg/kg; 100 µCi/kg) alone and three groups of animals received the same one hr after oral administration of probenecid (90 mg/kg). **Results:** are described in Table 12 and 13. Highest concentrations of radioactivity were present in kidney (700 µg-eq/g at 30 min postdose). The concentrations in all tissues declined over 24 hr period. Initial clearance from the kidney was slower than from other tissues. No phosphorylated metabolites of HPMPC were detected in kidney or liver samples, regardless of probenecid regimen. Analysis of urine and kidney tissue revealed an unknown metabolite, apparently co-eluting with the unknown metabolite observed previously in monkey urine following iv administration of HPMPC.

**Table 12**

Effect of probenecid on distribution of radioactivity following iv administration of <sup>14</sup>C-HPMPC to rabbits

Tissue	Concentration of total radioactivity (µg-eq/g) in tissues					
	Without probenecid			with probenecid		
	30 min	6 hr	24 hr	30 min	6 hr	24 hr
Kidney	671.3	498.8	267.8	731.8	598.6	125.4
Liver	9.2	3.4	2.0	7.4	2.7	1.9
Skin	20.6	0.9	0.6	18.6	2.9	0.8
Skeletal muscle	7.4	0.2	0.3	11.5	1.12	0.7
Testes	3.0	1.3	0.4	3.1	1.7	0.4
Plasma	28.2	1.0	0.2	40.4	1.1	0.2
Urinary recovery	0	83.9	98.3	0	111.2	109.4

Table 13

Relative abundance of  $^{14}\text{C}$ -HPMPC metabolites in urine and kidney of rabbits

Location	Species (retention time in min)	Mean % dose present as species					
		Without probenecid			with probenecid		
		30 min	6 hr	24 hr	30 min	6 hr	24 hr
Urine	unknown (6.4)	-	1.2	2.2	-	0.9	1.5
	HPMPC (11.3)	-	98.8	97.8	-	99.1	98.5
Kidney	unknown (6.4)	1.1	2.7	2.3	3.0	4.2	2.0
	HPMPC (11.3)	98.9	97.4	97.8	97.1	95.8	98.1

**Comments:** Autoradiography indicated that initial levels (30 min post-dose) of radioactivity achieved in the cortex of the kidney were reduced almost 2-fold by the presence of concomitant probenecid. This suggested that the initial high concentrations of probenecid were sufficient to block active tubular secretion of HPMPC, leading to a decreased accumulation of the drug in the proximal tubular cell of the renal cortex. These data supported the clinical use of probenecid as a nephro-protectant during the HPMPC therapy.

### 13. Oral bioavailability of HPMPC and cyclic HPMPC in beagle dogs (94-DDM-0930-006).

Two groups of dogs received a single oral dose of cHPMPC (10 mg/kg) or HPMPC (10 mg/kg); followed by a two weeks of washout period, the same animals received a single iv dose of cHPMPC (10 mg/kg) or HPMPC (10 mg/kg) for the determination of the oral bioavailability in dogs. Plasma concentrations of cHPMPC and HPMPC were monitored over the course of 24 hr by a validated HPLC method. **Results:** following the iv administration of cHPMPC, concentrations of cHPMPC declined in an apparent mono-exponential manner. Following the oral or iv cHPMPC administration, there were no quantifiable levels of HPMPC present in any of the plasma samples. The mean pharmacokinetic parameters are summarized in Table 14.

Table 14

Mean Pharmacokinetic parameters for cHPMPC (10 mg/kg) and HPMPC (10 mg/kg) following iv and oral administrations to dogs (n=5)

Parameters	cHPMPC	HPMPC
Oral, F (%)	21.9	21.3
Oral, C <sub>max</sub> (µg/ml)	1.51	1.37
Oral, T <sub>max</sub> (hr)	1.5	1.3
MRT (hr)	0.79	2.59
Cl <sub>r</sub> (l/hr/kg)	0.35	0.29
V <sub>dss</sub> (l/kg)	0.27	0.76
T <sub>1/2</sub> (hr)	0.68	5.7

**Comments:** The total body clearance of cHPMPC was slightly greater than that observed for HPMPC. Both drugs were actively secreted by the kidney. Although, cHPMPC was less nephrotoxic than HPMPC in the animals. This may be the result of a more efficient efflux of cHPMPC from the kidney cells into the tubular lumen. Conversion of cHPMPC to HPMPC was undetectable in the present study. However, data in rats and monkey suggested that 5-10% of an iv dose of cHPMPC was ultimately converted to HPMPC within cells. These data suggested a low extent of conversion from cHPMPC to HPMPC in dogs.

14. **A Summary of Single-Dose and Multiple-Dose Pharmacokinetics of <sup>14</sup>C-HPMPC, Lot # 28648-7 and 28701-18-1 in the African Green Monkey**  
 -257/904      -41671-01/02)  
 July 31, 1991,

#### Single Dose study in Monkeys

This was a three part study to determine the pharmacokinetics of the drug after IV, SC and oral administration, as well as to determine the concentration of the drug in the kidneys at various times after the IV administration. Thirteen fasted and lightly sedated monkeys were administered a single dose of <sup>14</sup>C-HPMPC at 50 mg (26.1 µCi)/kg. Blood samples were taken at 0, 5, 10, 20, 40 min, and 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hr after the dosing. After the IV dosing, one monkey was randomly selected at 1, 6, 24 and 72 hr after the dosing and sacrificed for the kidney removal. The drug concentration was determined in the plasma and kidney samples by a combination of combustion and a liquid scintillation counting method. **Results:** the terminal half-life was estimated from a least squares, linear regression calculation of blood concentration vs time. The AUC was calculated by the trapezoidal rule and the clearance was

estimated from the dose divided by the AUC after the IV administration of the drug. The Vd was estimated from the clearance multiplied by  $t_{1/2}/0.693$ . The mean single dose pharmacokinetics parameters for the drug in monkeys are summarized in Table 15 and the concentration of the drug measured in the kidney and corresponding plasma sample after a single IV administration are shown in Table 16. The oral bioavailability of the drug is low (23%); whereas, sc administered is almost completely bioavailable (91%). The concentrations of the drug measured in the kidney and corresponding plasma samples after a single IV dose (Table 16) show that the concentration of the drug in the kidney decreased from 1212  $\mu\text{g-eq/ml}$  at 1 hr to a mean concentration of 17.2  $\mu\text{g-eq/ml}$  at 120 hr; whereas, the plasma concentration of the drug decreased from 34.7  $\mu\text{g-eq/ml}$  at 1 hr to a mean concentration of 0.015  $\mu\text{g-eq/ml}$  at 120 hr.

**Comments:** Subcutaneous administration of HPMPC may be a viable alternative to the IV for frequent parenteral administration. The clearance (216 ml/hr.kg) of the drug after the IV dose is higher than the nominal GFR in the monkey (180 ml/hr.kg). The terminal half-life after oral and SC administrations are about 0.7 times that determined after IV dosing (32.9 hr), suggesting an increased metabolism of the drug via these routes. Also, these data suggest that the elimination of the drug in the kidney occurs with a half-life of (23 hr), which approximates the plasma half-life. No drug-related histopathologic changes were observed in the monkeys when dosed with the either routes.

**Table 15**  
Summary of Pharmacokinetics Parameters for  $^{14}\text{C}$ -HPMPC after the Single Dose Administration to African Green Monkey

Route & Dose (mg/kg)	$C_{\text{max}}$ ( $\mu\text{g-eq/ml}$ )	$T_{\text{max}}$ (hr)	$T_{1/2}$ (hr)	$Cl_r$ (ml/hr.kg)	Vd (L/kg)	AUC <sub>0-∞</sub> ( $\mu\text{g-eq.hr/ml}$ )	F (%)
Oral 44.0	2.6± 2.0	14± 9.3	24. 2.2	206	10.2	47.9± 11.4	23
SC 43.4	76.9± 17.8	0.42± 0.08	22± 6.6	204.9	6.5	171.1± 25.8	91
IV 43.4	174.6± 12.9	0.08	33± 4.3	216± 17	10.3± 2.1	209.9± 15.3	100

NDA-020638

FIRM: GILEAD

TRADE NAME: VISTIDE (CIDDOFOVIR)

GENERIC NAME: CIDDOFOVIR

4 OF 4

Table 16

Concentration of  $^{14}\text{C}$ -HPMPC in Plasma and Kidney after the Single IV Dose (43.4 mg/kg) Administration to African Green Monkey

Time (hr)	Conc. in Kidney ( $\mu\text{g}\cdot\text{eq/g}$ )	Conc. in Plasma ( $\mu\text{g}\cdot\text{eq/ml}$ )	Ratio Kidney/Plasma
1	1212	35	35
6	464	3.9	122
24	308	0.3	986
72	49	0.03	1617
120	20	0.02	1040

#### Multiple Dose Study in Monkey

In the multiple dose study, 4.9 mg (16.6  $\mu\text{Ci}$ )/kg for 10 days) of the drug was injected to the monkeys. Blood samples were taken at 0, 5, 15 and 30 min, and 1, 1.5, 2, 4, 6, 8, 12 and 24 hr after administration of the first dose. On days 4, 6, 8 and 10 a single blood sample was taken immediately before administration of the next dose. In addition on day 10 of the study, blood samples were taken from 4 monkeys at the same time points used on the first day of the study. Results: the pertinent information from the study is summarized in Table 17 and a summary of the mean multiple dose pharmacokinetics parameters are summarized in Table 18.

Comments: The mean trough concentrations ( $C_{\min}$ ) of the drug measured and the AUCs on day 1 and 10 indicated that there is an accumulation of the drug. The mean AUC on day 10 of the study is about twice that determined for the first day of the study (13.4  $\mu\text{g}\cdot\text{eq}\cdot\text{hr/ml}$ ). The total clearance in the multiple IV dose study (275 ml/hr.kg) is about 33% greater than the  $Cl$  in the single dose IV study (207 ml/hr.kg). In the multiple IV dose study, the increase in  $Cl$ , and the decrease in elimination  $t_{1/2}$  (27 hr) are not, however, consistent with the observed accumulation (elevated  $C_{\min}$  and AUCs) of HPMPC. Secondly, the concentration of the drug in the kidneys and corresponding plasma samples from the monkeys administered multiple IV doses, on day 1 and 10 of the study, are comparable; suggesting that there appeared to be no additional accumulation of the drug in the kidney. However, in the monkeys that were sacrificed after the 10th dose, renal toxicity was observed in 2/2 monkeys. Thus, there is a discrepancy in the data presented and it is not clear which factor(s) contribute to the accumulation.

Table 17

Mean Trough, Plasma and Kidney Concentrations, and AUC of <sup>14</sup>C-HPMPC after the Multiple IV Administration (4.9 mg/kg/day) to the African Green Monkeys for 10 days

Day	Trough Conc. (µg-eq/ml)	AUC (µg-eq.hr/ml)	Conc. in Kidney (µg-eq/ml)	Conc. in Plasma (µg-eq/ml)
1	0.024	13.4, N=6	52.6, N=1	0.031
4	0.033	.	.	.
6	0.036	.	.	.
8	0.043	.	.	.
10	0.044	22.6, N=6	53.8, N=2	0.044
11	0.051	.	.	.

