CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-790

PHARMACOLOGY REVIEW

MEMORANDUM
April 25, 2006

TO: File
FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-790

I have reviewed the pharmacology/toxicology information in the proposed label for Dacogen (decitabine for injection) and concur. No additional changes are needed.

_____
Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director for Pharmacology and Toxicology

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/s/

Kenneth Hastings
4/25/2006 03:57:34 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-790
SERIAL NUMBER:	002
DATE RECEIVED BY CENTER:	8/21/2004
PRODUCT:	Decitabine (Dacogen™)
INTENDED CLINICAL POPULATION:	Patients with Myelodysplastic Syndrome
SPONSOR:	SuperGen, Inc. 4140 Dublin Blvd., Suite 200 Dublin, CA 94568.
DOCUMENTS REVIEWED:	Electronic submission
REVIEW DIVISION:	Division of Oncology Drug Products (HFD-150)
PHARM/TOX REVIEWER:	M. Anwar Goheer, Ph.D.
PHARM/TOX SUPERVISOR:	John K. Leighton, Ph.D., D.A.B.T.
ACTING DIVISION DIRECTOR:	Robert Justice, M.D., M.S.
PROJECT MANAGER:	Nicholette Hemingway

Date of review submission to Division File System (DFS): Dec. 1, 2005

7 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

_____ § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

Withheld Track Number: Pharm/Tox- 2

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/s/

Anwar Goheer
12/1/2005 12:22:00 PM
PHARMACOLOGIST

John Leighton
12/9/2005 10:04:59 AM
PHARMACOLOGIST

MEMORANDUM
Aug. 24, 2005

TO: File
FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-790

I have read the pharmacology/toxicology review of Decitabine (Dacogen®) by Dr. M. Anwar Goheer as approved by Dr. John K. Leighton and concur that this application is approvable. No additional studies are needed. Reliance on published studies is acceptable given the indication.

Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director for Pharmacology and Toxicology

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/s/

Kenneth Hastings
8/24/2005 03:39:35 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-790
SERIAL NUMBER:	002
DATE RECEIVED BY CENTER:	8/21/2004
PRODUCT:	Decitabine (Dacogen™)
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REVIEW DIVISION:	Division of Oncology Drug Products (HFD-150)
PHARM/TOX REVIEWER:	M. Anwar Goheer, Ph.D.
PHARM/TOX SUPERVISOR:	John K. Leighton, Ph.D., DABT
DIVISION DIRECTOR:	Richard Pazdur, M.D.
PROJECT MANAGER:	Nicholette Hemingway

Date of review submission to Division File System (DFS): June 22, 2005

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: The non-clinical studies submitted to this NDA provide sufficient information to support the use of decitabine (Dacogen™) in patients with myelodysplastic syndromes (MDS).

B. Recommendation for nonclinical studies: None

C. Recommendations on labeling: See separate labeling review

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings: 5-Aza-2'-deoxycytidine (decitabine) has been investigated since the 1960s. It is an antimetabolite of deoxycytidine, a natural nucleoside, in which the 5-carbon of the cytosine ring has been replaced by nitrogen. Arabinosylcytosine (Ara-C) and gemcitabine do not have 5-aza substitution and do not inhibit methylation. DNA methylation has been associated with gene silencing and decitabine, as a hypomethylating agent, may restore gene expression. The activity of DNA methyltransferase may also be reduced after exposure to decitabine.

During traditional toxicity assessment, decitabine was administered to rodent (mice, rats) and non-rodents (dogs, rabbits) for 1, 5, 85 (3 times d x 3 q 28 d) and 168 days (3 times d x 3 q 42 d for 4 cycles). Single and multidose administration of decitabine resulted in hematologic toxicities (leukopenia, anemia and thrombocytopenia). A dose-dependent significantly decreased testicular and epididymal weights correlated with the observed morphological alterations in these organs of treated animals. Effects on testicular morphology were also seen in a study on male reproductive fertility.

Decitabine did not produce mutations in the Ames test in the absence of metabolic activator (S9). However, this study was not definitive. Decitabine was mutagenic in the *in vitro* TK mutation test in L5178Y mouse lymphoma cells and *Escherichia coli lac-I* transgene in colonic DNA from decitabine treated mice. Decitabine induced chromosomal rearrangements in larvae of the fruit fly, *Drosophila melanogaster*.

Several studies that examine the carcinogenic potential of decitabine have been conducted. The studies provided did not follow standard protocols; i.e., the 2 year bioassay or utilize an alternative model. The results are conflicting and thus no firm conclusions can be drawn. Given the therapeutic indication, these studies are not necessary.

The sponsor did not conduct any study to characterize the reproductive or developmental toxicity of decitabine. In a fertility study in which male mice were injected 3 times a week for 7 weeks with 0.3 or 0.45 mg/m^2, and subsequently bred to untreated females, sperm counts in male offspring were decreased. Pregnancy rate was decreased and preimplantation loss was increased in untreated females bred to these males. In a study in which mice were injected on day 10 of gestation with 3 mg/m^2 decitabine, it was apparent that male offspring exposed *in utero* were particularly susceptible to the toxic effects of decitabine. Male offspring showed a persistent decrease in body weight relative to controls, a decrease in the amount of spermatids found in testis, and a reduction in fertility in mated females when bred at 3 and 5 months of age.

Studies available in the scientific literature indicate decitabine is teratogenic in mice and rats. Teratogenicity was observed in the absence of maternal toxicity. There were significant increases in major skeletal or visceral malformations due to decitabine treatment in mice and rats from 0.9 and 2.4 mg/m^2, respectively. Of particular importance is the hindlimb defect in mice (routinely observed at 3 mg/m^2) that was also seen in rats, where both hindlimbs and forelimbs are affected. The teratogenicity is related to the timing of dosing relative to gestational day.

The recommended human dose is 15 mg/m^2 every eight hours (45 mg/m^2/day) for three days. Therefore, it is important that sexually active patients be aware that there is no safety margin and should take precautions to avoid pregnancy.

B. Pharmacologic activity: Decitabine is a prodrug that is phosphorylated by deoxycytidine kinase (dC-kinase) to 5-aza'-deoxycytidine-5-monophosphate (5-aza-dCMP). The phosphorylation of 5-aza-dCMP to 5-aza-dCDP is catalyzed by deoxycytidylate kinase (dCMP kinase) and the diphosphate is further converted to decitabine triphosphate (5-aza-dCTP) by nucleoside diphosphonucleoside kinase (NDP kinase). Decitabine is specific for DNA and does not incorporate into RNA, in contrast to 5-azacytidine.

Cell cycle studies have shown that decitabine produces a preferential kill of S-phase cells. Its antineoplastic activity appears to result of the inhibition of cell proliferation (cytotoxicity) observed at higher doses and induction of hypomethylation that promote cell differentiation at lower doses. It is not clear from the studies in the literature whether the primary activity is cytotoxic or as a hypomethylating agent. Inhibition of DNA synthesis does not appear to play a role in the cytotoxicity of decitabine.

Reisistance to decitabine has been observed in *in vitro* studies. Possible mechanisms include gene overexpression of the metabolic enzyme, deoxyctidylate kinase, or decreased activity through gene mutation of the key activating enzyme, deoxycytidine kinase. Cross-resistance in cultured cells has

been observed with decitabine and cytarabine, but evidence from clinical studies that resistance to cytarabine is due to either of these mechanisms is preliminary at this time.

The effects of decitabine on the metabolism of cytochrome P450 substrates and formal drug-drug interaction studies have not been conducted.

C. Nonclinical safety issues relevant to clinical use: The primary toxicity related to decitabine administration during multicycle intravenous infusion in rats, dogs and rabbits were myelosuppression. Teratogenicity in the absence of maternal toxicity has been observed at single doses one-fifth to one-tenth that of the intended clinical dose. Exposure *in utero* or to male mice prior to mating resulted in decreased fertility and increased preimplantation loses. Exposure information is not available at these doses, so comparisons to human clinical doses will be based on dose administered, adjusted for body surface area.

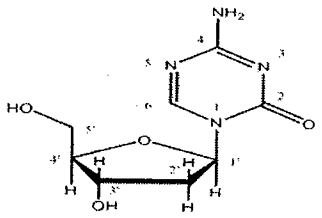
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number:	21-790
Review number:	1
Sequence number/date/type of submission:	002 / 8-21-2004 / NDA
Information to sponsor:	Yes (X) No ( )
Sponsor and/or agent:	SuperGen, Inc. 4140 Dublin Blvd., Suite 200 Dublin, CA 94568.
Manufacturer for drug substance:	[redacted]
Manufacturer for drug product:	[redacted]
Reviewer name:	M. Anwar Goheer, Ph.D.
Division name:	Division of Oncology Drug Products
HFD #:	150
Review completion date	June 20, 2005

Drug:

Trade name:	Dacogen™
Generic name:	Decitabine
Chemical name:	4-Amino-1-(2-deoxy-β-D-erythro-pento-furanosyl)-1,3,5-triazin-2(1H)-one
Other Names:	NSC- 127716; DAC; AzDC; Deoxyazacytidine; 5-Azadeoxycytidine; 5-Aza-2'-deoxycytidine; 5-azadCyd; s-Triazin-2(1H)-one, 4-amino-1-(2-deoxy-ß-D-erythro-pentofuranosyl-(8CI); 1,3,5-Triazin-2(H)-one, 4-amino-1-(2-deoxy-ß-D-erythro-pentofuranosyl-(9CI)
CAS registry number:	2353335
Molecular formula/molecular weight:	$C_8H_{12}N_4O_4$ / 228.21 daltons
Structure:	



Relevant INDs/NDAs/DMFs: IND 33,929
Drug class: Antimetabolite and DNA hypomethylating agent
Intended clinical population: Myelodysplastic Syndromes (MDS)
Clinical formulation: Lyophilized Powder for Injection

Composition of decitabine for injection, 50 mg vial

Ingredients	Reference to Quality Standard	Amount Per Vial	Function
Decitabine	In-house standard	50.0 mg	Active ingredient
Monobasic Potassium Phosphate	NF/EP	68.0 mg	Buffering agent
Sodium Hydroxide	NF/EP	11.6 mg	Buffering agent
Water for Injection*	USP/EP	q.s. to 10 mL	Solvent

(Excerpted from the sponsor's submission)

Route of administration: Intravenous

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 21-790 are owned by SuperGen, Inc., or are data for which SuperGen has obtained a written right of reference. Any information or data necessary for approval of NDA 21-790 that SuperGen does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that SuperGen does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 21-790.