Table 18

Summary of Multiple Dose Pharmacokinetics Parameters (after administration of the last dose) after the IV Administration of <sup>14</sup>C-HPMPC (4.6 mg/kg/day) to African Green Monkeys for 10 days

Route & Dose (mg/kg)	t <sub>1/2</sub> (hr)	Cl <sub>T</sub> (ml/hr.kg)	Vd (L/kg)	AUC <sub>0-∞</sub> (µg-eq.hr/ml)
IV, 4.6	26.5	275	10.5	18.2

15. Cidofovir concentrations in serum samples from a 13-week iv toxicity study in cynomolgus monkeys (a report on partial analysis of samples from toxicity study 2-P51), Lot # 504B93-01, Gilead Science, Inc., Foster City, CA, March 8, 1995, (93-TOX-2-P51-BA/PO504-00036)

Four groups of normal male and female cynomolgus monkeys (weight: 2.9-3.6 kg males and 2.7-3.4 kg females; age: unknown; 3-5 animals/sex/group) were administered HPMPC via intravenous injection at dose levels of 0 (vehicle control), 1.0 (low), 5 (mid) or 20 mg/kg once per week (high) for a period of 13 weeks. Plasma samples were obtained from surviving animals at 1, 2, 3, 4, 6 and 12 hr post dose on day 1 of weeks 1, 4, 8 and 12. The samples were analyzed from 2 male and 2 female monkeys for each low (weeks 1 and 12) and high (weeks 1 and 4) dosage groups only. Results are summarized in Table 19 and 20.

Table 19

Non-compartmental Pharmacokinetic Parameters for Cidofovir  
IV Administration in Monkeys (Mean of 2 Animals)

Parameters	1 mg/kg/week			20 mg/kg/week		
	Week 1;σ	Week 1;♀	Week 12;σ	Week 1;σ	Week 1;♀	Week 4;σ
AUC <sub>0-∞</sub> (μg·hr/ml)	3.7	1.87	3.56	104	69.2	199
MRT (hr)	1.73	1.12	1.21	1.45	1.49	2.92
CL <sub>r</sub> (ml/hr/kg)	280	348	284	194	306	101
V <sub>dis</sub> (ml/kg)	472	626	339	282	473	295
T <sub>1/2</sub> (hr)	1.29	0.93	0.93	1.07	1.04	1.56
C <sub>max</sub> (μg/ml)	1.85	1.64	2.45	64.4	39.2	82.0

Table 20

Dose Normalized AUC Values

Dose (mg/kg/week)	Sex	Week	AUC/Dose
1	male	1	3.7
1	female	1	1.87
1	male	12	3.56
20	male	1	3.18
20	female	1	3.46
20	male	4	9.92

Comments: Clearance of Cidofovir (high) apparently decreased by week 4 of the study; at the same time, the AUC value was 1.9 times greater than week 1. The apparent decrease in clearance of Cidofovir following 4 weeks of dosing (high) may be a consequence of the nephrotoxicity observed in this dose group.

16. HPMPC concentrations in serum samples from a 30-day sc toxicity study of HPMPC in monkeys, April 18, 1995, (94-TOX-2-N13-BA/P0504-0038)

Groups of male and female cynomolgus monkeys (weight: 2.9-3.4 kg; 3 animals/sex/group) were administered HPMPC via subcutaneous injection at dose levels of 0 (vehicle control), 0.5 (low), 1.5 (mid) or 7.5 mg/kg (high) twice per week (total of 9 injections) for a period of 4 weeks. Serial blood samples (high) were obtained at day 1 and day 28. Results: are shown in Table 21.

Mean AUC values showed no apparent sex difference at the high dose.

Table 21

Non-compartmental Pharmacokinetic Parameters for Cidofovir SC Administration in Monkeys at 7.5 mg/kg bi weekly

Parameters	Day 1		Day 28	
	♂	♀	♂	♀
AUC <sub>0-24</sub> (µg <sup>h</sup> /ml)	36.5	33.4	79.7	60.9
MRT (hr)	2.87	2.47	3.01	2.76
CL <sub>T</sub> (ml/hr/kg)	210	234	101	142
V <sub>dss</sub> (ml/kg)	603	583	297	357
T <sub>1/2</sub> (hr)	2.98	2.95	2.17	2.49
C <sub>max</sub> (µg/ml)	16	18	27.3	23.1

Comments: There was a significant increase in exposure to HPMPC at day 28 in both male and female animals. This increase in exposure appeared to have resulted from a significant decrease in total body clearance of the drug at day 28.

17. Effect of probenecid on the urinary excretion of <sup>14</sup>C-HPMPC following iv administration to monkeys, April 22, 1994, (2-T03/P0504-00012)

Two groups of male and female monkeys (1-2 animal/sex/group) received <sup>14</sup>C-HPMPC (100 µCi) at a dose level of 10 mg/kg via a single bolus iv injection. One of the groups received an oral administration of 30 mg/kg probenecid (G2) one hr prior to the iv administration of HPMPC. Results: in G1, 4 hr following iv administration of HPMPC, circulating HPMPC plasma levels were 1.37 and 1.03 µg-eq/ml in male and female animals, respectively. In the G2 animals, circulating HPMPC levels were 1.91 and 0.86 µg-eq/ml in male and female monkey, respectively. The urine excretion data (G1) showed that the total radioactivity recovered after 168 hr of urine collection following the administration of HPMPC were 102.96% and 85% for male and female monkeys, respectively. In G2, the total recoveries were 80.47 and 103% for male and female animals, respectively.

18. Effect of probenecid on the urinary excretion of <sup>14</sup>C-HPMPC following iv administration to monkeys, June 27, 1994, (93-DDM-0504-003/P0504-00018)

The effect of concomitant oral probenecid on the urinary

excretion of  $^{14}\text{C}$ -HPMPC was examined in monkeys. Animals were dosed iv with  $^{14}\text{C}$ -HPMPC (10 mg/kg; 100  $\mu\text{Ci}/\text{kg}$ ) alone or one hr after oral administration of probenecid (30 mg/kg). Urine samples were cage collected over 7 days post-dose. Results: are summarized in Table 22-24. Analysis of urine samples by HPLC revealed the presence of an unknown metabolite of HPMPC in urine of all animals. Excretion of the unknown metabolite was essentially complete by about 72 hr post-dose. This metabolite accounted for 1.9% and 2.2% of the administered dose recovered in urine of animals given HPMPC alone or with probenecid, respectively.

Table 22

Effect of probenecid on urinary recovery of total radioactivity following iv administration of  $^{14}\text{C}$ -HPMPC

Animals	% $^{14}\text{C}$ -dose recovered in urine			
	24 hr		7 days	
	(-) probenecid	(+) probenecid	(-) probenecid	(+) probenecid
1 male	87.1	74.7	102.9	80.5
2 male	72.1	118.7	80.3	128.6
3 female	79.7	64.6	91	74
Mean $\pm$ SD	79.6 $\pm$ 7.5	86 $\pm$ 28.8	91.4 $\pm$ 11.3	94.4 $\pm$ 29.8

Table 23

Effect of probenecid on terminal elimination half-life of total radioactivity in urine following iv administration of  $^{14}\text{C}$ -HPMPC

Animal	Terminal half-life (hr)	
	without probenecid	with probenecid
1 male	44.8	30.1
2 female	31.3	20.9
3 female	23.9	22.2
Mean $\pm$ SD	33.3 $\pm$ 10.6	24.4 $\pm$ 5

Table 24  
Relative abundance of <sup>14</sup>C-HPMPC metabolites in urine

Species (retention time in min)	Mean % dose excreted as species	
	without probenecid	with probenecid
unknown (6.4)	1.9	2.2
HPMPC (11.3)	89.5	92.1

**Comments:** The present study represented the first time that an extracellular metabolite of HPMPC had been formed in vivo. Probenecid had no statistically significant effect on the rate or extent of recovery of total radioactivity in urine following iv administration of radiolabelled HPMPC. In addition, probenecid had no effect on the rate and extent of urinary recovery of HPMPC or its unknown metabolite.

19. Cidofovir concentrations in serum samples from a 13-week iv toxicity study in cynomolgus monkeys (a report on partial analysis of samples from toxicity study 2-P51), Lot # 504B93-01, Gilead Science, Inc., Foster City, CA, March 8, 1995, (93-TOX-2-P51-BA/PO504-00036)

Four groups of normal male and female cynomolgus monkeys (weight: 2.9-3.6 kg males and 2.7-3.4 kg females; age: unknown; 3-5 animals/sex/group) were administered HPMPC via intravenous injection at dose levels of 0 (vehicle control), 1.0 (low), 5 (mid) or 20 mg/kg once per week (high) for a period of 13 weeks. Plasma samples were obtained from surviving animals at 1, 2, 3, 4, 6 and 12 hr post dose on day 1 of weeks 1, 4, 8 and 12. The samples were analyzed from 2 male and 2 female monkeys for each low (weeks 1 and 12) and high (weeks 1 and 4) dosage groups only. Results are summarized in Table 25 and 26.

Table 25

Non-compartmental Pharmacokinetic Parameters for Cidofovir  
IV Administration in Monkeys (Mean of 2 Animals)

Parameters	1 mg/kg/week			20 mg/kg/week		
	Week 1;♂	Week 1;♀	Week 12;♂	Week 1;♂	Week 1;♀	Week 4;♂
AUC <sub>0-∞</sub> (μg·hr/ml)	3.7	1.87	3.56	104	69.2	199
MRT (hr)	1.73	1.12	1.21	1.45	1.49	2.92
CL <sub>r</sub> (ml/hr/kg)	280	548	286	194	306	101
Vdss (ml/kg)	472	624	339	282	473	295
T <sub>1/2</sub> (hr)	1.29	0.93	0.93	1.07	1.04	1.56
Cmax (μg/ml)	1.85	1.64	2.45	64.4	39.2	82.0

Table 26

Dose Normalized AUC Values

Dose (mg/kg/week)	Sex	Week	AUC/Dose
1	male	1	3.7
1	female	1	1.87
1	male	12	3.56
20	male	1	5.18
20	female	1	3.46
20	male	4	9.95

Comments: Clearance of Cidofovir (high) apparently decreased by week 4 of the study; at the same time, the AUC value was 1.9 times greater than week 1. The apparent decrease in clearance of Cidofovir following 4 weeks of dosing (high) may be a consequence of the nephrotoxicity observed in this dose group.

21. Concentration of HPMPC in plasma samples from a 26-week repeated dose subcutaneous toxicity study in Sprague-Dawley rats, Gilead Sciences, Inc., Foster City, CA, September 8, 1994, (2-T-70/94-TOX-0504-003-BA)

HPMPC concentrations were determined in plasma samples obtained from a 26-week sc toxicity study in rats. Groups of rats were administered HPMPC via sc route at dose levels of 0 (vehicle control), 0.6 (low), 3.0 (mid) or 15.0 mg/kg/weekly (high). Plasma samples were obtained at 0, 1, 2, 3, 4, 6 and 12 hr post-

dose at week 1, 5 and 14 using 4 rats per time point. Samples were analyzed using a validated bioanalytical method for HPMPC in rat plasma. Results are shown in Table 27.

**Table 27**  
Pharmacokinetic Parameters for HPMPC following Repeated Subcutaneous Administration to Male Rats

Dose (mg/kg/weekly)	Parameter (Plasma)	Week of Study		
		1	5	14
0.6	AUC ( $\mu\text{g}^{\text{hr}}/\text{ml}$ )	0.14	0.49	0.55
	C <sub>max</sub> ( $\mu\text{g}/\text{ml}$ ), Mean $\pm$ SD	0.28 $\pm$ 0.03	0.30 $\pm$ 0.21	0.44 $\pm$ 0.09
	T <sub>max</sub> (hr)	1	1	1
3.0	AUC ( $\mu\text{g}^{\text{hr}}/\text{ml}$ )	1.31	4.11	3.18
	C <sub>max</sub> ( $\mu\text{g}/\text{ml}$ ), Mean $\pm$ SD	1.06 $\pm$ 0.10	1.72 $\pm$ 0.27	1.94 $\pm$ 0.11
	T <sub>max</sub> (hr)	1	1	1
15.0	AUC ( $\mu\text{g}^{\text{hr}}/\text{ml}$ )	8.83	7.32	8.71
	C <sub>max</sub> ( $\mu\text{g}/\text{ml}$ ), Mean $\pm$ SD	6.70 $\pm$ 1.26	2.38 $\pm$ 0.14	4.60 $\pm$ 0.41
	T <sub>max</sub> (hr)	1	2	2

**Comments:** This study demonstrated that repeated sc administration of HPMPC over the course of 14 weeks to rats, did not result in an accumulation of the test compound at the high dose. However, the sponsor contended that the results demonstrated an alteration of the expected pharmacokinetic profile of the compound, and such might have attributed to altered physiology and absorption at or near the site of injection.

**22. Protein Binding of Cidofovir, Cyclic HPMPC, PMEA and PMPA in Human Plasma and Serum, Cidofovir Lot # 1966-C-9P, Gilead Sciences, Inc., Foster City, CA, June 9, 1995 (P0504-00039/95-DDM-XXXX-001)**

The protein binding of cidofovir, cyclic HPMPC, 9-(2-phosphonylmethoxyethyl) adenine [PMEA] and 9-(2-phosphonylmethoxypropyl) adenine [PMPA] was determined in human plasma and serum using a centrifugation and ultrafiltration method. Five concentrations of each compound were prepared in phosphate buffered saline (PBS), human plasma and serum over the range of 0.6 to 25.6  $\mu\text{g}/\text{ml}$  for cidofovir and cyclic HPMPC, 0.1 to 25.1  $\mu\text{g}/\text{ml}$  for PMEA and 0.01 to 25.01 for PMPA. Results: are shown in Table 28. All the four compounds showed a very low protein binding (< 6%) in either human plasma or serum.

Table 28

Percent Unbound (SD) Cidofovir, Cyclic HPMPC, PMEA and PMPA in Human Plasma and Serum

Compound	Mean Percent Unbound (SD)		
	Human Plasma	Human Serum	PBS
Cidofovir	94.8 (3.8)	99.9 (3.3)	100.6 (1)
Cyclic HPMPC	96.7 (3.9)	95.7 (5.2)	99.3 (4.2)
PMEA	98.2 (6.3)	100.8 (7.2)	100 (1.3)
PMPA	99.3 (3.3)	92.8 (3.6)	99.8 (2.3)

### PHARMACOLOGY STUDIES

Cardiovascular System Safety in the Rat, BMY-41671 HPMPC, Lot # 27408-69, July 2, 1991, (50017/PSA 89203)\*

One group of normal male and female Sprague-Dawley (OFA) rats (weight: 263 - 323 g males and 253 - 302 g females; age: eight weeks; 5 animals/sex/group) were administered HPMPC by an intravenous injection (via lateral tail vein) with 5.0 mg/kg in sterile saline (0.1 ml/100 g) at the rate of approximately 0.05 ml/sec (bolus dose). Twenty-four to 48 hr before the day of the experiment, the rats were anesthetized with an intraperitoneal injection of chloral hydrate (300 mg/kg) and surgically prepared for recording arterial blood pressure and heart rate. The femoral artery was cannulated with an indwelling polyvinyl tube which exited externally at the nape of the neck. During the recovery period, rats were monitored for general health and satisfactory arterial pressure pulse and heart rate. A control group received sterile saline under similar experimental conditions. This study was performed to investigate the potential acute effect of a single intravenous dose of HPMPC on the cardiovascular system in the conscious rat. There were no substantial or consistent effects noted in systemic pressure and heart rate in the combined male and female rat groups with vehicle or HPMPC. Conclusion: HPMPC administered to rats by a single intravenous dose of 5 mg/kg did not produce effects on the cardiovascular function.

## Appendix # 2

Tabulated summary of animal toxicity studies.

**Table 1**  
Summary of acute iv toxicity studies

Species	Dose level (mg/kg)	Approximate LD <sup>50</sup> (mg/kg)	Approx. NOAEL (mg/kg)
Mice	400	>800	400
	800		
Rats	400	>800	400
	800		
Rabbits $\sigma$	10	>100	25
	25		
	50		
	100		
Monkeys	40	>75	40
	75		

**Table 2**  
Summary of subchronic/chronic iv toxicity studies

study	Dose level (mg/kg/weekly)	Laboratory findings	Post mortem findings & NOAEL
1-month rats (without probenecid)	0.3 mg/kg/day	High: reduced RBC, hematocrit, hemoglobin; decreased total protein, albumin; increased BUN, creatinine, glucosuria & proteinuria	Mid & High: nephropathy (tubular necrosis); bone marrow hypoplasia & lymphoid atrophy  NOAEL: 0.3 mg/kg/day
	1.0		
	5.0		
3-month rats (without probenecid)	3	Mid & High: increased albumin/globin ratio, creatinine, K, Na; depression in myeloid & erythroid parameters	Mid & High: bone marrow erythroid depletion; renal tubular necrosis; testicular degeneration  NOAEL: 3.0 mg/kg/weekly
	15		
	60		
6-month rats (without probenecid)	0.6	High: lower testes weights; reduced RBC, hematocrit, hemoglobin; increased WBC, creatinine, Cl, Ca; lower bilirubin, K	High: ♀ mammary adenocarcinomas 22/44; ♂ Zymbal's gland carcinomas 3/44; Low, Mid & High ♂: kidney-karyomegaly, testes degeneration/atrophy NOAELs ♀: 3; ♂: <0.6 mg/kg/weekly
	3		
	15		
6-month rats 19-wk interim; sc (without probenecid)	0.6	Low, Mid & High: decreased MCHC; High: increased creatinine, Ca, ALT	♀: Dose-related mammary adenocarcinomas 4/20-Low; 7/20-Mid; 12/30-High; ♂: 1 Zymbal's-Mid & High; High: zonal bone marrow hypoplasia NOAEL: not identified.
	3		
	15		
1-month monkeys sc (without probenecid)	0.5 mg/kg/twice weekly	♂ High: decreased RBC, hematocrit, hemoglobin; ♀ & ♂ increased AST, Cl, creatinine	Mid & High: nephropathy; High: bone marrow depletion  NOAEL: 0.5 mg/kg/twice a week
	1.5		
	7.5		
1-month monkeys (with probenecid)	0.1	No significant effect on hematology or clinical chemistry parameters	High: tubular nephropathy; decreased spermatogenesis; minimal severity testicular effect at Mid  NOAEL: 1 mg/kg/weekly
	0.25		
	1.0		
3-month monkeys (without probenecid)	1	Mid & High: increased BUN, creatinine; decreased Cl, K, albumin; decreased erythroid parameters (RBC, hemoglobin, hematocrit)	Mid & High: renal tubular necrosis; bone marrow erythroid depletion; testicular degeneration, thymic depletion, lymph node depletion; hepatocellular hypertrophy NOAEL: 1 mg/kg/once weekly
	5		
	20		
12-month monkeys: 6 month interim (with probenecid-High)	0.1	No significant effect	No significant effect  NOAEL: 2.5 mg/kg/once weekly
	0.5		
	2.5		

Table 3

Carcinogenic potential of HPMPC in animal chronic toxicity studies

Species	Route	Dose (mg/kg/week)	Duration (week)	♀: incidence of mammary adenocarcinoma + Zymbal's gland carcinoma	♂: Zymbal's gland carcinoma
Rat	sc	0.6	19	4/20	-
		3.0	19	7/20 + 1/20 Zymbal's gland carcinoma	1/20
		15.0	19	12/30	-
	iv	0.6	26	0/20	-
		3.0	26	0/20	-
		15.0	26	22/44 + 3/44 Zymbal's gland carcinoma	6/44
Monkey	iv	0.1	26	None	None
		0.5	26	None	None
		2.5	26	None	None

Table 4

Reproductive toxicity of HPMPC in rats and rabbits

Study	Dose level (mg/kg/weekly)	Laboratory findings	NOAEL & (Body surface equivalent dose in humans)
Segment I (♂ rat)	1	High: reduced body weight gains & food consumption; no effect on fertility in the study	Paternal NOAEL: 5 mg/kg/weekly (humans = 0.71 mg/kg/weekly)  Developmental NOAEL: 15 mg/kg/weekly (humans = 2.1 mg/kg/weekly)
	5		
	15		
Segment I (♀ rat)	1.2	Low, Mid & High: decreased maternal body weights and food consumption; increased resorptions, dead fetuses	Maternal & Developmental NOAEL could not be identified; <1.2 mg/kg/weekly (humans: 0.17 mg/kg/weekly significant toxic effects on conception rate & early & late gestation.
	6		
	30		
Segment II (pregnant rats)	0.5	Mid & High: maternal effects reduced body weight gains, reduced food consumption; fetal effects: reduced ossification (High)	Maternal & Developmental NOAELs: 0.5 mg/kg/day (humans = 0.07 mg/kg/day)
	1.5		
	3		
Segment II (pregnant rabbits)	0.05	High: Maternal-reduced body weights, food consumption; fetal-increased resorption, reduced weights, retarded fetal ossification; increased incidence of soft tissue & skeleton malformat. on (maternal toxic dose)	Maternal & Developmental NOAELs: 0.25 mg/kg/day (humans = 0.036 mg/kg/day)
	0.25		
	1.0		
Segment III (rats)	0.1	No toxic effects	Maternal, Developmental & Reproductive NOAELs: 1.0 mg/kg/day (humans = 0.33 mg/kg/day)
	0.3		
	1.0		

## Appendix # 3

Tabulated summary of animal pharmacokinetic studies.

**Table 1**  
Single dose pharmacokinetics of iv HPMPC in nonclinical studies

Species	Dose (mg/kg)	AUC (µg <sup>2</sup> hr/ml)	C <sub>0</sub> (µg/ml)	Vd (l/kg)	Cl <sub>r</sub> (l/hr/kg)	T <sub>1/2</sub> (hr)
Rat	3	5.09	10.2	0.87	0.6	6.6
	5	6.47	12.62.96	2.96	0.79	9.26
	15	11.6	27.4	2.33	1.29	5.2
Rabbit	1	3.66	3.55	0.71	0.26	5.4
Dog	10	34.4	33.9	0.76	0.29	5.7
African green monkey	4.9	13.4	15.3	-	0.37	-
	43	210	175	-	0.28	32.9
Cynomolgus monkey	1	3.7	-	0.47	0.28	1.3
	20	104	-	0.28	0.19	1.1

**Table 2**  
Repeated dose pharmacokinetics of iv HPMPC in nonclinical studies

Species	Dose (mg/kg)	Period (week)	C <sub>0</sub> (µg/ml)	Vd (l/kg)	Cl <sub>r</sub> (l/hr/kg)	T <sub>1/2</sub> (hr)
Rat	15	1	-	2.33	1.19	5.16
		12	-	6.83	1.17	11.2
	60	1	-	1.26	1.29	3.63
		9	-	0.71	0.93	4.08
	0.6	1	1.55	-	-	-
		26	2.36	-	-	-
	3.0	1	6.27	-	1.15	-
		26	11.4	-	0.61	-
	15.0	1	27.4	-	1.29	2.23
		26	55.5	-	0.49	2.07
Cynomolgus monkey	1	1	-	0.47	0.28	1.29
		12	-	0.34	0.29	0.93
	20	1	-	0.28	0.19	1.07
		4	-	0.30	0.10	1.56
	5	1	-	-	0.6	3.56
		13	-	-	0.29	2.97

**Table 3**  
Bioavailability of HPMPC

Species	Dose (mg/kg)	% F (sc)	% F (oral)
Mouse	100	-	10
Rat	3	91.5	-
	5	-	3
Dog	10	-	21.3
African Green monkey	43	95	23

**Table 4**  
Urinary recovery of iv HPMPC in nonclinical studies

Species	Dose (mg/kg)	Time (hr)	Urinary Recovery (% of dose)	% of recovered drug unchanged
Mouse	100	6	80	nd
Rat	5	24	60	nd
	3	24	39.1	nd
Rabbit	15	6	83.9	98.8
	15	24	98.3	97.8
	1	24	77.5	nd
Cynomolgus monkey	10	24	79.6	99.38
	10	168	91.4	99.2

**Table 5**  
Effect of probenecid (30 mg/kg; oral) on pharmacokinetics of HPMPC in monkeys

Dose	Parameter	Time	Mean value (with probenecid)	Mean value (without probenecid)
5 mg/kg/weekly	C <sub>0</sub> (µg/ml)	week 1	4.81	8.04
		week 13	7.48	7.54
	AUC <sub>0-6</sub> (µg*hr/ml)	week 1	6.93	11.6
		week 13	11.6	10.8
	Half-life (hr)	week 1	3.32	1.99
		week 13	2.19	2.45

## Appendix # 4

Tabulated summary of human pharmacokinetic studies.