Studies reviewed within this submission:

2.6.2 PHARMACOLOGY

2.6.2.2 Primary pharmacodynamics

1. Preclinical antitumor activity of 5-aza-2'-deoxycytidine against human head and neck cancer xenografts.
2. Effect of intravenous infusion of 5- aza- 2'- deoxycytidine on survival time of mice with L1210 leukemia.
3. Synergistic action of 5- aza- 2'- deoxycytidine and 3- deazauridine on L1210 leukemic cells EMT6 tumor cells.
4. Transformation of 5-aza-2'-deoxycytidine- ^{3}H and its incorporation in different systems of rapidly proliferating cells..
5. Antileukemic activity of 5-aza-2'-deoxycytidine and cytarabine against intracerebral L1210 murine leukemia.
6. Antagonism of 5-aza-2'-deoxycytidine antileukemic activity by concomitant treatment with cytarabine.
7. Comparison of the in vitro cytotoxicity (L1210) of 5- aza- 2'- deoxycytidine with its therapeutic and toxic effects in mice.
8. Kinetics of phosphorylation of 5- aza- 2'- deoxycytidine by deoxycytidine kinase.
9. 5- Aza- 2'- deoxycytidine: an overview. From: 5- Aza- 2'- deoxycytidine: preclinical and clinical studies.
10. Induction of cytidine deaminase in HL- 60 myeloid leukemic cells by 5- aza- 2'- deoxycytidine.
11. DNA methyltransferase inhibitors- state of the art.
12. DNA alkali- labile sites induced by incorporation of 5- aza- 2'- deoxycytidine into DNA of mouse leukemia L1210 cells.
13. Incorporation of a potent antileukemic agent, 5- aza- 2'- deoxycytidine, into DNA of cells from leukemic mice.
14. Incorporation of 5- aza- 2'- deoxycytidine- 5'- triphosphate into DNA. Interactions with mammalian DNA polymerase α and DNA methylase.
15. 5- Aza- 2'- deoxycytidine: preclinical studies in mice.
16. Comparison of the antileukemic activity of 5-aza-2'-deoxycytidine, 1-β-D-arabinofuranosylcytosine and 5-azacytine against L1210 leukemia.
17. Inhibition of DNA methyltransferase and induction of Friend erythroleukemia cell differentiation by 5-azacytidine and 5-aza-2'deoxycytidine.
18. Toxicity of 5-aza-2'-deoxycytidine to mammalian cells is mediated primarily by covalent tapping of DNA methyltransferase rather than DNA demethylation.

2.6.2.3 Secondary Pharmacodynamics

1. 5- Azacytidine and 5- azadeoxycytidine inhibit human immunodeficiency virus type 1 replication in vitro.
2. Expression of HLA class 1 antigens and restoration of antigen- specific CTL response in melanoma cells following 5- aza- 2'- deoxycytidine treatment.

2.6.2.4 Safety pharmacology

No sponsor initiated studies were conducted.

2.6.2.5 Pharmacodynamic Drug Interactions

1. Deoxycytidine kinase and deoxycytidine deaminase values correspond closely to clinical response to cytosine arabinoside remission induction therapy in patients with acute myelogenous leukemia.
2. Tetrahydrouridine, cytidine analogues, and haemoglobin F.
3. Transformation of 5- aza- 2'- deoxycytidine- ^{3}H and its incorporation in different systems of rapidly proliferating cells.
4. Reversal of drug resistance in human tumor xenografts by 2'- deoxy- 5- azacytidine-induced demethylation of the hMLH1 gene promoter.
5. De novo induced mutations in the deoxycytidine kinase (dck) gene in rat leukemic clonal cell lines confers resistance to cytarabine (AraC) and 5-aza-2'-deoxycytidine (DAC).
6. Transfection of wild-type deoxycytidine kinase (*dck*) cDNA into an AraC- and DAC-resistant rat leukemic cell line of clonal origin fully restores drug sensitivity.
7. Drug resistance to 5-aza-2'-deocycytidine, 2',2'-difluorodeoxycytidine, and cytosine arabinoside conferred by retroviral-mediated transfer of human cytidine deaminase cDNA into murine cells.
8. Association of decreased uridine and deoxycytidine kinase with enhanced RNA and DNA polymerase in mouse leukemic cells resistant to 5-azacytidine and 5- aza- 2'- deoxycytidine.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.3 Absorption

1. Plasma and cerebrospinal fluid pharmacokinetics of 5- aza- 2'-deoxycytidine in rabbits and dogs.
2. Antileukemic activity of 5- aza- 2'- deoxycytidine and cytarabine against intracerebral L1210 murine leukemia.
3. Comparison of the in vitro cytotoxicity (L1210) of 5- aza- 2'- deoxycytidine with its therapeutic and toxic effects in mice.

4. Effect of intravenous infusion of 5- aza- 2'- deoxycytidine on survival time of mice with L1210 leukemia.
5. Bioavailability study of [^{3}H] 5- aza- 2'- deoxycytidine in mice and the effect of tetrahydrouridine.
6. Pharmacokinetics of decitabine in an IVAP rabbit model.

2.6.4.4 Distribution:

1. Plasma and cerebrospinal fluid pharmacokinetics of 5- aza- 2'- deoxycytidine in rabbits and dogs.
2. Transformation and metabolic effects of 5- aza- 2'- deoxycytidine in mice.
3. Comparison of the in vitro cytotoxicity (L1210) of 5- aza- 2'- deoxycytidine with its therapeutic and toxic effects in mice.
4. Comparison of the metabolism and inhibitory effects of 5- azacytidine and 5- aza- 2'- deoxycytidine in mammalian tissues.

2.6.4.5 Metabolism:

1. Kinetics of deamination of 5- aza- 2'- deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3- deazauridine, thymidine or uracil arabinoside.
2. Transformation of 5- aza- 2'- deoxycytidine- 3H and its incorporation in different systems of rapidly proliferating cells.
3. Transformation and metabolic effects of 5- aza- 2'- deoxycytidine in mice.
4. Kinetics of phosphorylation of 5- aza- 2'- deoxycytidine by deoxycytidine kinase.
5. Kinetic interaction of 5- aza- 2'- deoxycytidine- 5'- monophosphate and its 5'-triphosphate with deoxycytidylate deaminase.
6. Kinetics of 5- aza- 2'- deoxycytidine phosphorylation in mouse spleen and L1210 leukemic cell extracts.
7. Bioavailability study of [^{3}H] 5- aza- 2'- deoxycytidine in mice and the effect of tetrahydrouridine.

2.6.4.6 Excretion

1. Transformation and metabolic effects of 5- aza- 2'- deoxycytidine in mice.
2. Comparison of the in vitro cytotoxicity (L1210) of 5- aza- 2'- deoxycytidine with its therapeutic and toxic effects in mice.
3. Comparison of the metabolism and inhibitory effects of 5- azacytidine and 5- aza- 2'- deoxycytidine in mammalian tissues.

2.6.4.8 Other Pharmacokinetic Studies

1. Preclinical toxicology of 5- aza- 2'- deoxycytidine in mice and dogs.
2. Chemotherapy of L1210 and L1210/ ARA- C leukemia with 5- aza- 2'- deoxycytidine and 3- deazauridine.

2.6.6 TOXICOLOGY

2.6.6.3 Repeat-dose toxicity

1. Intravenous toxicity (range finding) study with decitabine by daily administration (5 days per week) in mice.
2. A multicycle intravenous infusion toxicity study in Sprague Dawley rats followed by 25 day recovery periods.
3. A multicycle intravenous infusion toxicity study in Beagle dogs followed by 25- day recovery periods.
4. A multicycle intravenous infusion toxicity study in rabbits.

2.6.6.4 Genetic toxicology

1. Evaluation of the mutagenic activity of DAC (pure) in Salmonella / microsome test.
2. Evaluation of the mutagenic activity of DAC (pure) in an *in vitro* mammalian cell gene.
3. Mutagenicity of 5-aza-2'-deoxycytidine is mediated by the Mammalian DNA methyltransferase.
4. Somatic recombination: a major genotoxic effect of two pyrimidine antimetabolitic chemotherapeutic drugs in *Drosophila melanogaster.*
5. Induction of a step in carcinogenesis that is normally associated with mutagenesis by nonmutagenic concentrations of 5-azacytidine.
6. Mutagenicity of 5- azacytidine and related nucleosides in C3H/ 10T 1/ 2 clone 8 and V79 cells.
7. DNA demethylation and pericentromeric rearrangements of chromosome 1.

2.6.6.5 Carcinogenicity

1. Carcinogenicity and haemoglobin synthesis induction by cytidine analogues.
2. The demethylating agent 5-aza-2'-deoxycytidine is a multipotent carcinogen in rats.
3. 5-Aza-2'-deoxycytidine is chemopreventive in a 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone-induced primary mouse lung tumor model.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

1. 5-aza-2'-deoxycytidine induces alterations in murine spermatogenesis and pregnancy outcome.

Embryo- fetal development

1. Teratogenic effects of the demethylating agent 5- aza- 2'- deoxycytidine in the Swiss Webster mouse.
2. Cell death and cell cycle perturbation in the developmental toxicity of the demethylating agent, 5-aza-2'-deoxycytidine.
3. Differentially expressed genes associated with 5-Aza-2'-Deoxycytidine induced hindlimb defects in the Swiss Webster Mouse
4. 5-Aza-2'-deoxycytidine-induced dysmorphogenesis in the rat.

Prenatal and postnatal development, including maternal function

1. 5-Aza-2'-deoxycytidine-induced inhibition of differentiation of spermatogonia into spermatocytes in the mouse.
2. Susceptibility to postnatal growth retardation induced by 5- aza- 2'-deoxycytidine in utero: gender specificity and correlation with reduced insulin-like growth factor.
3. 5-AZA-2'Deoxycytidine (5-AZA-CdR): a demethylating agent affecting development and reproductive capacity.

Studies <u>not</u> reviewed within this submission:

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.2 Methods of Analysis:

1. Plasma and cerebrospinal fluid pharmacokinetics of 5- aza- 2'-deoxycytidine in rabbits and dogs.
2. High- performance liquid chromatographic analysis of chemical stability of 5- aza- 2'- deoxycytidine.
3. Sample preparation for the determination of 5- aza- 2'- deoxycytidine in plasma by high performance liquid chromatography.
4. In vitro cytotoxic and biochemical effects of 5- aza- 2'- deoxycytidine.
5. Effect of intravenous infusion of 5- aza- 2'- deoxycytidine on survival time of mice with L1210 leukemia.

6. Comparison of the metabolism and inhibitory effects of 5- azacytidine and 5- aza- 2'- deoxycytidine in mammalian tissues.
7. Analytical method validation of decitabine in rat plasma.
8. Analytical method validation of decitabine in dog plasma.
9. Sample analysis for the determination of decitabine in rabbit plasma by LC/ MS/ MS assay.

2.6.6 TOXICOLOGY

2.6.6.2 Single- Dose Toxicity

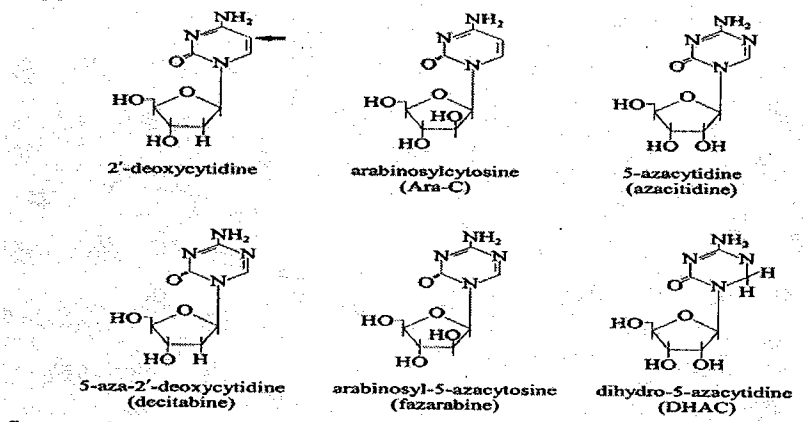
1. Effect of intravenous infusion of 5- aza- 2'- deoxycytidine on survival time of mice with L1210 leukemia.
2. Toxicology in mice of the antileukemic agent 5- aza- 2'- deoxycytidine.
3. 5- Aza- 2'- deoxycytidine: preclinical studies in mice.
4. Assessment of acute intravenous toxicity with decitabine in the mouse.
5. Assessment of acute intravenous toxicity with decitabine in the mouse.
6. Assessment of acute intravenous toxicity with decitabine (_____) in the mouse.
7. Preclinical toxicology of 5- aza- 2'- deoxycytidine in mice and dogs.

2.6.6.3 Repeat-dose toxicity

1. Subacute intravenous toxicity study, after 5 daily administrations of decitabine per week over 4 weeks by hematology, histopathology, and bone marrow cytology in the mouse and followed by a 28 day recovery period.
2. Intravenous infusion dose range finding toxicity study of a test article in rats.
3. Intravenous infusion dose range finding toxicity study of decitabine in rabbits.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary: Decitabine (5-aza-2'-deoxycytidine) is a pyrimidine nucleoside analog. It differs from the natural nucleoside deoxycytidine by the presence of nitrogen at position 5 of the cytosine ring (arrow in figure below). Decitabine is an inhibitor of the DNA methyltransferases. Like arabinosylcytosine (cytarabine, Ara-C), 5-axacytidine and arabinosyl-5-azacytosine (fazarabine), it must be phosphorylated to be activated.



Above figure shows Ara-C and the DNA methyltransferase inhibitors, as compared to the natural nucleoside, deoxycytidine.

Primary Pharmacodynamics: Decitabine is phosphorylated by deoxycytidine kinase to form an active intermediate, 5-aza-deoxycytidine monophosphate (5-aza-dCMP). The monophosphate is rapidly converted to 5-aza-deoxycytidine triphosphate. This analog is incorporated into DNA and not RNA. The substitution at the 5' position prevents methylation at this site, consequently affecting transcriptional activity of genes sensitive to silencing through methylation. The principal metabolic route inactivating decitabine is cytidine deaminase. The activating and metabolic routes are shown schematically below.

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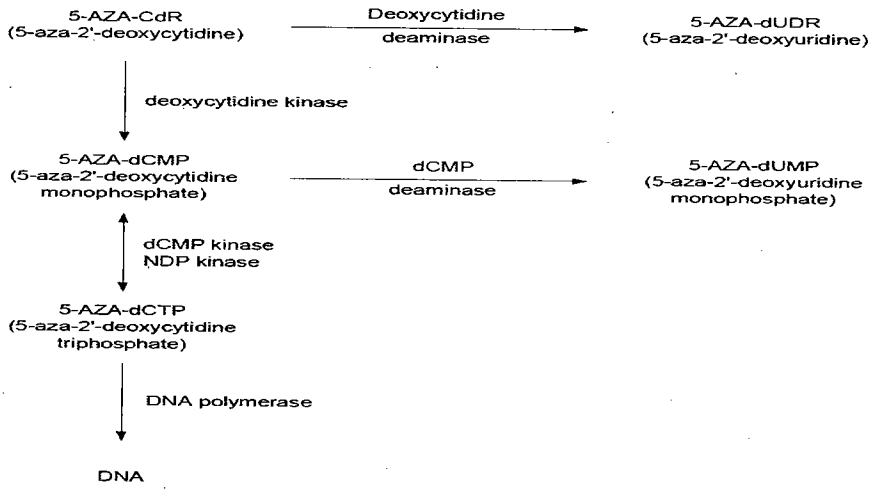


Decitabine is a cytotoxic agent *in vivo* and in *vitro*. It was active against murine tumors AKR, P388, and L1210; human head and neck tumor xenografts; and other cell lines. The magnitude of anti-tumor activity depended on the dose, route and schedule of drug administration. Optimal concentration for *in vitro* activity as measured by cytotoxicity in L1210 cells was 0.5-1.0 μg/mL. Decitabine showed synergistic cytotoxicity with 3-deazuridine (an agent that depletes intracellular pools of cytosine nucleotides) on L1210 leukemic cells *in vivo*. The combination decitabine and cytarabine, antineoplastic agents with similar modes of action, is less effective than decitabine alone in L1210 cells in an *in vivo* tumor model. It remains unclear whether the primary activity of decitabine is through cytotoxicity or hypomethylation.

Secondary Pharmacodynamics: Decitabine can be used *in vivo* to induce re-expression of MLH1 in MMR-deficient cells. This in turn sensitizes xenografts to cisplatin, carboplatin, epirubicin, and temozolomide. Decitabine treatment alone had no significant effect on the growth rate of the drug resistant ovarian (A2780/cp70) and colon (SW48) tumor xenografts. This sensitization by decitabine may be related to re-expression of MLH1 and not re-expression of the tumor suppressor gene. Thus, decitabine may have a role in increasing the efficacy of chemotherapy for patients with tumors that lack MLH1 expression. Other examples of sensitization of tumors to decitabine are available in the scientific literature.

Pharmacodynamic drug interactions: Drug resistance to decitabine was reported for cells transfected with cytosine deaminase cDNA. Alternatively, cells resistant to decitabine were generated by sequential step-up of decitabine concentrations. Mutations in deoxycytidine kinase gene were found to be necessary for the resistance phenotype, and transfection of the wild-type gene conferred resistance to cytarabine and decitabine.

2.6.2.2 Primary pharmacodynamics

Note: No sponsor initiated studies were conducted. Relevant literature references provided by the sponsor are reviewed here.

1. **Preclinical antitumor activity of 5- aza- 2'- deoxycytidine against human head and neck cancer xenografts.** Boudewijn et al., Investigational New Drugs; 6:299-304 (1988).

Female nude mice (~10 weeks old) were implanted subcutaneously with tumor cells in the lateral thoracic region on both sides. Treatment was started when the tumors reached a volume between 50 and 150 mm^3. Decitabine showed anti-tumor activity in solid tumor model, human head and neck tumor xenografts transplanted in nude mice as shown below.

Line	Dose (mg/kg)	Injections on day	Growth delay factor[a]	Complete regression[b]
HNX-DU	2	0,4,8	2.9	1/6
HNX-HE	2	0,4,8	0.0	0/8
HNX-HEp-2	2	0,4,8	0.9	0/5
HNX-KE	2	0,4,8	0.1	0/7
HNX-14C	2	0,4,8	2.3	1/6
HNX-DU	0.25	0–4,7–11	0.2	0/7
HNX-HE	0.25	0–4,7–11	0.0	0/9
HNX-KE	0.25	0–4,7–11	0.0	0/7
HNX-14C	0.25	0–4,7–11	3.1	0/5

[a] growth delay factor (GDF): $\frac{TDt - TDc}{TDc}$ (TD: tumor volume doubling time, t: treated, c: control).

Underlined values mean significant difference (Student's t-test, $p < 0.05$) in tumor volume doubling times between tumors from treated and control animals.

[b] no. completely regressed / no. total tumors.

(Excerpted from the publication)

A significant response was noted when the drug was injected intraperitoneally at a maximum tolerated dose of 2 mg/kg every four days for three doses.

2. **Effect of intravenous infusion of 5- aza- 2'- deoxycytidine on survival time of mice with L1210 leukemia.** Momparler and Gonzales. Cancer Research; 38:2673-2678 (1978).

Male CD2F1 (BALB/c x DBA/2 F1) mice (24-27 g, ~9 weeks old) were given 5 x 10^4 L1210 leukemic cells (IV) on day 0. Decitabine was infused (0.16 mL/hr) for the time indicated on day 3. The effect of a continuous infusion of decitabine on the survival time of leukemic mice is shown below.

Duration of i.v. infusion (hr)	5-aza-dCyd dosage (mg/kg)	Mean survival time (days)	% ILS	Estimated leukemic cell kill (log)	% wt change (Day 8)
0	0	7.2 ± 1.0[a]			
0.5	0.34	8.3 ± 1.1	15	0.6	
1.0	0.64	9.1 ± 1.0	25	1.1	
2.0	1.24	9.9 ± 1.3	36	1.6	+3.0
4.0	2.46	11.4 ± 1.2	58	2.5	+4.9

[a] Mean ± S.D.

(Excerpted from the publication)

The survival time of the leukemic mice increased with the duration of the infusion of decitabine.

3. **Synergistic Action of 5- Aza- 2'- deoxycytidine and 3- Deazauridine on L1210 Leukemic Cells and EMT6 Tumor Cells.** Momparler et al., Cancer Research; 39:3822-3827 (1979).

Male CD2F1 mice (27-28 g body weight) were given IV injections of 10^3 (experiment A) or 10^4 (experiment B) L1210 leukemic cells on day 0. Decitabine (5-aza-dcyd) and/or 3-deazauridine (3-DU) were infused IV at 0.22 mL/hour for the indicated times on day 3 and results are shown below.

Experiment	Chemotherapy	Dosage (mg/kg)	Infusion time (hr)	Survival time (days)	ILS (%)	40-day survivors	Wt. change[a] (%)
A	None		6	7.6 ± 0.4[b]	0	0/5	+3
	4-aza-dCyd	0.97	6	10.0 ± 0.3	32	0/5	0
	3-DU	45.1	6	8.1 ± 0.3	7	0/5	0
	5-aza-dCyd + 3-DU	0.97 + 46.0	6	13.1 ± 0.7	72	0/5	0
B	None		12	7.1 ± 0.5	0	0/4	
	5-aza-dCyd	9.8	12	22.6 ± 2.3	218	0/5	−5
	3-DU	94.7	12	8.2 ± 0.4	16	0/5	−12
	5-aza-dCyd + 3-DU	9.7 + 97.2	12	29.6 ± 4.8	317	1/5	

[a] Body weight on Day 7 (Experiment A) and Day 10 (Experiment B).
[b] Mean ± S.D.

(Excerpted from the publication)

The combination of decitabine and 3-deazauridine (an agent that depletes intracellular pools of cytosine nucleotides) increased the survival time of leukemic mice at both the 6 and 12 hour time points.

4. **Transformation of 5- aza- 2'- deoxycytidine- ^{3}H and its incorporation in different systems of rapidly proliferating cells.** Cihak, A. Europ J Cancer; 14:117-124 (1978).

Preferential incorporation of decitabine-^{3}H into DNA in the spleen and the thymus of AKR mice with lymphatic leukemia are shown below. Decitabine-^{3}H was taken up into the spleen and thymus nucleic acid more effectively than in the liver and kidney.

Incorporation of decitabine-^{3}H in tissues of AKR mice with lymphatic leukemia

Tissue	Incorporation (%) dis/min 10^{-3}/g tissue ± S.E. Control AKR	Lymphatic leukemia		dis/min 10^{-3}/organ ± S.E. Control AKR	Lymphatic leukemia	
Liver	254 ± 35	753 ± 93	(296)	267 ± 31	1037 ± 86	(389)
Kidney	298 ± 28	309 ± 33	(104)	75 ± 6	74 ± 7	(99)
Spleen	6439 ± 385	6120 ± 510	(95)	545 ± 44	1420 ± 117	(261)
Thymus	3088 ± 270	2720 ± 304	(88)	248 ± 30	250 ± 21	(100)

Groups of 5 female AKR mice (26 g) 7 days after s.c. inoculation with 2.10^{7} leukemic cells were injected i.p. 2 hr before killing with 5-aza-2′-deoxycytidine-^{3}H (125 μCi/0.2 μmole per animal). Controls are taken as 100%.