Table 1

Mean single dose pharmacokinetic parameters for iv HPMPC without probenecid

Parameters	Dose (mg/kg)			
	1.0	3.0	5.0	10.0
C <sub>max</sub> (µg/ml)	3.12	7.34	11.5	23.5
AUC <sub>0-∞</sub> (µg*hr/ml)	8.35	19.96	28.34	68.8
Cl <sub>r</sub> (ml/hr/kg)	130	152	177	148
Cl (ml/hr/kg)	129	129	149	124
MRT (hr)	2.7	3.5	3.2	3.5
V <sub>ss</sub> (ml/kg)	339	533	556	516
T <sub>1/2</sub> β (hr)	1.4	2.7	2.4	3.14
24 hr urinary recovery (% of dose)	98.5	83.9	85.9	94.7

Table 2

Mean multiple dose pharmacokinetic parameters for iv HPMPC with probenecid and hydration

Parameters	Dose (mg/kg)		
	3.0	5.0	7.5
C <sub>max</sub> (µg/ml)	8.08	24.07	42.95
AUC <sub>0-∞</sub> (µg*hr/ml)	19.87	50.56	79.67
Cl <sub>r</sub> (ml/hr/kg)	134	100	107
Cl (ml/hr/kg)	-	72	90
MRT (hr)	3	2.6	2.8
V <sub>ss</sub> (ml/kg)	457	261	301
T <sub>1/2</sub> β (hr)	2.2	2.2	2.6
24 hr urinary recovery (% of dose)	-	66.7	72.4

Table 3

Mean multiple dose pharmacokinetic parameters for iv HPMPC with probenecid and hydration in the maintenance phase

Parameters	Dose (mg/kg)	
	3.0	5.0
C <sub>max</sub> (µg/ml)	11	16
AUC <sub>0-∞</sub> (µg*hr/ml)	29.9	36.1
Cl <sub>T</sub> (ml/hr/kg)	107	147
Cl <sub>r</sub> (ml/hr/kg)	80.1	112
MRT (hr)	3.76	2.86
V <sub>ss</sub> (ml/kg)	395	415
T <sub>1/2</sub> β (hr)	2.96	2.08
24 hr urinary recovery (% of dose)	74.3	76.3

Table 4

Mean multiple dose pharmacokinetic parameters for iv HPMPC at 5 mg/kg dose level with or without probenecid and hydration in the maintenance phase

Parameters	5 mg/kg	
	With probenecid	Without probenecid
C <sub>max</sub> (µg/ml)	9.79	11.6
AUC <sub>0-∞</sub> (µg*hr/ml)	25.7	28.3
Cl <sub>T</sub> (ml/hr/kg)	126	177
Cl <sub>r</sub> (ml/hr/kg)	80.1	149
MRT (hr)	3.44	3.2
V <sub>ss</sub> (ml/kg)	421	556
T <sub>1/2</sub> β (hr)	2.64	2.42
24 hr urinary recovery (% of dose)	74.3	85.9

Appendix # 5

Comparison of animal doses with the human therapeutic dose.

Table 1

Comparison of kinetic data from subchronic/chronic rat and monkey toxicity studies with the human therapeutic dose [5 mg/kg/once weekly; AUC = 27.9 µg\*hr/ml; Cmax = 9.79 µg/ml]

Study	Dose level (mg/kg/once weekly)	C <sub>0</sub> or C <sub>max</sub> (µg/ml)	AUC (µg*hr/ml)	NOEL/NOAEL	BSA: Equivalent dose in man	AUC: Equivalent dose in man
Rat 1-month (without probenecid)	0.3	-	-	0.3	0.04	-
	1.0	-	-			
	5.0	-	-			
Rat 3-month (without probenecid)	3.0	0.87	4.86	3.0	0.43	0.52
	15.0	2.6	12.66			
	60.0	14.64	64.22			
Rat 6-month (without probenecid)	0.6	2.36	-	9: 3.0	9: 0.43	9: 0.52
	3.0	11.4	4.89	9: <0.6	9: <0.09	
	15.0	59.5	30.5/926			
Rat 6-month 19-wk interim; sc; (without probenecid)	0.6	0.44	0.55	not identified	-	-
	3	1.94	3.18			
	15	4.6	8.71			
Monkeys 1-month; sc; (without probenecid)	0.5	-	-	0.5	0.17	-
	1.5	-	-			
	7.5	25	70			
Monkeys 1-month (with probenecid)	0.1	-	-	1.0	0.33	-
	0.25	-	-			
	1.0	-	-			
Monkeys 3-month (without probenecid)	1	2.5	3.36	1.0	0.33	0.12
	5	-	-			
	20	82	199			
Monkeys 12-month; 6-month interim sacrifice (with probenecid-High)	0.1	-	-	2.5	0.83	-
	0.5	-	-			
	2.5	-	-			

## Appendix # 6

Comparison of human pharmacokinetic parameters with rat and monkey

Table 1

Comparison of repeated dose pharmacokinetics of animals with man

Parameters	Species & Dose		
	Rat (15 mg/kg/week)	Human (5 mg/kg/weekly)	Monkey (5 mg/kg/weekly)
C <sub>max</sub> (µg/ml)	-	11.6	-
AUC <sub>0-∞</sub> (µg <sup>h</sup> /ml)	4.86	28.3	11.6
Cl <sub>r</sub> (ml/hr/kg)	1.17	177	0.23
Cl <sub>t</sub> (ml/hr/kg)	-	149	-
MRT (hr)	-	3.2	-
V <sub>ss</sub> (ml/kg)	6.83	556	0.30
t <sub>1/2</sub> β (hr)	11.2	2.42	2.45
24 hr urinary recovery (% of dose)	60	85.9	79.6

## PHARMACOLOGIST'S REVIEW

NDA 20-638.011

Date Submitted: January 11, 1996

Date Assigned: January 23, 1996

Date Review Completed: January 31, 1996

Assigned Reviewer: Pritam S. Verma, Ph.D.

KPD-530

**SPONSOR:** Gilead Sciences, Inc.  
353 Lakeside Drive  
Foster City, CA 94404

**DRUG:** Trade Name: Vistide™

Generic Name: Cidofovir

Other Designations: CDV,  
HPMPC

Code #: GS-0504

Chemical Name: (S)-1-(3-  
hydroxy-2-  
(phosphonyl-methoxy)propyl)  
cytosine

Molecular Formula:

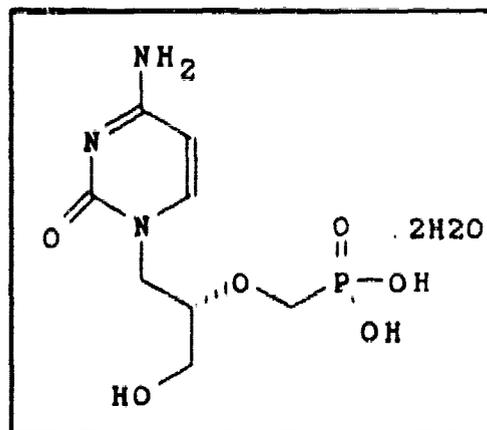
$C_8H_{14}N_3O_6P \cdot 2H_2O$

Molecular Weight: 315.21 as  
dihydrate

Melting Point: 204-212°C

Physical Appearance: White  
crystalline powder

Solubility: > 75 mg/ml in water with pH near neutrality



**DOSAGE FORM:** Vistide is supplied

as a sterile, clear solution in vials containing 375 mg of anhydrous cidofovir in 5 ml at concentration of 75 mg/ml. The formulation is pH adjusted to 7.4 with sodium hydroxide and/or hydrochloric acid and contains no preservatives. Each single-use vial must be diluted prior to intravenous administration.

**ROUTE OF ADMINISTRATION:** Parenteral administration by intravenous infusion.

**DOSAGE:** Vistide must be diluted in 100 ml of 0.9% saline prior to administration. To minimize potential nephrotoxicity, probenecid and intravenous saline pre-hydration must be

administered with each Vistide infusion.

**Induction Treatment:** The recommended dose of Vistide is 5 mg/kg body weight (given as an intravenous infusion at a constant rate over 1 hr) administered once weekly for two consecutive weeks.

**Maintenance Treatment:** Following completion of induction treatment, the recommended maintenance dose of Vistide is 5 mg/kg body weight (given as an intravenous infusion at a constant rate over 1 hr) administered once every two weeks.

**INDICATION:** Treatment of CMV Retinitis in Patients with AIDS.

**RELATED INDs**

**DMFs:**

**INTRODUCTION:**

Cidofovir [HPMPC] is a phosphonate nucleotide analog of deoxycytidine triphosphate (dCTP). The compound has been shown to

s. The active intracellular metabolite HPMPC diphosphate (HPMPCpp) is formed by cellular enzymes and has a long intracellular half-life (17-65 hr) in cultured cells. The anti-CMV activity of HPMPC appears to result from the high affinity of HPMPCpp for inhibition of CMV DNA polymerase ( $K_i = 6.6 \mu\text{m}$ ). In so doing, high concentrations of HPMPC and its phosphorylated metabolites may potentially inhibit cellular DNA polymerases thus leading to cytotoxicity. This is one possible mechanism for HPMPC-mediated nephrotoxicity. Within the kidney, high concentrations of HPMPC may interfere with other cellular functions, possibly by inhibiting the transport of endogenous anions or by depleting the intracellular pool of adenosine triphosphate. Presently, the sponsor has submitted an Information Amendment-Pharmacology/Toxicology.

**BACKGROUND:**

HPMPC is a member of a class of compounds termed phosphonomethylether nucleotide analogues. Unlike acyclovir and

ganciclovir which require intracellular activation by the viral enzymatic machinery, phosphorylation of HPMPC in cells to HPMPCpp is independent of virus infection. The two principal systemic toxicities observed in animals are dose-limiting nephrotoxicity and carcinogenicity. Tumors associated with HPMPC administration have been observed in rats and were primarily limited to mammary tissues. Additional tumors were observed on the neck, Zymbal's gland and uterus. Other toxicities included effects on bone marrow (erythroid and myeloid depletion) and testes (hypospermia). In preclinical studies, the tissue distribution, pharmacokinetics and nephrotoxic potential of HPMPC were all affected by the dose and schedule of HPMPC administration and by concomitant administration of probenecid.