(Excerpted from the publication)

High incorporation of thymidine and deoxycytidine, and low incorporation of decitabine by proliferating hepatocytes in regenerating rat livers are shown below.

Differences in incorporation of thymidine-^{3}H, deoxycytidine-2-^{14}C, and 5-aza-2′-deoxycytidine-^{3}H in the liver of intact and partially hepatectomized rats

Administered	Incorporation, dis/min 10^{-3}/g tissue ± S.E. (%) Intact	Partially hepatectomized	
Thymidine-^{3}H	31.1 ± 5.2	289.6 ± 28.8	(930)
Deoxycytidine-2-^{14}C	6.0 ± 0.7	73.2 ± 9.4	(1220)
5-Aza-2′-deoxycytidine-^{3}H	207.0 ± 22.5	218.2 ± 22.5	(105)

Groups of 6 intact or partially hepatectomized female rats (175–180g) 24 hr after operation were given 2 hr before killing thymidine(methyl-^{3}H) (100 μCi/0.2 μmole per animal), deoxycytidine-2-^{14}C (6 μCi/0.2 μmole per animal) or 5-aza-2′-deoxycytidine-^{3}H (100 μCi/0.2 μmole per animal). Intact animals are taken as 100%.

(Excerpted from the publication)

The treatment of rats with 5-azacytidine prior to partial hepatectomy increased 3H-thymidine but not ^{3}H-5-aza-2'deoxycytidine incorporation into DNA in 24 hours regenerating livers. 5-Azacytidine depressed thymidine but especially 5-aza-2'deoxycytidine incorporation into spleen DNA.

Changes in the incorporation of thymidine-^{3}H and 5-aza-2'deoxycytidine-^{3}H in 5-azacytidine-treated rats subjected to partial hepatectomy.

Tissue	5-Azacytidine treatment	Incorporation, dis/min 10^{-3}/organ ± S.E. (%) Thymidine-^{3}H		5-Aza-2′-deoxycytidine-^{3}H	
Regenerating liver	−	452.5 ± 43.8	(100)	342.7 ± 27.2	(100)
	+	678.3 ± 41.9	(150)	360.0 ± 16.8	(105)
Spleen	−	76.3 ± 12.0	(100)	354.0 ± 42.6	(100)
	+	41.8 ± 4.9	(54.8)	75.7 ± 10.4	(21.4)

Groups of 5 female rats (175–185g) were given i.p. at 24 hr intervals two doses of 5-azacytidine (6 μmole per 100g) or 0.9% NaCl and 24 hr later partial hepatectomy was carried out. Incorporation of thymidine(methyl-^{3}H) (100 μCi/0.2 μmole per animal) or 5-aza-2′-deoxycytidine-^{3}H (100 μCi/0.2 μmole per animal) into the fraction of nucleic acids was measured 24 hr after operation during a 2 hr pulse.

(Excerpted from the publication)

5. **Antileukemic activity of 5- aza- 2'- deoxycytidine and cytarabine against intracerebral L1210 murine leukemia.** Chabot and Momparler, Cancer Treat Rep; 68:1483-1487 (1984).

CD2F1 mice were given intracerebral inoculation of 10^3 L1210 cells. Animals were treated on day 6 with increasing doses of decitabine (5-aza-dCyd) or cytarabine (Ara-C) as a 12-hour IV infusion and results are shown below.

Drug	Dose (mg/kg)	Mean survival time (days ± SD)	ILS (%)	Body weight loss† (%)
5-Aza-dCyd	0	10.7 ± 0.6	0	
	1	11.1 ± 0.4	4	
	5	15.2 ± 1.7	43	-35‡
	20	23.8 ± 3.9	122	-20
	30	22.8 ± 4.5	113	-24
	45	23.0 ± 4.5	115	-24
	67	23.6 ± 3.8	121	-28
Ara-C	0	11.2 ± 0.4	0	
	48	14.5 ± 2.4	30	
	231	14.0 ± 2.2	25	-19‡
	1000	17.6 ± 0.6	57	-18
	1500	17.2 ± 1.3	54	-17
	2300	19.4 ± 4.7	73	-20
	3400	17.6 ± 1.7	57	-18

* On Day 0, 10^3 leukemic cells were injected ic, and on Day 6, the chemotherapy was administered at the indicated total dose as a 12-hr iv infusion. There were 5 mice/group.

†Body weight loss as determined 7 days after chemotherapy.

‡These means contain dying animals, and the body weight loss is in large part due to the advanced leukemia itself. In the case of ara-C, only 3 of 5 animals were weighed.

(Excerpted from the publication)

Decitabine (5-Aza-dCyd) in this model was more effective than Ara-C as shown above.

6. **Antagonism of 5- aza- 2'- deoxycytidine antileukemic activity by concomitant treatment with cytarabine.** Colombo et al., Cancer Treat Rep; 70:1451-1453 (1986).

The administration of cytarabine (Ara-C) on different days simultaneously with decitabine antagonized decitabine activity against L1210 mouse leukemia in this *in vivo* model as shown below.

Survival Time

Treatment day	Control	Ara-C	Aza-dC	Ara-C + Aza-dC
3	9.0±0.5	11.0±0.3	25.6±1.9	14.2±0.5
5	8.7±0.4	11.1±0.3	15.4±1.2	11.8±0.2
3, 6, & 9	9.0±0.5	14.7±0.2	25.2±1.8	18.9±0.5

7. **Comparison of the in vitro cytotoxicity (L1210) of 5- aza- 2'-deoxycytidine with its therapeutic and toxic effects in mice.** Covey and Zaharok. Eur J Cancer Clin Oncol; 21:109-117 (1985).

Following IV administration of 100 mg/kg, Cmax was 130 µg/mL, falling to a less than an effective concentration of 0.05 µg/mL (based on an *in vitro* cytotoxicity assay) by 4 hours. Optimally effective concentrations *in vitro* were between 0.5-1.0 µg/mL. Alzet minipumps were used to provide sustained plasma levels of decitibine and compared to intravenous administration in CDF1 mice (20-25 g body weight) bearing L1210 (10^4 cells). An increase in life span using an infusion of 2 mg/kg/h for 8 hours (Css approx 1 µg/mL) starting 3 days after tumor implant was as effective as a single dose of 225 mg/kg, without the host animal toxicity observed at longer infusion times (e.g., 12 h).

Effects of various doses and schedules on day 3 staged L1210 tumor in mice

Treatment and dose*	Toxic day of death	% Body weight (+, gain; -, loss)	Tumor day of death	Median day of death Toxic	Median day of death Tumor	Survivors at 60 days	Log cell Δ	Comments
Control								
$L1210^{i.v.}$ 1×10^4 cells	-	-0, +5	7[5],8	-	7	0	+5	
$DAC_{10}^{i.v.}$ d3q3hr × 3	-	-5, +2	23,24,25,36 37,45	-	25	0	-6	
$DAC_{10}^{i.v.}$ d3q3hr × 4	59	-11, +5	26,27,30[2],52	59	30	0	-6	
$DAC_{Alza}^{s.c.}$ d3 2 mg/kg/hr × 8 hr	-	-4, +5	20[2],23,24,27 30	-	23	0	-6	
$DAC_{Alza}^{s.c.}$ d3 2 mg/kg/hr × 12 hr	-	-19, +6	27, 28, 36[2],50 59	-	36	0	-6	large weight loss
$DAC_{Alza}^{s.c.}$ d3 2 mg/kg/hr × 16 hr	13[2]	-21, +6	24,25,26,43 30	13	25	0	-6	toxic
$DAC_{50}^{i.v.}$ d3q × 1	-	-0, +7	18[2],19[3],24	-	19	0	-6	
$DAC_{150}^{i.v.}$ d3q × 1	-	-5, +3	20[2],22,23 24,26	-	22	0	-6	
$DAC_{225}^{i.v.}$ d3q × 1	-	-7, +4	20,24,25,27 34,51	-	25	0	-6	

(Excerpted from the publication)

8. **Kinetics of phosphorylation of 5- aza- 2'- deoxycytidine by deoxycytidine kinase.** Momparler and Derse. Biochemical Pharmacology; 28:1443-1444 (1979).

The interactions of decitabine (5-aza-2'-deoxycytidine, 5-AZA-CdR) and its triphosphate form (decitabine triphosphate) with deoxycytidine (CdR) kinase are reported in this short communication.

Inhibition of CdR (deoxycytidine) kinase by dCTP (2'-deoxycytidine triphosphate)
or
5-AZA-dCTP (decitabine 5'-triphosphate)

Substrate	Addition	Concn (μM)	Nucleotide formed (nmole)	Inhibition (%)
CdR	None		0.95	0
	dCTP	5	0.81	15
	dCTP	10	0.68	28
	dCTP	20	0.53	44
	5-AZA-dCTP	20	0.83	13
	5-AZA-dCTP	40	0.67	29
	5-AZA-dCTP	80	0.50	47
5-AZA-CdR	None		0.98	0
	dCTP	5	0.96	2
	dCTP	10	0.67	32
	dCTP	20	0.40	59
	5-AZA-dCTP	20	0.74	25
	5-AZA-dCTP	40	0.57	42
	5-AZA-dCTP	80	0.37	62

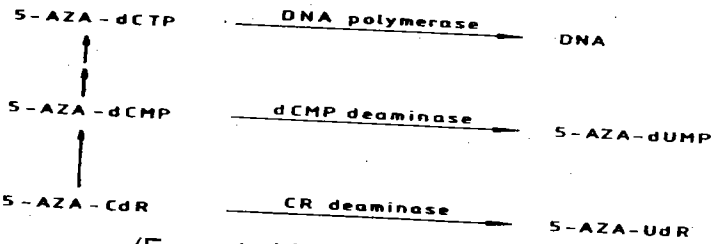
* The standard reaction mixture contained 100 mM imidazole–HCl, pH 6.8, 0.05 μCi of 20 μM [^{14}C]CdR or [6–^{14}C]5AZA-CdR, as indicated, and the indicated concentrations of dCTP or 5-AZA-dCTP. The reaction mixture was incubated for 5 min at 37° in the presence of 0.3 unit CdR kinase.

(Excerpted from the publication)

The inhibition produced by decitabine triphosphate was less than at equimolar concentration of 2'deoxycytidine triphosphate, possibly due to a higher affinity of deoxycytidine kinase for dCTP compared to aza-dCTP.

9. **5- Aza- 2'- deoxycytidine: an overview. From: 5- Aza- 2'- deoxycytidine: preclinical and clinical studies.** Momparler and De Vos. Mol Pharmacol; 25:436-440 (1984).

This review article is from the proceedings of the workshop on 5-aza-2'-deoxycytidine held in Amsterdam on March 11, 1989. Preclinical studies indicated that decitabine (5-Aza-CdR) is a more effective antineoplastic agent than the cytosine arabinoside, a related deoxycytidine analogue. The metabolism of decitabine is outlined below. Decitabine is first phosphorylated by deoxycytidine kinase to 5-aza-2'-deoxycytidine monophosphate (5-Aza-dCMP). 5-Aza-dCMP is rapidly converted to its 5'-triphosphate form (5-Aza-dCTP) by other kinases. 5-Aza-dCTP is an excellent substrate for DNA polymerase α. The deamination of decitabine by cytidine deaminase or the deamination of 5-Aza-dCMP by dCMP deaminase results in complete loss of antileukemic activity.



(Excerpted from the publication)

10. Induction of Cytidine Deaminase in HL- 60 Myeloid Leukemic Cells by 5- aza- 2'- deoxycytidine. Momparler and LaLiberte. Leukemia Research; 14:751-754 (1990).

5-Aza-2'-deoxycytidine produced a loss of clonogenicity and DNA synthesis in human HL-60 myeloid leukemic cells as shown below.

Treatment	Concentration (μM)	Growth inhibition (%)	Colony formation (%)	DNA synthesis inhibition (%)
Control	—	0*	100	0†
5-AZA-CdR	0.1	32.6 ± 4.2	<1	25.9 ± 0.9
5-AZA-CdR	1.0	57.7 ± 1.1‡	<1	48.1 ± 1.8§

5×10^4 cells/ml (50 ml) were incubated with the indicated concentrations of 5-AZA-CdR for 72 h and cell number and DNA synthesis determined. For colony formation the cells were placed in soft agar after 72 h.
* Mean ± S.E., $n = 4$.
† Control cells (10^5) incorporated 25,942 ± 2016 cpm.
‡ Significantly different (Student's t-test) from 0.1 μM 5-AZA-CdR ($p < 0.005$).
§ Significantly different (Student's t-test) from 0.1 μM 5-AZA-CdR ($p < 0.0005$).

(Excerpted from the publication)

Decitabine also increased cytidine deaminase activity in these cells.

Treatment	Concentration (μM)	Cytidine deaminase (units/mg)	NBT (%)
Control	—	0.65 ± 0.16*	5.5 ± 0.6
5-AZA-CdR	0.1	2.09 ± 0.22	19.3 ± 1.7
5-AZA-CdR	1.0	4.31 ± 0.35†	35.9 ± 1.1‡

5×10^4 cells/ml (50 ml) were incubated with the indicated concentrations of 5-AZA-CdR for 72 h and assayed for cytidine deaminase activity or reduction of nitroblue tetrazolium (NBT) as described under Materials and Methods.
* Mean ± S.E., $n = 4$.
† Significantly different (Student's t-test) from 0.1 μM 5-AZA-CdR ($p < 0.005$).
‡ Significantly different (Student's t-test) from 0.1 μM 5-AZA-CdR ($p < 0.0005$).

(Excerpted from the publication)

Induction of cytidine deaminase by decitabine shows its importance in the catabolism of cytosine nucleoside analogues.

11. DNA methyltransferase inhibitors- state of the art. Goffin and Eisenhauer. Annals of Oncology; 13:1699-1716 (2002).

DNA methylation is the addition of a methyl group to the 5 position of cytosine. Methylation plays a role in genomic stability and carcinogenesis. DNA methylation inhibitors have demonstrated the ability to inhibit hypermethylation, restore suppressor genes expression and exert antitumor effects in *in vivo* and *in vitro* models. The role of methylation in cancer and four methylation inhibitors, which are analogs of the nucleoside deoxycytidine, (5- azacytidine, 5- aza- 2'-

deoxycytidine, 1- ß- D- arabinosyl- 5- azacytosine and dihydro-5-azacytidine) are reviewed in this article.

12. **DNA alkali- labile sites induced by incorporation of 5- aza- 2'-deoxycytidine into DNA of mouse leukemia L1210 cells.** D'Incalci et al., Cancer Research 1985; 45:3197-3202

Decitabine treatment of L1210 cells produced alkali-labile sites in the DNA that was synthesized during exposure to the drug. Alkali-labile sites increased in a dose dependent manner as shown below. The labile sites were not seen double stranded DNA in cells treated to 10 µg/mL.

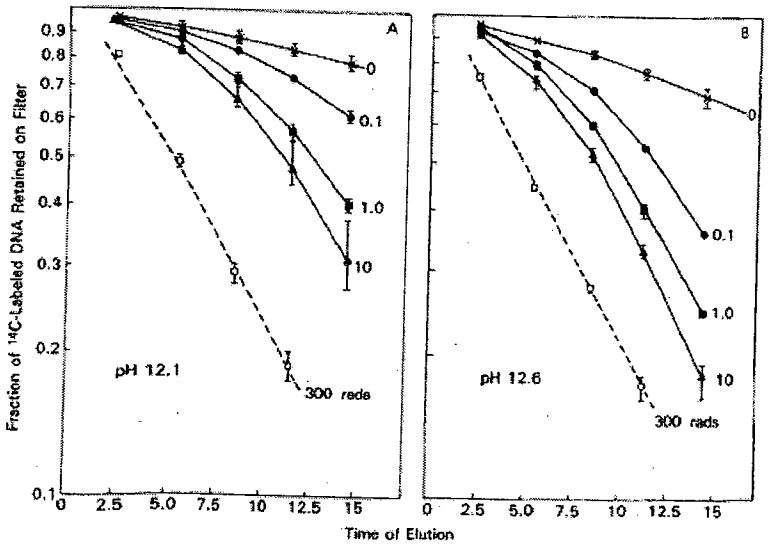


Chart 1. Alkaline elution profiles of [¹⁴C]DNA of L1210 cells treated for 24 h with aza-dCyd, 0 (×), 0.1 (●), 1.0 (■), or 10 µg/ml (▲). Elutions were carried out at pH 12.1 (A) and pH 12.6 (B). Labeling with [¹⁴C]thymidine, 0.02 µCi/ml, and treatment with aza-dCyd were simultaneous. Each point, mean of 3 values; bars, range. – – –, elution of [³H]DNA of L1210 after irradiation with 300 rads in the cold; 0.1 µCi [³H]-thymidine for 24 h was used to label these cells. Each point, mean of 60 values; bars, SE.

Assay for DNA double-strand breaks in L1210 treated for 24 h with Aza-dCyd, 0.1 to 100 µg/ml

Elution was carried out at pH 9.6 after lysing the cells on polyvinyl chloride filters. Values are the –log (retention) of DNA after 12 h of elution (fourth fraction) considering the retention after collecting the first fraction as 1.0.

		Aza-dCyd (µg/ml)			
Control	3000 rads	0.1	1	10	100
0.024[a] (0.02–0.03)[b]	0.11 (0.10–0.12)	0.025 (0.01–0.03)	0.04 (0.02–0.05)	0.025 (0.02–0.03)	0.036 (0.03–04)

[a] Mean of 3 replications.
[b] Numbers in parentheses, range.

13. **Incorporation of a potent antileukemic agent, 5- aza- 2'-deoxycytidine, into DNA of cells from leukemic mice.** Vesely and Cihak. Cancer Research; 37:3684-3689 (1977).

5-Aza-2'-deoxycytidine (aza-dCyd, decitabine) administration increased the life-span of P388 leukemic-bearing mice as shown below.

The i.p. administration of both drugs on 5 consecutive days was started 24 hr after inoculation of 5×10^6 leukemic cells i.p.; each group included 8 female mice.

Daily dose (mg/kg)	5-Azacytidine		aza-dCyd	
	Survival (days)	% survival	Survival (days)	% survival
0.5	6.5 ± 0.7[a]	100	6.5 ± 0.7	100
1.0	9.5 ± 1.1	146	12.5 ± 1.0	192
1.5	10.0 ± 1.4	154	13.2 ± 1.6	203
2.4	11.5 ± 0.9	177	16.4 ± 1.2	252
4.0	12.3 ± 1.9	189	9.6 ± 2.0	148
	11.4 ± 2.0	176	6.6 ± 1.6	102

[a] Average ± S.E.

(Excerpted from the publication)

14. **Incorporation of 5- aza- 2'- deoxycytidine- 5'- triphosphate into DNA. Interactions with mammalian DNA polymerase α and DNA methylase.** Brouchard and Momparler. Molecular Pharmacology; 24:109-114 (1983).

DNA polymerase α catalyzed the incorporation of 5-aza-2'-deoxycytidine 5'-triphosphate (decitabine triphosphate) into DNA as shown below.

Substrate	Template	Incorporation produced by DNA polymerase
		pmoles/20 min/mg protein
dTTP	poly(dIC)	<2
dCTP		23
5-AZA-dCTP		16
dTTP	poly(dAT)	107
dCTP		<2
5-AZA-dCTP		<2

(Excerpted from the publication)

These experiments showed that [^{3}H] 5-AZA-dCTP incorporation into poly(dAT) was negligible as compared with its incorporation into poly(dIC). [^{3}H] dTTP was incorporated into poly (dAT), but not into poly (dIC). The data suggests that the incorporation follows the rules of Watson-Crick base pairing since the nucleotide analogue was incorporated into poly (dIC) but not into poly (dAT).

15. **5- Aza- 2'- deoxycytidine: preclinical studies in mice.** Vesely and Cihak. Neoplasma; 27:113-119 (1980).

Intraperitoneal administration of 5-aza-2'-deoxycytidine (4 mg/kg) affected the synthesis of DNA in mouse spleen resulting in depression initially followed by the enhancement of thymidine uptake reaching the maximum on day 8.

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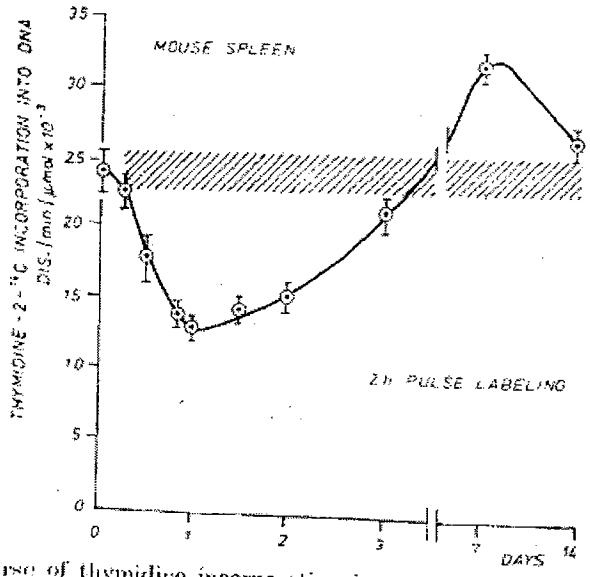


Fig. 4. Time-course of thymidine incorporation into spleen DNA of mice treated with AzadCyd. Groups of 4–5 mice received ip the drug (4 mg/kg) and 2 h before killing thymidine-2-^{14}C [37 KBq/0.01 μmol (1 μCi/0.01 μmol) per animal] was injected. Dashed, incorporation of thymidine in untreated controls.

(Excerpted from the publication)

16. **Comparison of the antileukemic activity of 5-aza-2'-deoxycytidine, 1-β-D-arabinofuranosylcytosine and 5-azacytine against L1210 leukemia.** Momparler et al., Leukemia Res 8 1043-1049 (1984).

The growth inhibitory and cytotoxic effects, effects on DNA synthesis methylation are reported in this publication. Shown below are comparative growth inhibition and cytotoxicity for the three compounds measured after 48 hour exposure (IC_{50}) or 6 hours (LC_{50}).