#### NON-CLINICAL TOXICOLOGY

**Toxicity Studies Summary:** The following study was conducted in accordance with the FDA Good Laboratory Practice Regulations.

A 12-month chronic toxicity study of HPMPC following once weekly iv administration in cynomolgus monkeys with an interim sacrifice at 6 months, Lot # 504-B93-01,

January 11, 1996, (94-TOX-0504-015\86540\T0504-00069)

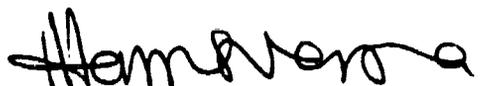
Groups of male and female monkeys (5 animals/group/sex with an additional 2 animals/sex/group in the control and high groups as recovery animals), received an iv bolus injection of HPMPC at dose levels of 0 (vehicle control), 0.1 (low), 0.5 (mid) or 2.5 mg/kg/once weekly (high); an additional group received probenecid (30 mg/kg/dose) via oral gavage approximately 1 hr prior to being given an iv bolus injection of HPMPC at a dose level of 2.5 mg/kg/once weekly for 52 consecutive weeks. The control animals received an oral dose of sterile water approximately 1 hr prior to being given the iv bolus dose of the saline vehicle. **Results:** there were no deaths. Incidental clinical signs seen in the control or treated animals included liquid feces, emesis, skin scabs or lesions, thin fur cover of the hindlimb, forelimb, dorsal thoracic or lumbar regions or tail or swollen hindlimb/hindpaw. There were no clinical signs seen which were considered treatment-related. There were no treatment-related effects on appetency, ophthalmology, hematology or bone marrow parameters. An overall lower body weight gain was seen in males (high without probenecid). **Clinical Biochemistry:** treatment-related increases were seen in BUN and creatinine values of males and females (high without probenecid) and ALP values of males (high without probenecid). **Urinalyses:** treatment-related increases in glucose and protein concentrations were seen in males and females (high without probenecid) when compared to the controls. **Organ weights:** a statistical significant decrease in spleen

weights (absolute) was seen in males (high without probenecid) and testes (absolute and relative to brain weight) of males (high with/without probenecid). A statistically significant increase in liver weights relative to body weight was seen in males (high without probenecid) and in kidney weights relative to body weight of males and females (high without probenecid) when compared to the controls. Gross pathology: included bilateral pallor of the kidneys of the 5/5 males and 4/5 females (high without probenecid). Small testes and/or epididymides were also seen in some animals.

**Comments:** Toxicokinetic and histopathology data are pending. Based on these preliminary results, the NOAEL for HPMPC when treated in combination with orally administered probenecid given to monkeys, was 2.5 mg/kg/week. On the basis of body surface area conversion, an equivalent dose of HPMPC in humans would be 0.83 mg/kg/once weekly. The dosage of 0.83 mg/kg/once weekly is approximately 3-fold lower than the proposed human dosage of 5 mg/kg twice weekly. The NOEL for HPMPC when given without an oral dose of probenecid was 0.5 mg/kg/once weekly. On the basis of body surface area conversion, an equivalent dose of HPMPC in humans would be 0.17 mg/kg/once weekly.

**CONCLUSIONS:**

No regulatory actions or communications are necessary at this time.

  
Pritam S. Verma, Ph.D.  
Reviewing Pharmacologist

# Chemist Review

**DIVISION OF ANTIVIRAL DRUG PRODUCTS**  
**Review of Chemistry, Manufacturing and Controls Section**

**NDA #**                      20-638

**CHEMISTRY REVIEW #** #1

**DATE REVIEWED:** 3/20/96

<u>SUBMISSION TYPE</u>	<u>DOCUMENT DATE</u>	<u>CDER DATE</u>	<u>ASSIGNED DATE</u>
Original	9/29/95	10/4/95	10/20/95
NC	11/10/95	11/13/95	11/15/95
BC	12/13/95	12/14/95	12/20/95
Amendment	2/16/96	2/16/96	2/20/95
NC	2/29/96	3/4/96	3/16/96
BZ	3/8/96	3/11/96	3/13/96

**NAME/ADDRESS OF APPLICANT:**

Gilead Sciences, Inc.  
346 Lakeside Drive  
Foster City, CA 92037

**DRUG PRODUCT NAME**

Proprietary:

Vistide

Non proprietary:

Cidofovir

Code Name/#:

GS-0504

**PHARMACOLOGICAL CATEGORY:**

Antiviral

**INDICATION:**

CMV Retinitis Infection

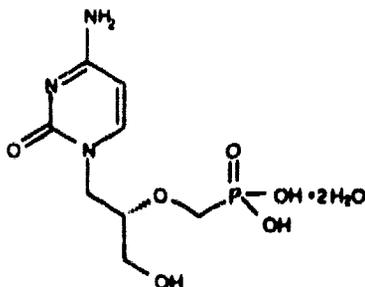
**DOSAGE FORM/STRENGTH:**

Injection; 75.0 mg/mL, 5 mL per vial (375 mg of anhydrous Cidofovir).

**ROUTE OF ADMINISTRATION:**

Intravenous infusion

**CHEMICAL NAMES/STRUCTURAL FORMULA:**



HPMPC: 1-(S)-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine:  
Cidofovir

**Molecular formula:**

$C_8H_{14}N_3O_6P \cdot 2H_2O$

**Molecular weight:** 315.22

**Anhydrous Molecular Weight:** 279.19

**Theoretical Water Content:** 11.4%

**CAS:**149394-66

**PATENTS:**

Patent # 5,142,051, Exp. date  
8/25/2009 for drug substance  
Cidofovir, and for drug product  
Vistide. Patent is owned by Institute  
of Organic Chemistry and Bio-  
Chemistry, Riga, Represented by  
Gilead Sciences, CA.

**SUPPORTING DOCUMENTS:**

Letter of Authorization from \_\_\_\_\_ dated 4/10/95  
to review Type I DMF # \_\_\_\_\_  
Letter of Authorization from \_\_\_\_\_ dated 6/28/95 to review Type II DMF # \_\_\_\_\_

Facimile dated 12/12/96 (from Gilead on Microbial Contamination Lots)  
Facimile dated 12/13/96 (from Gilead on Microbial Contamination Lots)  
Facimile dated 2/13/96 (from FDA to Gilead on Methods and General Product  
Clarifications )  
Facimile dated 3/6/96 (from Gilead to FDA on Established Name)  
Facimile dated 3/6/96 (from Gilead to FDA on proposed Established Name)  
Facimile dated 3/6/96 (from Gilead to FDA on proposed Carton Label)  
Facimile dated 3/6/96 (from Gilead to FDA on Established Name)  
Facimile dated 3/8/96 (from Gilead to FDA on Analytical Methods)  
Facimile dated 3/10/96 (from Gilead to FDA on Final Carton Label)  
Facimile dated 3/11/96 (from Gilead to FDA on Acceptance Established Name)  
Facimile dated 3/12/96 (from Gilead to FDA on Analytical Methods)  
Facimile dated 3/18/96 (from FDA to Gilead on Specifications for Drug Substance and  
Drug Product)  
Facimile dated 3/19/96 (from Gilead on to FDA Package Insert)  
Facimile dated 3/19/96 (from Gilead on Package Insert, Handling and Disposal)  
Facimile dated 3/20/96 (from Gilead to FDA on Specifications)  
Facimile dated 3/21/96 (from Gilead to FDA on Specifications)  
Facimile dated 3/22/96 (from Gilead to FDA on Commitment on Impurity Profile and  
Residual Solvents)  
Facimile dated 3/22/96 (from Gilead to FDA on Specifications)  
Facimile dated 3/22/96 (from Gilead to FDA on Final Specifications for Drug Substance  
and Drug Product)  
Telecon dated 3/28/96 (FDA and Gilead agree on final specifications, storage temperature  
conditions drug product and red dilution statement on carton and label)

**RELATED DOCUMENTS:**

Pre-IND letter dated 11-25-91  
IND  
IND  
IND  
IND  
IND  
IND

**CONSULT REVIEWS:**

Consult Review of Established and Trade Name by Daniel Boring, Ph.D., R.Ph., dated  
11/8/95  
Consult Review on Sterilization Validation by Microbiologist Paul Stinavage, Ph.D., dated  
11/22/95)

Consult Review-1 on Environmental Assessment (EA) by Nancy Sager, EA Team Leader, dated 10/22/95

Consult Review-2 on Environmental Assessment (EA) by Nancy Sager, dated 2/12/96

➤ **COMMENTS:**

### **Background**

During a pre-IND consultation with DAVDP, the sponsor was provided a letter detailing our requirements and recommendations for their proposed IND on HPMPC (cidofovir). The sponsor submitted IND (March 1992), which incorporated almost all of our requests regarding chemistry, manufacturing, and controls. Chemistry recommendations on the IND were provided to the Applicant

All of these recommendations were taken into account while submitting this NDA and the Treatment IND. In between the NDA and the IND the following changes have occurred:

### **Changes Since Initial IND:**

3) Two new analytical laboratories are added: Gilead Sciences, Foster City, CA, and NAmSA, Irvine, CA.

4) Two new warehouses are also included.

5) The formulation strength has changed from \_\_\_\_\_ to 75 mg/mL (5 mL/vial) from the first submission of the IND to the current NDA.

6) The container and closure

addressed it adequately.

he

**Drug substance Satisfactory**

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**Drug Product *Satisfactory***

Vistide™ (cidofovir injection) is a clear solution packaged in single-use 5 mL USP Type I glass vials. Each 5 mL vial contains 375 mg (75 mg/mL) of cidofovir (anhydrous).

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## Environmental Assessment (EA): *Satisfactory*

The EA review was completed, and the deficiencies were communicated to the applicant (reviewer, Nancy Sager, EA Team Leader, HFD-004). The responses were reviewed and found to be acceptable. The EA (with FONSI) was completed on 2/8/96.

## Methods Validation: *Pending*

The analytical methodology is adequately described and includes adequate validation. The methods validation package, covering both the drug substance and the drug product is being submitted to the San Francisco District and to the Division of Drug Analysis (St. Louis). As of March 25, 1996, validation of analytical methods is not complete.

## Labeling: *Satisfactory*

The CDER Labeling and Nomenclature Committee have found "Vistide (cidofovir injection)" to be acceptable. The Applicant has agreed to provide carton labels for cidofovir drug substance, which should reflect the new storage conditions of -10 to -20 °C. As of 3/29/96, no label has been provided. A facsimile of drug product carton and vial labels have been provided. In telecon dated 3/28/96, the applicant agreed to add a dilution statement, (Dilute before use in a red box) on the carton and container label in the next printing (labels are currently printed). The issue concerning the storage condition for the drug product needs to internally resolved within CDER. The carton label indicates storage of (15-30 °C), while the current stability protocol is for 25 °C. In telecon dated 3/28/96, the applicant agreed to change the storage statement to 20-25 °C on the vial label, carton and in the package insert at the next printing.

## Establishment Inspection: *Pending*

The following facilities were inspected and found acceptable under the Treatment IND in September-October 1995:

3) Gilead Sciences, California (analytical testing laboratories).

An acceptable EIR (EER submitted 10/30/95) for all three facilities for this NDA was received on 3/16/96. The facilities were found acceptable based on profile.

Microbial contamination was reported under the IND [redacted] for one lot at [redacted]. On further probing at the time of the treatment IND and under the current NDA, the following actions and observations are noted: (1) Thomas Arista, the inspector, had been briefed by this reviewer to investigate the microbial contamination at the treatment IND inspection; (2) the inspector had some reservations about [redacted] in his 9/95 Treatment IND inspection; but under urgent timeline he didn't have the time to go back to see the implementation of certain corrective measures that [redacted] promised to make; (3) the microbial contamination had continued to occur in [redacted] according to an amendment to Gilead's IND [redacted] not their NDA (applicant has been asked to submit all relevant information to the NDA); and (4) Compliance had not ordered a re-inspection of [redacted] for the NDA (apparently not knowing the seriousness of the situation).