Compound	Growth inhibition IC_{50} (nM)	Cytotoxicity LC_{50} (nM)
Decitabine	10	100
Cytarabine	20	1000
Azacytidine	200	1000

The inhibition of DNA synthesis, as measured by ^{3}H-thymidine uptake, after a 4 hour exposure is shown in the table below.

Drug	Concentration (μM)	% inhibition DNA synthesis
Decitabine	100	<5
cytarabine	0.1	33
	1	77
	10	95
Azacytidine	10	<5
	100	16

17. **Inhibition of DNA methyltransferase and induction of Friend erythroleukemia cell differentiation by 5-azacytidine and 5-aza-2'deoxycytidine**. Creusot et al. J Biol Chem 257: 2041-2048 (1982).

Maximal differentiation as measured by staining positively with benzidine of Friend erythroleukemia cells occurred in the concentration range of 0.1-0.4 μM decitabine at a 24 h exposure. The mechanism through which decitabine and azacytidine inhibit DNA methyltransferase activity requires that the analog be incorporated into DNA. A DNA content of azacytidine of less than 0.1% has approximately 90% loss of methyltransferase activity. At 48 hours after removal of the analog, no differences are seen between treated and untreated cells in the acceptance rate of methyl groups, indicating that normal levels of methyltransferase had been restored.

18. **Toxicity of 5-aza-2'-deoxycytidine to mammalian cells is mediated primarily by covalent trapping of DNA methyltransferase rather than DNA demethylation.** Juttermann et al. Proc Natl Acad Sci USA 91: 11797-11801 (1994).

The article provides support for the concept that trapping of the DNA methyltransferase rather than hypomethylation of DNA is the critical factor in cytotoxicity. The investigators used embryonic stem (ES) cells from wild type, heterozygous (50% ↓ in enzyme levels vs wt) and homozygous (90%↓) mice with a mutation in the methyltransferase gene to assess the relationship of transferase activity, hypomethylation (30%↓ in 5-methylctyosine content in DNA relative to heterozygous and wt) and cytotoxicity. Homozygous ES cells were 10x more resistant to decitabine than wt cells, as measured by % survival of cells after a 24 h exposure, followed by 9 days of drug-free growth.

Wild type females were mated to heterozygous males, followed by a single IP injection of saline or decitabine (200 or 300 μg/25 g bw; 400 μg/25 g was lethal to dams) on gestation day 14. Dams were allowed to deliver and pups were genotyped at weaning for frequency of mutation in methyltransferase gene. In dosed mice, there was an increased frequency at birth for the heterozygous genotype and that these animals appeared healthier than sicker wild type liter mates (runts/severe skin ulceration).

2.6.2.3 Secondary Pharmacodynamics

Note: No sponsor initiated studies were conducted. Relevant literature references provided by the sponsor are reviewed here.

1. **5- Azacytidine and 5- Azadeoxycytidine Inhibit Human Immunodeficiency Virus Type 1 Replication *In Vitro*.** Bouchard et al., Antimicrob Agents Chemother; 34:206-209 (1990).

The *in vitro* inhibition of the replication of HIV type 1 in human CEM T cell by 5-azacytidine (5-AZAC), 5-azadeoxycytidine (5-AZAdC), and 5-azadeoxycytidine (AZT) are shown below.

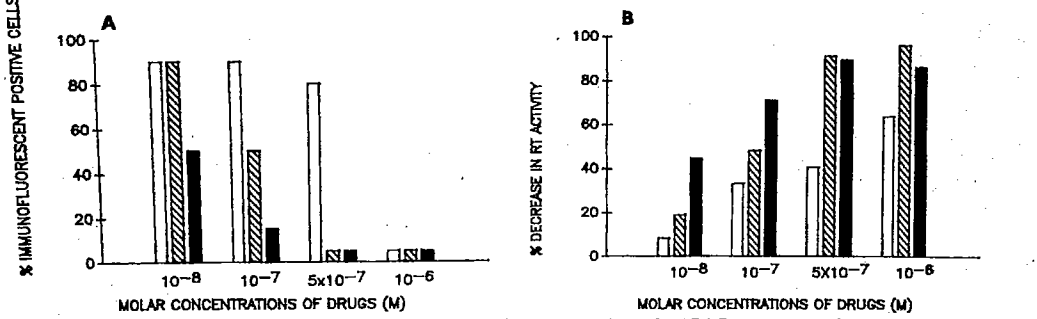


FIG. 2. Inhibition of HIV-1 replication in CEM cells by different molar concentrations of 5-AZAC (□), 5-AZAdC (▨), and AZT (■). (A) Average percentage of viral antigen expression by each group of drug-treated and untreated HIV-infected CEM cells 3 days after infection as determined by indirect immunofluorescence. A total of 90 to 95% of untreated, infected cells were observed to be fluorescent 3 days after infection. (B) Mean percent decrease in RT activity in supernatants of each of the drug-treated, infected CEM cells compared with activity in supernatants of untreated, infected cells. There was ±2% variation in replicate RT activity determinations. In these experiments, 1 ml of the supernatant of the uninfected, non-drug-treated CEM cells (cell control) incorporated 1,000 cpm of the [^{3}H]dTTP, and the HIV-infected, non-drug-treated CEM cells (virus-cell control) incorporated between 5×10^6 and 10×10^6 cpm of the [^{3}H]dTTP.

(Excerpted from the publication)

Both 5-azacytosine derivatives were more effective when added at the time of infection as shown below.

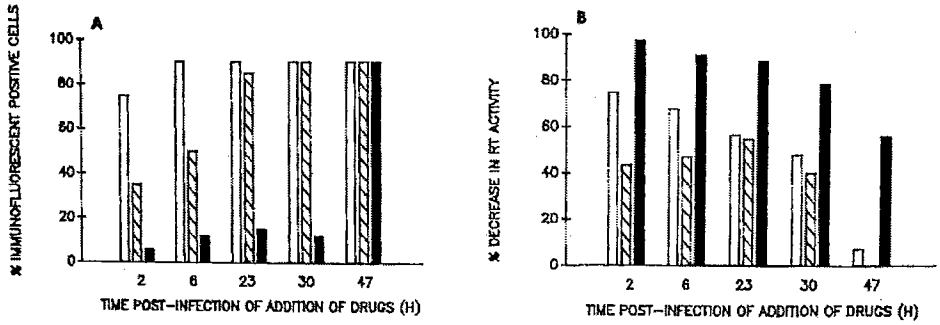


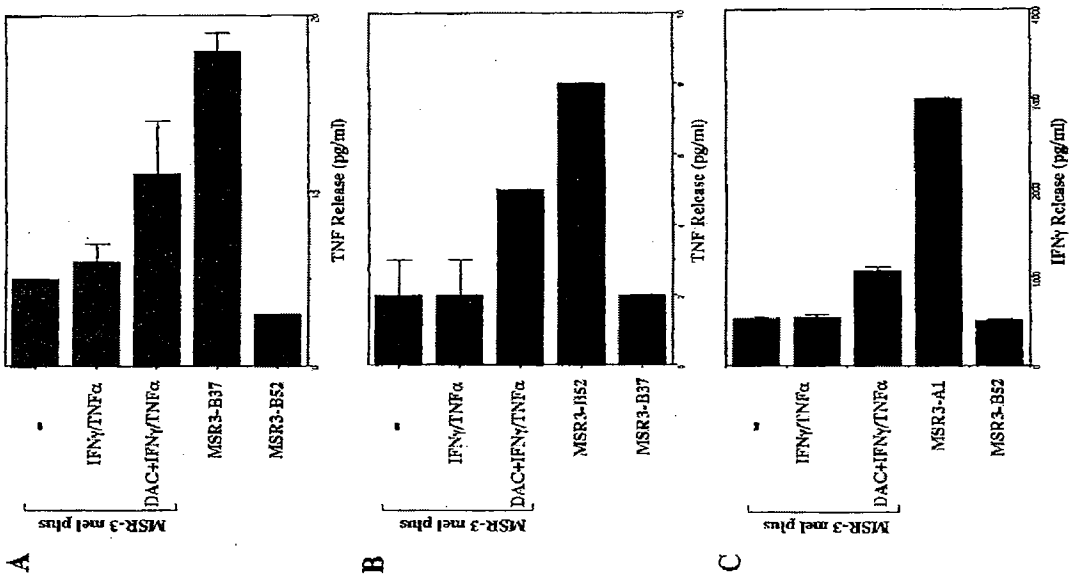
FIG. 3. Effects of addition of 5-AZAC, 5-AZAdC, or AZT at various times after HIV-1 infection on the replication of the virus in CEM cells. At 2, 6, 23, 30 and 47 h after infection, 1 μM of 5-AZAC (□), 5-AZAdC (▨), or AZT (■) was added to quadruplicate infected CEM cell cultures. The experiment was repeated three times. (A) Average percentage (variability, ±5% or less) of each group of drug-treated, HIV-1-infected cells expressing viral antigens at each time point, as determined by immunofluorescence; (B) average percent decrease in RT activity (variability, ±2%) in the supernatants of each group of treated, infected CEM cells at each time point compared with the RT activity in the supernatants of untreated, infected cells 3 days postinfection. The average amount of [^{3}H]dTTP incorporated by 1 ml of the supernatants of uninfected, non-drug-treated CEM cells was 1,000 cpm, and the average amount incorporated by supernatants of HIV-infected, non-drug-treated CEM cells was between 5×10^6 and 10×10^6 cpm.

(Excerpted from the publication)

2. **Expression of HLA Class I Antigens and Restoration of Antigen-Specific CTL Response in Melanoma Cells Following 5- Aza- 2'-Deoxycytidine Treatment.** Serrano et al., Int J Cancer; 94: 243-251 (2001).

The MSR3-mel line, obtained from a metastatic lesion of a nonresponding patient, treatment with decitabine successfully induced HLA class 1 expression in this line. This *in vitro* demethylation restored the recognition of MSR3 mel cells by HLA-A and HLA-B restricted tumor specific T cells. Hypermethylation-induced

lack of HLA class 1 expression may be the cause of impaired response to vaccination in this melanoma patient.



(Excerpted for the publication)

5-Aza-2'-deoxycytidine (decitabine) treatment restored tumor recognition by tumor-antigen specific CTL clones. MSR3-mel cells were treated with 10 μM decitabine for 6 days and then with IFN-γ (20 U/ml) and TNF-α (10 ng/ml) for 2 additional days and used as stimulators in a cytokine release assay. MSR3-mel treated and untreated with IFN-γ and TNF-α were used as control. Decitabine restored expression of HLA-A1, B37 and B52 that was monitored as recognition by the effectors (a) CTL 337 (b), MSR3-M3 and (c) CTL4.A1, These effector cells recognized (a) the $MAGE_{127-136}$-HLA-B37-restricted peptide,[27]" (b) the MAGE-$3_{143-151}$ peptide presented by HLA-B52 molecules" and a yet unidentified tumor antigen expressed by MSR3-mel and recognized in an HLA- AI-restricted fashion (c). MSR3-B37, MSR3-B52 and MSR3-AI cell lines were included as positive controls.