As of 3/22/96, four of the eight representative lots of drug substance have been found contaminated; three with microbes and one chemically. Information pertaining to microbial contamination has been submitted to the IND and not the NDA. (see attached telecons, and

E-mails). The cause of the contamination is unknown. The inspection at \_\_\_\_\_ for the treatment IND was satisfactory. Based on the recurrence of this problem the following specifications and storage conditions have been put in place for didanosine drug substance:

- a) tight microbial specification for bacteria
- b) tighter control for moisture
- c) storage conditions of

The following lots were contaminated:

Lot # 1966-C-10P contaminated with *aspergillus fumigatus*;

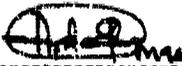
Lot # 1966-C-7P contaminated with *Cephalosporium*, *Trichoderma*, *Penicillium*;

Lot #1966-C-9P contaminated chemically; and

Lot # 1966-C-13P contaminated with *Penicillium fellutanum* (different species).

## CONCLUSIONS AND RECOMMENDATIONS:

The NDA application and accompanying amendments provide adequate information on the chemistry, manufacturing and controls for Vistide (didanosine injection). The sterilization validation is adequate and satisfactory. The Environmental Impact Assessment is complete and satisfactory. The manufacturing facilities have acceptable current GMP status based on profile of the facilities. However, \_\_\_\_\_ needs to be reinspected to verify implementation of process validations for cleaning and environmental monitoring to determine the cause of and to prevent microbial contamination in the drug substance. The new tighter microbial and moisture specifications, coupled with sub-zero ( \_\_\_\_\_ ) storage conditions for the drug substance do minimize microbial contamination. Pending satisfactory reinspection of \_\_\_\_\_ this NDA, as amended, is recommended for approval from the chemistry perspective.

 3/29/96

Albinus M. D'Sa, Ph. D., Review Chemist

Concurrence:

HFD-530/CChen *CChen 3/29/96*

cc:

Orig. NDA 20-638  
HFD-530/Div. File  
HFD-830/ Div. File  
HFD-830/ E Sheinin

HFD-530/DFeigal  
HFD-530/CChen  
HFD-530/DPratt  
HFD-530/WDempsey

HFD-530/PVerma  
HFD- 530/KStruble  
HFD-530/  
File: 020-638.002

\*\*\* SENSITIVE \*\*\*

REVIEW  
OF  
ENVIRONMENTAL ASSESSMENT  
FOR

NDA 20-638

VISTIDE™

(cidofovir injection)

75 mg/mL

DIVISION OF ANTI-VIRAL DRUG PRODUCTS  
(HFD-530)

CENTER FOR DRUG EVALUATION AND RESEARCH

DATE COMPLETED: OCTOBER 21, 1995

**ENVIRONMENTAL ASSESSMENT**

**1. Date:**

EA dated: August 1995  
Consult: October 12, 1995

CSO: Kim Struble

**2. Name of applicant/petitioner:**

Gilead Sciences

**ADEQUATE**

**3. Address:**

353 Lakeside Drive  
Foster City, California 94404

**ADEQUATE**

**4. Description of the proposed action:**

**a. Requested Approval:**

The applicant is filing a new drug application for FDA approval. The drug will be administered by injection and contains 75 mg/mL.

The applicant has filed an abbreviated EA under 21 CFR § 25.31a(b) (3) based on the infrequent use of Vistide which would result in low maximum yearly market volume. The estimated U.S. patient population is 1 which would result in approximately of drug substance being used.

**ADEQUATE**

**b. Need for Action:**

Used in the treatment of peripheral retinitis caused by cytomegalovirus (CMV) infection in patients with Acquired Immunodeficiency Syndrome (AIDS).

**ADEQUATE**

c. Production Locations:

i. Proprietary Intermediate(s):

Based on the list of starting materials, it does not appear that any proprietary intermediates are used.

ii. Drug Substance:

iii. Finished Dosage Form:

Facility Description & Adjacent Environment:  
A brief description has been provided for each of the facilities.

ADEQUATE

d. Expected Locations of Use (Drug Product):

Hospitals and homes. ADEQUATE

e. Disposal Locations:

Returned drug goods will be incinerated. The facilities and permitting information have been provided.

ADEQUATE

5. Identification of chemical substances that are the subject of the proposed action:

Drug Substance: Cidofovir

Chemical Name: 1-[(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]cytosine dihydrate (IUPAC)

(S)-[[2-(4-amino-2-oxo-1(2H)-pyrimidinyl)-1-(hydroxymethyl)ethoxy]methyl]phosphonic acid, dihydrate (CAS)

CAS #: 149394-66-1

Molecular Weight: 315.22

Molecular Formula: C<sub>8</sub>H<sub>14</sub>N<sub>3</sub>O<sub>6</sub>P · H<sub>2</sub>O

Structural Formula: provided in EA

Physical Descrip.: White to off-white crystalline powder

Additives: No information provided

Impurities: no information provided

DEFICIENT Information regarding impurities and additives should be provided

6. Introduction of substances into the environment: For the site(s) of production:

a. Potential Emitted substances:

A list of chemical substances associated with the drug substance and drug product production is provided. There are no chemicals listed that are of unusual concern.

ADEQUATE.

b. Controls (Air, Liquid Effluent, Solid):

Information has been provided for both facilities. The information provided adequately describes the air, liquid and solid emission controls used to limit entry into the environment.

ADEQUATE

c. Compliance with Federal, State and Local Emission Requirements:

Compliance statements from both manufacturers are provided.

ADEQUATE

d. **Effect of Approval on Compliance with Current Emissions Requirements:**

This is not addressed for either manufacturing site.

**DEFICIENT**

e. **Estimated Expected Emitted Concentration/Quantities:**

Only a production estimate is required for an abbreviated EA filed pursuant to 21 CFR § 25.31a(b)(3). An estimate of \_\_\_\_\_ was provided. There is no expectation that production, disposal or use of this small quantity will adversely impact the environment.

**ADEQUATE**

- 7. **Fate of emitted substances in the environment:**
- 8. **Environmental effects of released substances:**
- 9. **Use of resources and energy:**
  - a. **Production:**
  - b. **Effect on Endangered/Threatened Species:**
  - c. **Effect on Properties Listed/Eligible for National Register of Historic Places:**
- 10. **Mitigation measures:**
- 11. **Alternatives to the proposed action:**

7-11 N/A

- 12. **List of preparers, & their qualifications (expertise, experience, professional disciplines) and consultants:**

The preparer is identified. **ADEQUATE**

- 13. **Certification:**

An appropriate certification is provided. **ADEQUATE**

- 14. **References:**

References are provided. **ADEQUATE**

- 15. **Appendices:**

6 Appendices are listed. An MSDS is provided.

**DRAFT DEFICIENCY LETTER**

1. Format item 5: Any additives or impurities in the drug substance that are found at environmentally significant levels should be listed.
2. Format item 6: Although both manufacturers indicate that they are in compliance with the appropriate emission/environmental requirements, the effect of approval on compliance with current emissions requirements was not addressed (see 21 CFR § 25.31a(b)(3) "format item 6").
3. As required by regulations issued by the Council on Environmental Quality, the environmental assessment will be made public by FDA. You should provide a non-confidential version of your environmental assessment suitable for public release. Confidential business information such as production volumes, marketing estimates, impurities and the chemicals used to manufacture the drug substance may be redacted from the document. Information that may be redacted is that considered confidential by CDER under Freedom of Information Act procedures.

EA Review #1, NDA 20-659

Page 6

Endorsements:

HFD-004/NBSager

*2508 11/2/95*

HFD-004/RLWilliams

*PLW 10/27/95*

CC: Original NDA 20-638/KStruble copy to NDA/HFD-530

EA File 20638

File: 20628e00.rns

\*\*\*SENSITIVE\*\*\*

REVIEW

OF

ENVIRONMENTAL ASSESSMENT

FOR

NDA 20-638

VISTIDE™

(cidofovir injection)

75 mg/mL

DIVISION OF ANTI-VIRAL DRUG PRODUCTS  
(HFD-530)

CENTER FOR DRUG EVALUATION AND RESEARCH

DATE COMPLETED: February 12, 1996

Summary:

A FONSI is recommended.

Cidofovir is a synthetic drug which will be administered intravenously in the treatment of peripheral retinitis caused by cytomegalovirus (CMV) infection in patients with acquired immunodeficiency syndrome (AIDS). Based on the low expected patient population (patients) and active moiety use an abbreviated EA was submitted pursuant to 21 CFR § 25.31a(b)(3). There is no evidence that extraordinary circumstances exist that would require the submission of additional environmental information.

Precautions taken at the sites of manufacture and the methods of disposal are expected to minimize occupational exposures and environmental release.

**ENVIRONMENTAL ASSESSMENT**

**1. Date:**

EA dated: August 1995  
Consult: October 12, 1995  
Review #1: October 21, 1995  
EA dated: December, 1995  
Consult: December 21, 1995

CSO: Kim Struble

**2. Name of applicant/petitioner:**

Gilead Sciences

**3. Address:**

353 Lakeside Drive  
Foster City, California 94404

THE FOLLOWING ARE IN RESPONSE TO THE DEFICIENCY "FAX" OF OCTOBER 23, 1995:

1. Format item 5: Any additives or impurities in the drug substance that are found at environmentally significant levels should be listed.

**RESPONSE:** There are no impurities that are found at environmentally significant levels. In response to the request regarding additives the applicant responded that all materials are listed in format 5.

2. Format item 6: Although both manufacturers indicate that they are in compliance with the appropriate emission/environmental requirements, the effect of approval on compliance with current emissions requirements was not addressed (see 21 CFR § 25.31a(b)(3) "format item 6").

**RESPONSE:** Based on the production volume, the approval will not effect the state of compliance. **ADEQUATE**

3. As required by regulations issued by the Council on Environmental Quality, the environmental assessment will be made public by FDA. You should provide a non-confidential version of your environmental assessment suitable for public release. Confidential business information such as production volumes, marketing estimates, impurities and the chemicals used to manufacture the drug substance may be redacted from the document. Information that may be redacted is that considered confidential by CDER under Freedom of Information Act procedures.

**RESPONSE:** An acceptable FOI copy is provided. **ADEQUATE**

**ENVIRONMENTAL ASSESSMENT**  
**AND**  
**FINDING OF NO SIGNIFICANT IMPACT**  
**FOR**

**NDA 20-638**

**VISTIDE™**

**(cidofovir intravenous)**

**75 mg/mL**

**FOOD AND DRUG ADMINISTRATION**  
**CENTER FOR DRUG EVALUATION AND RESEARCH**  
**DIVISION OF ANTI-VIRAL DRUG PRODUCTS**  
**(HFD-530)**

**FINDING OF NO SIGNIFICANT IMPACT**

**NDA 20-638**

**Vistide™**

**(cidofovir intravenous)**

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. FDA is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process.

The Food and Drug Administration, Center for Drug Evaluation and Research has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

In support of their new drug application for Vistide™, Gilead Sciences, Inc. has prepared an abbreviated environmental assessment in accordance with 21 CFR 25.31a(b)(3) (attached) which evaluates the potential environmental impacts of the manufacture, use and disposal of the product.