2.6.2.4 Safety pharmacology: No sponsor initiated studies were conducted.

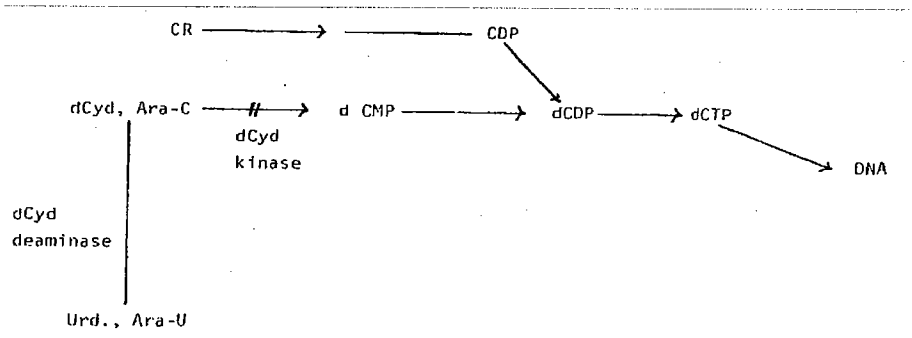
Neurological effects:	Not studied
Cardiovascular effects:	Not studied
Pulmonary effects:	Not studied
Renal effects:	Not studied
Gastrointestinal effects:	Not studied
Abuse liability:	Not studied
Other:	None

2.6.2.5 Pharmacodynamic drug interactions

No sponsor initiated studies were conducted.

1. **Deoxycytidine Kinase and Deoxycytidine Deaminase Values Correspond Closely to Clinical Response to Cytosine Arabinoside Remission Induction Therapy in Patients With Acute Myelogenous Leukemia.** Colly et al., Semin Oncol 1987; 14:267-261

The metabolic pathways for deoxycytidine (dCyd) and cytosine arabinoside (Ara--C) are shown below based on evaluation of data from 21 patients.



(Excerpted from the publication)

The phosphorylation of deoxycytidine (dCyd) or cytosine arabinoside (Ara- C) to monophosphate (d CMP) cannot occur in the absent of deoxycytidine kinase (dCyd kinase). The cells deficient in this kinase can synthesize deoxycytidine triphosphate (dCTP) necessary for DNA replication and thus cell proliferation by the presence of an enzyme that reduces cytidine diphosphate (CDP) to deoxycytidine diphosphate (dCDP). The dCyd deaminase enzyme causes deamination of dCyd and Ara-C to uridine (Urd) and uridine arabinoside (Ara-U), respectively. Patients with Ara-C resistant disease had either low dCyd kinase activity or a high deaminase activity.

2. **Tetrahydrouridine, Cytidine analogues, and Haemoglobin F.** DiSimone et al., Am J Hematology 18: 283-288 (1985).

Baboons (1.5-6 years old) were treated parenterally with 5-azacytidine (azaC) and 2-deoxy-5-azacytidine (d-azaC, decitabine) with or without tetrahydrouridine (THU) and results are shown below.

Baboon	Drug	Dose (mg/kg)	THU	Granulocyte numbers: Before treatment	Granulocyte numbers: Nadir	Maximal Hb F level (5)	Reticulocyte percentages[a]
4756	d-azaC	0.06	+	3,000	1,000	64.4	13–14
	azaC	0.25	+	3,000	1,000	69.4	16–19
4612	d-azaC	0.04	+	2,800	2,400	31.3	20–30
	azaC	0.25	+	2,100	1,600	67.8	13–15
4625	d-azaC	1.5	—	3,000	500	61.5	14–13
	azaC	4.0	—	3,200	1,000	49.8	11–14
4624	d-azaC	0.75	—	3,200	800	43.4	9–10
	azaC	4.0	—	4,200	1,600	39.0	12–10
4626	d-azaC	0.3	—	3,000	1,200	39.2	11–11
	azaC	4.0	—	3,500	1,300	68.0	15–12

*THU was given in two 10-mg/kg doses, the first one alone intravenously and the second 5 minutes later subcutaneously with d-azaC.
[a]First number indicates reticulocyte percentage after bleeding and prior to use of drug, the second at the time of maximal Hb F response. Corresponding erythrocyte counts varied between 2.0 and 2.4 × $10^6/\mu l$.

(Excerpted from the publication)

The administration of THU, (an inhibitor of the enzymatic conversion of azaC to 5-azauridine catalyzed by cytidine deaminase), reduced the amount of azaC and d-azaC more than 90% needed to achieve maximum HbF elevations. All regimens, azaC and d-azaC with or without THU were associated with a decrease of the absolute granulocyte count. This toxic effect of both analogues may be due to their incorporation into DNA.

3. **Transformation of 5- aza- 2'- deoxycytidine- ^{3}H and its incorporation in different systems of rapidly proliferating cells.** Cihak, A. Euro J Cancer 14: 117-124 (1978).

Male AKR mice (~25 g body weight) were injected i.p. with cytosine arabinoside (3 µmol) simultaneously with thymidine (methyl-^{3}H, 50 µCi/0.2 µmole per animal) or 5-aza-2'-deoxycytidine-^{3}H (150 µCi/0.2 µmole per animal) 7 days after inoculation with 2x10^7 leukemic cells. The animals were killed 2 hours after injection and results are shown below.

Inhibition of thymidine-^{3}H and 5-aza-2'-deoxycytidine-^{3}H incorporation in various tissues of leukemic mice with cytosine arabinoside

Tissue	Incorporation, dis/min 10^{-3}/g tissue ± S.E. (%): Control	Cytosine arabinoside	
	Thymidine-^{3}H		
Liver	771 ± 78	362 ± 65	(46.9)
Spleen	851 ± 68	51 ± 5	(6.0)
Thymus	161 ± 20	20 ± 3	(12.4)
	5-Aza-2'-deoxycytidine-^{3}H		
Liver	720 ± 108	423 ± 36	(58.8)
Spleen	9350 ± 415	1066 ± 53	(11.4)
Thymus	3036 ± 216	712 ± 102	(25.8)

(Excerpted from the publication)

The incorporation of both thymidine and 5-aza-2'-deoxycytidine was depressed by the simultaneous administration of cytosine arabinoside.

4. **Reversal of drug resistance in human tumor xenografts by 2'- deoxy-5- azacytidine- induced demethylation of the hMLH1 gene promoter.** Plumb et al., Cancer Research 60: 6039-6044 (2000).

2-Deoxy-5-azacytidine (DAC) treatment alone has no effect on the growth rate of mismatch repair-deficient (MMR), drug resistant ovarian (A2780/cp70) and colon (SW48; data not reproduced) tumor xenografts. However, DAC treatment sensitized the xenografts to cisplatin, carboplatin, temozolomide, and epirubicin. Sensitization was comparable with that obtained by reintroduction of the hMLH1 gene by chromosome 3 transfer. Loss of MMR did not affect *in vitro* sensitivity to taxol, and dacitabine did not affect the sensitivity of the xenograft to this agent. CP70-ch3 is a derivative of A2780/cp70 in which chromosome 3 is introduced micelle-mediated chromosome transfer.

Effects of DAC pretreatment on the drug sensitivity of MMR-deficient xenografts are shown in the next table.

Treatment	Time to double initial tumor volume (days)[a]			
	A2780/cp70		CP70-ch3	
Control	2.4 ± 0.2	NS	2.9 ± 0.2	NS
DAC (5 mg/kg × 3)	2.5 ± 0.3		3.3 ± 0.4	
Carboplatin (80 mg/kg)	2.9 ± 0.2	[b]	5.4 ± 0.2	NS
DAC + carboplatin	6.1 ± 0.5		5.6 ± 0.2	
Cisplatin (6 mg/kg)	2.9 ± 0.2	[b]	5.1 ± 0.2	[c]
DAC + cisplatin	6.0 ± 0.3		6.1 ± 0.2	
Temozolomide (200 mg/kg)	2.1 ± 0.2	[b]	4.7 ± 0.4	NS
DAC + temozolomide	3.6 ± 0.2		4.6 ± 0.4	
Epirubicin (10 mg/kg)	4.3 ± 0.4	[c]	4.8 ± 0.5	NS
DAC + Epirubicin	6.0 ± 0.6		5.3 ± 0.7	
Taxol (15 mg/kg)	4.5 ± 0.2	NS	5.2 ± 0.4	NS
DAC + Taxol	4.8 ± 0.6		5.2 ± 0.03	

[a] Growth delay is quantified as the time taken for the tumor to double the initial volume (day 0), and the results are the mean ± SE of six mice. NS, not significant.
[b] $P < 0.001$.
[c] $P < 0.05$.

(Excerpted from the publication)

5. **De novo induced mutations in the deoxycytidine kinase (dck) gene in rat leukemic clonal cell lines confers resistance to cytarabine (AraC) and 5-aza-2'-deoxycytidine (DAC).** Stegmann et al., Leukemia 9: 1032-1038 (1995).

Rat leukemic cell lines were exposed to cytarabine or decitabine at gradually increasing concentrations form 0.1 to 10 μM over a period of 140 or 180 days. All clones were cross-resistant and deficient in deoxycytidine kinase activity. Cytarabine induced gene rearrangements and point mutations in the DCK gene over the 140 and 180 days, respectively. Decitabine induced point mutations only. Mutations were randomly distributed throughout the coding region.

6. **Transfection of wild-type deoxycytidine kinase (*dck*) cDNA into an AraC- and DAC-resistant rat leukemic cell line of clonal origin fully restores drug sensitivity.** Stegmann et al. Blood 85: 1188-1194 (1995).

Rat cell lines containing point mutations in *dck* were used in this study to assess mechanism of resistance to cytarabine and decitabine. Transfection of rat cell lines with a plasmid containing *dck* cDNA restored RNA expression and DCK enzymatic activity. The IC_{50} for Ara-C was similar in transfected cells and original controls (without point mutations in *dck*)

7. **Drug resistance to 5-aza-2'-deocycytidine, 2',2'-difluorodeoxycytidine, and cytosine arabinoside conferred by retroviral-mediated transfer of human cytidine deaminase cDNA into murine cells.** Eliopoulos et al., Cancer Chemother Pharmacol 42: 373-378 (1998).

Transfection of cells with a retroviral vector that expresses cytidine deaminase conferred resistance to 5-AZA-CdR. Drug resistance can be reversed by THU.

8. **Association of decreased uridine and deoxycytidine kinase with enhanced RNA and DNA polymerase in mouse leukemic cells resistant to 5- azacytidine and 5- aza- 2'- deoxycytidine.** Cihak and Sorm. Cancer Research; 30:2180-2186 (1970).

The development of resistance of mouse leukemic cells to 5-azacytidine (AzCR) and 5-aza-2'-deoxycytidine (AzCdR, decitabine) is associated with the reduction of deoxycytidine kinase activity as shown below.

Uridine and deoxycytidine kinase of mouse leukemic cells resistant to 5-azacytidine and 5-aza-2'-deoxycytidine

Incubation was for 10 min at 37° in a total volume of 0.2 ml with partially purified uridine kinase or deoxycytidine kinase, respectively. The experiment was repeated 3 times with enzyme preparations from different transplant generations with essentially the same results. Enzyme activity is expressed in μμmoles of nucleoside 5'-phosphate/100 μg of protein.