Cidofovir is a synthetic drug which will be administered intravenously in the treatment of peripheral retinitis caused by cytomegalovirus (CMV) infection in patients with acquired immunodeficiency syndrome (AIDS). The drug substance will be manufactured by \_\_\_\_\_ and the drug product will be manufactured at \_\_\_\_\_

The finished drug product will be used in hospitals and home care settings.

Cidofovir may enter the environment from excretion by patients, from disposal of pharmaceutical waste or from emissions from manufacturing sites. The projected maximum yearly market volume is low based on the expected patient population and therefore no adverse environmental effects are expected.

Disposal may result from production waste such as out of specification lots, returned goods and user disposal of empty or partly used product and packaging. Pharmaceutical waste will be disposed of by the manufacturer at a licensed incineration facility. At U.S. hospitals or in home care situations, empty or partially empty packages will be disposed of by health care professionals in accordance with standard procedures.

Precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release.

The Center for Drug Evaluation and Research has concluded that the product can be manufactured, used and disposed of without any expected adverse environmental effects. Adverse effects are not anticipated upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places.

2/12/96  
DATE

Nancy B. Sager

PREPARED BY

Nancy B. Sager

Acting Supervisor

Environmental Assessment Team

Center for Drug Evaluation and Research

2/12/96  
DATE

R. L. Williams, M.D.

CONCURRED

Roger L. Williams, M.D.

Deputy Center Director for Pharmaceutical Science

Center for Drug Evaluation and Research

Attachment: Environmental Assessment

**ENVIRONMENTAL ASSESSMENT REPORT FOR  
VISTIDE™ (cidofovir intravenous)  
Abbreviated Assessment**

**NON-CONFIDENTIAL VERSION**

**1. Date**

December, 1995

**2. Name of Applicant**

Gilead Sciences, Inc.

**3. Address**

353 Lakeside Drive  
Foster City, California 94404

**4. Description of Proposed Action**

This abbreviated environmental assessment report is submitted in support of the New Drug Application for Vistide™ (cidofovir intravenous). This submission is pursuant to 21 CFR 25.31a(b)(3) on the basis of an infrequent use of Vistide™ (cidofovir intravenous) which would result in a low maximum yearly market volume.

Vistide™ (cidofovir intravenous) contains 75 mg/mL equivalent of anhydrous cidofovir. It is intended for the treatment of peripheral retinitis caused by cytomegalovirus (CMV) infection in patients with acquired immunodeficiency syndrome (AIDS). Currently, ganciclovir is the leading therapy for the treatment of CMV reinitis in AIDS patients in the United States. A low maximum yearly market volume of Vistide™ is determined based on an infrequent use of Vistide™ as specified in the product package insert, and an assumption that Vistide™ achieves a 100% replacement of the Ganciclovir I.V. treatment rate, which is derived from a confidential market place analysis.

2 Pages

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## 5. Identification of Chemical Substances That Are the Subject of the Proposed Action

### a. Drug Substance — Cidofovir

Generic Name (USAN): Cidofovir

Chemical Names: 1-[(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]cytosine dihydrate (IUPAC)

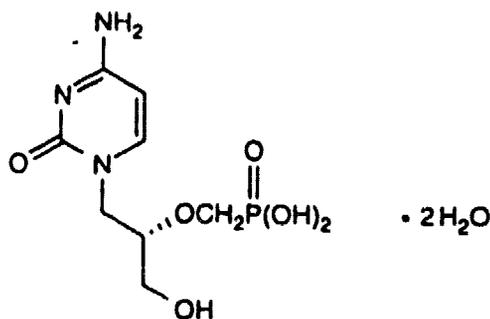
(S)-[[2-(4-amino-2-oxo-1(2H)-pyrimidinyl)-1-(hydroxymethyl)ethoxy]methyl]phosphonic acid, dihydrate (CAS)

CAS Reg. No.: 149394-66-1

Molecular Formula:  $C_8H_{14}N_3O_6P \cdot 2 H_2O$

Formula Weight: 315.22

Chemical Structure:



Physical Description: Cidofovir is a white to off-white crystalline powder.

Dissociation Constants:  $pK_{a1} = 1.7$   $pK_{a2} = 4.7$   $pK_{a3} = 6.9$

Partition Coefficient:  $\log P$  (octanol/aqueous buffer, pH 7.1) = 3.3

Solubilities: Cidofovir is sparingly soluble in dimethyl sulfoxide at 12 mg/mL and is practically insoluble (<0.1 mg/mL) in most organic solvents, including acetone, dichloromethane, methanol, ethanol, 2-propanol and N,N-dimethylformamide. It is slightly soluble in water at 8.4 mg/mL (apparent pH ~3.5). At pHs above 6, the solubility is greater than 170 mg/mL.

Acute Aquatic Toxicity: *Daphnia magna*,  $EC_{50}$ : 881 mg/L, 48 hours

**b. Starting Materials for the Cidofovir Drug Substance Manufacture**

The starting materials used in the manufacture of cidofovir drug substance are listed in the confidential version of the environmental assessment report for Vistide™ (cidofovir intravenous).

**c. Solvents/Reagents Used in Cidofovir Drug Substance Manufacture**

The solvents and reagents used in the manufacture of cidofovir drug substance are listed in the confidential version of the environmental assessment report for Vistide™ (cidofovir intravenous).

**d. Substances Used in Drug Product Formulation**

The following substances are used in the manufacture of Vistide™ (cidofovir intravenous) drug product.

Hydrochloric acid, NF	CAS[7647-01-0]
Sodium hydroxide, NF	CAS[1310-73-2]
Water for Injection, USP	CAS[7732-18-5]

## 6. Introduction of Substances into the Environment

Vistide™ will be used throughout the entire United States; however, concentrations of affected populations occur in several major urban areas. The approval of the application for this new drug will not significantly affect the quality of human health or the environment as defined in the National Environmental Policy Act (NEPA).

Manufacturing controls, applicable regulations and acts, and permit information are described below for the drug substance manufacturing site,

A letter of compliance from the management of \_\_\_\_\_ included in Appendix 2. Contained in the \_\_\_\_\_ Abbreviated Environmental Assessment Report (Appendix 1) are manufacturing controls, applicable regulations and acts, permit information, and compliance statements for the manufacture of Vistide™ drug product at \_\_\_\_\_

Based on the low maximum yearly market volume of Vistide™, the approval of this new drug application will not affect the state of compliance with respect to current emission and environmental requirements at both \_\_\_\_\_

The Material Safety Data Sheet for cidofovir is given in Appendix 3.

### a. Drug Substance Manufacturing Site —

Information pertaining to licenses or permits held by the waste disposal and transportation contractors providing services to \_\_\_\_\_ is provided in Appendix 4.

#### (1) Air Emissions

Potential air emissions consist primarily of solvent vapors, fugitive dust and acid vapors. Exhausted plant air from drying rooms is discharged through filters into the environment. Exhaust gases are scrubbed by two counter current gas scrubbing towers, run in series. Hydrogen evolved from the process is vented to the atmosphere with a nitrogen dilution.

Air emissions in \_\_\_\_\_ are regulated under \_\_\_\_\_ Environmental Protection and Enhancement Act, which encompasses the previously effective Clean Air Act. All air releases are reported to the Environmental Protection Services of Alberta Environment. Appendix 5 is a *License to Operate or Use* issued by \_\_\_\_\_ Environment for the \_\_\_\_\_

Chemicals site relating to the Clean Air Act. The license number is 93-AL-102 and the license expires on May 1, 1998.

## (2) Wastewater

The wastewater discharges from [redacted] are regulated by the [redacted] Protection and Enhancement Act, which encompasses the previously effective Clean Water Act. Wastewater from the cidofovir process is in two categories: nonhazardous and hazardous aqueous wastes. The former consists of mainly clean water used through condenser coils, and water used in equipment cleaning which may contain detergents. These nonhazardous aqueous wastes are discharged to the [redacted] city sanitary sewer following the guidelines set in the [redacted].

The hazardous aqueous wastes, which consist primarily of inorganic salts and minor quantities of solvents, include neutralized aqueous wastes (neutralized acids and bases, brine solutions, etc.), diluted suspensions of spent charcoal or Celite™ from the process, scrubbing tower effluents, and plant sumps effluents. Hazardous wastewater is packaged and shipped for deep-well disposal. This service is currently provided by the [redacted] a full member of the Environmental Services Association of Alberta (EISA).

## (3) Hazardous Chemical Wastes

Disposal of hazardous chemical wastes is regulated under the [redacted] Environmental Protection and Enhancement Act. Hazardous chemical wastes from the cidofovir process occur as halogenated liquid wastes, non-halogenated liquid wastes, and hazardous solid wastes. Halogenated and non-halogenated liquid wastes are mainly spent solvent streams from the process, and they are bulked separately and sent off-site for incineration. This service is currently provided by [redacted].

Hazardous solid wastes are mainly unused raw materials and by-products from the process, and they are packaged and shipped off-site for incineration. This service is currently provided by [redacted].

Both firms are full members of the EISA.

**(4) Solid Wastes**

Solid wastes such as candle filters and centrifuge cloths that have been exposed to chemicals are water washed before being disposed of off-site as non-hazardous solid waste in a commercial landfill. This service is currently provided by \_\_\_\_\_ which is also a full member of the EISA.

**(5) Occupational Exposure**

Health hazards control methods employed a \_\_\_\_\_ consist of engineering controls, administrative controls, and personal controls. Engineering controls include ventilation controls, process equipment selection, and isolation/enclosure of hazards. Administrative controls include sound safety programs, standard work procedures, training and educating the workers, and following preventative maintenance schedules. The personal controls include the use of personal protective equipment specified in operating instructions and the following of proper personal hygiene practices.

\_\_\_\_\_ follows guidelines set by \_\_\_\_\_ Statute and Regulations. Most prominent are the Chemical Hazards Regulations Regulation 393/88 with amendments up to and including \_\_\_\_\_ Regulation 303/92), and General Safety Regulations \_\_\_\_\_ Regulation 448/83 with amendments up to and including \_\_\_\_\_ Regulation 348/84).

**7. Fate of Emitted Substances in the Environment**

Under 21 CFR 25.31a(b)(3), information on the environmental fate of emitted substances is not required for an abbreviated Environmental Assessment Report.

**8. Environmental Effects of Released Substances**

Under 21 CFR 25.31a(b)(3), information on the environmental effects of released substances is not required for an abbreviated Environmental Assessment Report.

**9. Use of Resources and Energy**

Under 21 CFR 25.31a(b)(3), information on the use of resources and energy is not required for an abbreviated Environmental Assessment Report.

**10. Mitigation Measures**

Under 21 CFR 25.31a(b)(3), information on mitigation measures of released substances is not required for an abbreviated Environmental Assessment Report.

**11. Alternatives to the Proposed Action**

Under 21 CFR 25.31a(b)(3), information on alternatives to the proposed action is not required for an abbreviated Environmental Assessment Report.

**12. Preparer**

Appendix 6 contains the curriculum vitae of the preparer, Taiyin Yang, Ph.D., Director of Analytical and Quality Operations, Gilead Sciences, Inc.

**13. Certification**

The undersigned official certifies that the information presented in this Environmental Assessment is true, accurate, and complete to the best of the knowledge of Gilead Sciences, Inc.

Taiyin Yang

Taiyin Yang, Ph.D.  
Director  
Analytical and Quality Operations  
Gilead Sciences, Inc.

Dec. 5, 1995

Date

#### **14. References**

No references are included.

#### **15. Appendices**

Appendix 1: Abbreviated Environmental Assessment for

Appendix 2: Letter of Compliance for

Appendix 3: Material Safety Data Sheet for Cidofovir

Appendix 4: Permit Information for Disposal and Transportation Contractors

Appendix 5: License to Operate or Use for

Appendix 6: Curriculum Vitae of Preparer

13 Pages

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**Appendix 3**

**Material Safety Data Sheet for Cidofovir**

MATERIAL SAFETY DATA SHEET  
CIDOFOVIR

-----  
PRODUCT IDENTIFICATION

Synonyms:	(S)-1-[3-hydroxy-2-(phosphonylmethoxy)-propyl]cytosine, GS-0504 (HPMPC), HPMPC Drug Substance, Cidofovir
Formula CAS No.:	149394-66-1
Molecular Weight:	315.22
Chemical Family:	Nucleotide Analog
Chemical Formula:	$C_8H_{14}N_3O_6P \cdot 2H_2O$
Hazardous Ingredients:	Not Applicable

-----  
PRECAUTIONARY MEASURES

Wear gloves and protective clothing when handling material. Avoid direct contact with the substance. Promptly remove material on skin, eyes or clothing by rinsing thoroughly with water. Avoid breathing powder.

-----  
EMERGENCY FIRST AID

If skin contact occurs, wash with water and detergent. Remove contaminated clothing. If eye or mouth contact occurs, wash with plenty of water. Seek medical advice, if appropriate.

-----  
SECTION 1: Physical Data

Appearance:	White to off-white powder
Odor:	None
Solubility:	Less than 10 mg/ml in water as free acid >100mg/ml at neutral pH.
Boiling Point:	Not applicable
Melting Point:	Decomposes above 290° C
Density:	Not applicable
Vapor Pressure:	Not applicable
Evaporation Point:	Not applicable

-----  
SECTION 2: Fire and Explosion Hazard

Fire:	Not a fire hazard
Explosion:	Not an explosion hazard; may be flammable as a dust.
Fire Extinguishing Media:	Use appropriate means for extinguishing surrounding fire.
Special Information:	None

MATERIAL SAFETY DATA SHEET  
CIDOFOVIR

-----  
SECTION 3: Reactivity Data

Stability:	Stable under ambient conditions
Hazardous Decomposition Products:	None
Hazardous Polymerization:	Will not occur
Incompatibilities:	None known

-----  
SECTION 4: Spill Disposal Information

Spills:	Wear gloves and other suitable protective clothing. Rinse area with water and detergent.
Disposal:	Dispose of in accordance with local, state, and federal laws.

-----  
SECTION 5: Health Hazard Information

A. Exposure and Health Effects

Inhalation:	Health hazards have not been thoroughly investigated. No information available
Ingestion:	May be harmful if swallowed
Skin Contact:	Minimal effects; may cause irritation
Eye Contact:	Minimal effects; may cause irritation
Reproductive Effects:	Teratogenic in rabbits, embryotoxic
Mutagenic Effects:	Negative in Ames test. Positive in PBL clastogenesis and mouse micronucleus assays.
Chronic Exposure:	In humans, systemic exposures of 3 mg/kg weekly resulted in no obvious toxicities.
Aggravation of Pre-existing Condition:	No information available

B. First Aid

Inhalation:	If adverse symptoms occur, contact a physician
Ingestion:	No data available; avoid inhalation
Skin Exposure:	Avoid ingestion
Eye Exposure:	Immediately wash skin with soap and copious amounts of water
	Wash thoroughly with running water

C. Toxicity Data

Acute Toxicity:	The toxicological properties of this molecule have not been fully characterized. Because of this, it is suggested that conservative handling practices be employed at all times.
	Minimum lethal IV dose is >800 mg/kg in CD-1 mice and in Sprague-Dawley rats and >40 but less than 75 mg/kg in the cynomolgus monkey

**MATERIAL SAFETY DATA SHEET  
CIDOFOVIR**

**C. Toxicity Data (cont.)**

**Subacute Toxicity:** The major subacute toxicities target the kidney (renal tubular nephrosis) and testicles (reduced weight and spermatogenesis).

**Carcinogenicity:** Mammary tumors were induced in rats when treated subcutaneously or intravenously. Therefore, cidofovir should be considered a potential carcinogen for humans.

-----  
**SECTION 6: Occupational Control Measures**

<b>Airborne Exposure Limits:</b>	Not determined
<b>Ventilation System:</b>	Use with adequate ventilation
<b>Personal Respirators:</b>	Use if required to avoid inhalation
<b>Skin Protection:</b>	Required
<b>Eye Protection:</b>	Wear safety glasses

-----  
**SECTION 7: Storage and Special Information**

Store at room temperature.

-----

The above information is believed to be correct but is not purported to be all inclusive and shall be used only as a guide. Gilead Sciences shall not be held liable for any damage resulting from handling or from contact with the above product.

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3 of 3  
August 1995

7 Pages

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**Appendix 6**

**Curriculum Vitae of Preparer**

Curriculum Vitae of Preparer

TAIYIN YANG, Ph. D.

**TITLE** Director, Analytical and Quality Operations  
Gilead Sciences, Inc., Foster City, CA

**RESPONSIBILITIES** Analytical development, process control, quality control, stability studies, quality assurance and registration activities for new chemical entities and pharmaceutical products.

**EDUCATION** Ph.D., Organic Chemistry, 1980  
University of Southern California

B.S., Chemistry, 1974  
National Taiwan University

**EXPERIENCE**

1994 - present Director, Analytical and Quality Operations  
Gilead Sciences, Inc., Foster City, CA

1993 - 1994 Director, Analytical Chemistry  
Gilead Sciences, Inc., Foster City, CA

1992 - 1993 Director, Chemical Analysis  
Syntex Research, Palo Alto, CA

Responsible for analytical development, process control, quality control, stability studies, environmental analysis and registration activities for new chemical entities and recombinant proteins.

1987 - 1992 Department Head, Methods Development  
Syntex Research, Palo Alto, CA

Responsible for analytical development, quality control, stability studies and registration activities for new chemical entities.

1983 - 1987 Research Section Leader, Methods Development  
Syntex Research, Palo Alto, CA

1980 - 1983 Staff Researcher, Methods Development  
Syntex Research, Palo Alto, CA

**AFFILIATIONS** American Association of Pharmaceutical Scientists  
International Society for Pharmaceutical Engineering  
Society of Quality Control  
Drug Information Association  
American Chemical Society  
Chinese American Chemical Society  
Sigma Xi

**Curriculum Vitae of Preparer**

TAIYIN YANG, Ph.D.

**TITLE** Director, Analytical and Quality Operations  
Gilead Sciences, Inc., Foster City, CA

**RESPONSIBILITIES** Analytical development, process control, quality control, stability studies, quality assurance and registration activities for new chemical entities and pharmaceutical products.

**EDUCATION** Ph.D., Organic Chemistry, 1980  
University of Southern California

B.S., Chemistry, 1974  
National Taiwan University

**EXPERIENCE**

1994 - present Director, Analytical and Quality Operations  
Gilead Sciences, Inc., Foster City, CA

1993 - 1994 Director, Analytical Chemistry  
Gilead Sciences, Inc., Foster City, CA

1992 - 1993 Director, Chemical Analysis  
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Responsible for analytical development, process control, quality control, stability studies, environmental analysis and registration activities for new chemical entities and recombinant proteins.

1987 - 1992 Department Head, Methods Development  
Syntex Research, Palo Alto, CA

Responsible for analytical development, quality control, stability studies and registration activities for new chemical entities.

1983 - 1987 Research Section Leader, Methods Development  
Syntex Research, Palo Alto, CA

1980 - 1983 Staff Researcher, Methods Development  
Syntex Research, Palo Alto, CA

**AFFILIATIONS** American Association of Pharmaceutical Scientists  
International Society for Pharmaceutical Engineering  
Society of Quality Control  
Drug Information Association  
American Chemical Society  
Chinese American Chemical Society  
Sigma Xi

## CLINICAL PHARMACOLOGY/BIOPHARMACEUTICS LABELING COMMENTS

**NDA:** 20-638  
**DRUG:** Cidofovir intravenous  
**APPLICANT:** Gilead Sciences.

**REVIEWER:** Kellie S. Reynolds, Pharm.D.  
**DATE:** 12-15-95

The following is a suggested outline for the Pharmacokinetics section under the CLINICAL PHARMACOLOGY heading in the cidofovir intravenous label. The applicant should submit a revised label, following this outline for the Pharmacokinetics section.

General labeling comments follow the outline.

---

### **Pharmacokinetics**

State that cidofovir is administered with probenecid and that the effects of probenecid on cidofovir pharmacokinetics are described in the drug interaction section.

Population in whom cidofovir pharmacokinetics have been studied

Doses at which pharmacokinetics have been investigated

Brief comment regarding extent of distribution

Brief comment regarding mechanism of elimination

Dose (in)dependence of pharmacokinetics; state dose range

AUC at clinical dose

C<sub>max</sub> at clinical dose

Total clearance at clinical dose

Statement regarding (lack of) accumulation

### **Distribution:**

Volume of distribution

Protein binding

Blood/plasma ratio

Blood brain barrier penetration

### **Metabolism:**

Indicate any metabolites identified in human plasma and urine

Indicate any *in vitro* findings regarding cidofovir metabolism in humans

### **Excretion:**

Percent of dose excreted unchanged in urine

Percent of dose excreted as parent drug plus metabolites

Report renal clearance and the contribution of renal clearance to total clearance

Describe renal excretion (glomerular filtration, active secretion, etc.)

State that due to the fact that renal elimination is a significant (or major) pathway of cidofovir elimination, dose adjustments are needed. Include: (see DOSAGE AND ADMINISTRATION).

### **Drug-Drug Interactions**

Use this section to describe the effect of the 4 g probenecid regimen on the pharmacokinetics of cidofovir.

Describe the results of any drug-drug interaction investigations involving cidofovir. Provide reference to PRECAUTIONS or DOSAGE and ADMINISTRATION sections as needed.

**Special Populations:**

**Renal Insufficiency:**

State that cidofovir pharmacokinetics have not been investigated in patients with renal insufficiency.

State known/expected relationship between renal clearance and creatinine clearance.

State that the effect of dialysis on cidofovir pharmacokinetics is not known.

Please include the following four headings. If appropriate, state that the pharmacokinetics have not been investigated in a particular population.

**Geriatric:**

**Pediatric:**

**Gender:**

**Race:**

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**GENERAL COMMENTS:**

1. In the Pharmacokinetics section, indicate the cidofovir dose and the sample size for all parameters reported. Report parameters determined at the clinically relevant dose wherever possible. Do not include the study number within the text of the Pharmacokinetics section.

2. The review of the pharmacokinetic results is ongoing. Comments regarding the pharmacokinetic results will be submitted to the applicant as the review progresses. Changes to the general outline of the Pharmacokinetics section may also be made at a later date.

3. In the Drug Interactions section under the PRECAUTIONS heading, include the dose of probenecid.

4. Comments for DOSAGE and ADMINISTRATION will follow at a later date.

*Kellie S Reynolds 12-15-95*  
Kellie S. Reynolds, Pharm.D.  
Reviewer  
Antiviral Drugs Section, DPEIII  
Office of Clinical Pharmacology and Biopharmaceutics

Concurrence: Janice B. Jenkins, Ph.D. *Janice Jenkins 12/15/95*  
Acting Team Leader  
Antiviral Drugs Section, DPEIII  
Office of Clinical Pharmacology and Biopharmaceutics

cc: HFD-530 NDA 20638  
/MO/DPratt  
/CSO/KStruble  
/Biopharm/KReynolds  
/SBiopharm/JJenkins

**END**

**BT**

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J.H.M. Research & Development, Inc., 5776 Second Street, N.E., Washington, D.C. 20011