	Uridine kinase		Deoxycytidine kinase	
Leukemic cells	μμmoles	%	μμmoles	%
AKR/s	468.4	100	36.4	100
AKR/r-AzCR	120.2	26	10.7	29
AKR/r-AzCdR	421.3	90	9.2	25

(Excerpted from the publication)

2.6.3 PHARMACOLOGY TABULATED SUMMARY

DNA methylation and demethylation have been associated with the control of gene expression. The activity of DNA methyltransferases is reduced after exposure to decitabine, and incorporation of the aza analog of deoxycytidine prevents methylation at sites where the analog is present. Gene activation seen after exposure to decitabine may result with the induction of differentiation and the activation of tumor suppressor and other genes, leading to cytotoxicity. It has also been proposed that systemic administration of decitabine may enhance the immunogenic potential of tumors through expression of cell surface antigens. Representative studies and review articles were summarized above.

Conclusion: SuperGen did not perform any pharmacodynamic interaction studies. According to reports in the literature, decitabine has been shown to have several mechanisms of action including demethylation of certain genes allowing re-expression of silenced genes, immune mediated effects, and cytotoxicity. The cytotoxicity may be related to hypomethylation or trapping of the methyltransferase. Cross-resistance to other nucleoside inhibitors, such as cytarabine, has been described, but the clinical significance of this remains to be fully investigated.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 **Brief summary:** Decitabine is a nucleoside analog that must be phosphorylated a nucleotide in order to be active agent. The inhibitory action of decitabine depends on this metabolic conversion. Decitabine has been shown to cross the blood brain barrier through analytical measurement of CSF and activity against intracerebral tumors. The extent of tissue distribution has not been fully examined, but spleen and thymus have higher concentrations than liver. The volume of distribution is high, reported at 1230 mL/kg in dogs after a 3 hour intravenous infusion. Decitabine has a relatively short plasma half-life, the initial elimination phase being a few minutes (3-10) in mice, rabbits and dogs after an intravenous injection. It is metabolized by cytidine deaminase to a deoxyuridine derivative, an enzymatic step inhibited by tetrahydrouridine. Approximately 30% of a radiolabeled dose of decitabine was excreted in urine within 24 hours after an intravenous injection in mice. The elimination of the remaining 70% has not been investigated.

2.6.4.2 Methods of Analysis

[See under individual study reviews]

2.6.4.3 Absorption

1. **Plasma and Cerebrospinal Fluid Pharmacokinetics of 5- Aza- 2'-deoxycytidine in Rabbits and Dogs.** Chabot et al., Cancer Research 1983; 43:592-597

Decitabine was administered either as an IV bolus or as a continuous IV infusion after a loading dose. The decitabine concentrations in biological fluids (plasma and CSF; cisterna magna for rabbits, lumbar for dogs) were determined by bioassay (growth inhibition of L1210 cells *in vitro*) and HPLC and results are shown below.

5-Aza-dCyd pharmacokinetic parameters in rabbits

Rabbits received 5-aza-dCyd at the indicated doses either as an i.v. bolus or as a 2-step 180-min i.v. infusion. Plasma samples were collected at various time intervals, and 5-aza-dCyd concentration was determined by bioassay. Pharmacokinetic parameters are defined under "Materials and Methods."

Dose (mg/kg)	N	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (min)	K10 (min^{-1})	A (μg/ml)	B (μg/ml)	AUC 0→∞ (μg/ml/min)	Vdβ (ml/kg)	Vdss (ml/kg)	Vc (ml/kg)	CL (ml/min/kg)
Bolus (i.v.)											
1.25	3	3.1 ± 0.8[a]	43 ± 10	0.049 ± 0.012	2.6 ± 1.0	1.3 ± 0.2	88 ± 25	1038 ± 151	817 ± 100	360 ± 83	17.0 ± 4.9
2.5	7	3.4 ± 0.4	40 ± 5.2	0.041 ± 0.005	3.8 ± 0.6	2.8 ± 0.3	179 ± 29	1000 ± 130	758 ± 69	396 ± 31	15.9 ± 2.1
5.0	3	9.4 ± 5.0	49 ± 3.7	0.027 ± 0.0001	7.4 ± 1.2	5.1 ± 1.5	466 ± 26	1177 ± 352	629 ± 106	404 ± 25	10.8 ± 0.6
Mean		4.7	43	0.040				1050	742	390	15.0
Infusion (i.v.)											
15.4[b]	6		39 ± 3.7	0.018 ± 0.001		5.1 ± 0.5	287 ± 40[c]	845 ± 91			15.3 ± 1.6

[a] Mean ± S.E.
[b] Total dose for 180 min.
[c] AUC from the end of infusion to infinity.

(Excerpted from the publication)

5-Aza-dCyd pharmacokinetic parameters in dogs

Dogs received 5-aza-dCyd at the indicated doses either as an i.v. bolus or as a 2-step 180-min i.v. infusion. Plasma samples were collected at various time intervals, and 5-aza-dCyd concentration was determined by HPLC. Pharmacokinetic parameters are defined under "Materials and Methods."

Dose (mg/kg)	N	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (min)	K10 (min^{-1})	A (μg/ml)	B (μg/ml)	AUC 0→∞ (μg/ml/min)	Vdβ (ml/kg)	Vdss (ml/kg)	Vc (ml/kg)	CL (ml/min/kg)
Bolus (i.v.)											
4.0	3	5.5 ± 0.9[a]	69 ± 4.5	0.021 ± 0.004	5.3 ± 1.6	4.4 ± 0.7	478 ± 54	958 ± 164	779 ± 102	417 ± 40	8.6 ± 0.9
2.0	1	4.1	94	0.021	3.3	1.6	232	1273	1072	410	8.6
Mean		5.2	75	0.021				1037	852	415	8.6
Infusion (i.v.)											
3.28[b]	5	27.7 ± 4.9[c]	144 ± 22.1[c]	0.019 ± 0.005	3.2 ± 1.6	0.33 ± 0.02	163 ± 24[d]	2088 ± 395	1230 ± 314	672 ± 175	10.3 ± 1.9

[a] Mean ± S.E.
[b] Total dose for 180 min.
[c] $p < 0.05$ *versus* i.v. bolus; Student's *t* test.
[d] AUC from the end of infusion to infinity.

(Excerpted from the publication)

The decitabine concentrations in the CSF were 27% and 58% of the plateau plasma concentration in rabbits and dogs, respectively and followed the decline in plasma. These results showed that decitabine could cross blood-CSF barrier, producing cytotoxic concentrations in the CSF.

2. **Antileukemic activity of 5- aza- 2'- deoxycytidine and cytarabine against intracerebral L1210 murine leukemia.** Chabot and Momparler Cancer Treat Rep 1984; 68:1483-1487

In this study the authors have shown that decitabine was more effective than cytarabine against intracerebral L1210 murine leukemia at equitoxic doses (body weight loss).

3. **Comparison of the in vitro cytotoxicity (L1210) of 5- aza- 2'- deoxycytidine with its therapeutic and toxic effects in mice.** Covey and Zaharko. Eur J Cancer Clin Oncol 1985; 21:109-17

Decitabine was cleared from the plasma of the mice in a triexponential manner with an intermediate elimination half-life of 31.5 minutes as shown below. This value agrees as reported by Momparler and Gonzales (Cancer Res 1978; 38:2673-2678).

Pharmacokinetic parameters for decitabine in mice

$t_{1/2}^{\alpha} = 11.4$ min	$V_c = 594$ ml/kg
$t_{1/2}^{\beta} = 31.5$ min	$CL_p = 25.5$ ml/min/kg
$t_{1/2}^{\gamma} = 365$ min	$CL_t = 7.27$ ml/min/kg
AUC_{∞} 65 μg·hr/ml	$(CL_r/CL_p) \times 100 = 28.5\%$

*Determined following 100 mg/kg i.v.

(Excerpted from the publication)

4. **Effect of intravenous infusion of 5- aza- 2'- deoxycytidine on survival time of mice with L1210 leukemia.** Momparler and Gonzales. Cancer Research 1978; 38:2673-2678

Plasma concentration of decitabine during IV infusion to leukemic mice was determined and correlated with the antileukemic activity. The minimum effective dose of decitabine for an 8 hours IV infusion was about 0.4 mg/kg and produced an estimated average drug plasma concentration of 0.06 μg/mL.

5. **Bioavailability study of [^{3}H] 5- Aza- 2'- Deoxycytidine in mice and the effect of Tetrahydrouridine.** Southern Research (Study # 9189.15)

The objective of the present study was to assess the effect of tetrahydrouridine, cytidine deaminase inhibitor, on the kinetics of decitabine in mice. CD1 mice (~29 g body weight) were treated as shown below.

Group	THU			5-Aza-2′-Deoxycytidine		
	Route	Dose (mg/kg)	Dose Volume (mL/kg)	Route	Dose (mg/kg)	Dose Volume (mL/kg)
1	iv	0[a]	5	po	10	10
2	iv	25	5	po	10	10
3	iv	0	5	iv	10	10
4	iv	25	5	iv	10	10
5	po	25	5	po	10	10

[a]Mice received PBS

(Excerpted from the sponsor's submission)

Blood and urine samples were collected at different times and results are shown below.

Pharmacokinetic Parameters Calculated from Plasma Concentrations of 5-Aza-2′-Deoxycytidine: Non-Compartmental Analyses

PARAMETER	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
Tmax (hours)[a]	0.5	0.5	0	0	0.5
Cmax (ng/mL)[b]	1472	4564	9275[k]	12824[k]	2404
$t_{½e}$ (hours)[c]	NA	0.48	0.38	1.0	0.77
T_{last} (hours)[d]	4	4	2	8	8
C_{last} (ng/mL)[e]	138	50	174	54.6	26.4
$AUC_{0\text{-}last}$ (ng·hr/mL)[f]	1085	5803	4304	10584	4101
$AUC_{0\text{-}inf}$ (ng·hr/mL)[g]	NA	5838	4399	10666	4215
Clearance (ng/hr/kg)[h]	NR	NR	2273	938	NR
Vdss (mL/kg)[i]	NR	NR	1143	1104	NR
F (%)[j]	10[l]	55	--	100	40

NA: Pharmacokinetic parameter could not be calculated due to a limited number of data points
NR: Oral dose; parameter not reported
[a]Time of maximum plasma concentration
[b]Maximum plasma concentration
[c]Half-life of the terminal elimination phase
[d]Last time point 5-aza-2′-deoxycytidine was quantifiable in plasma
[e]Concentration of 5-aza-2′-deoxycytidine at last time point quantifiable
[f]Area under the plasma concentration versus time curve calculated from 0 to the last time point
[g]Area under the plasma concentration versus time point calculated from 0 to infinity
[h]Total body clearance
[i]Volume of distribution at steady state
[j]Bioavailability
[k]Extrapolated value
[l]Calculated from $AUC_{0\text{-}last}$ values for Group 1 and Group 4 mice

(Excerpted from the sponsor's submission)

Pharmacokinetic Parameters Calculated from Plasma Concentrations of Radioactivity: Non-Compartmental Analyses

PARAMETER	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
Tmax (hours)[a]	6	0.5	0	0	2
Cmax (nCi/mL)[b]	404	378	658[k]	612[k]	291
$t_{½e}$ (hours)[c]	NC	24.6	14.7	9.1	26.2
T_{last} (hours)[d]	8	8	8	8	8
C_{last} (nCi/mL)[e]	390	297	214	186	247
$AUC_{0\text{-}last}$ (nCi·hr/mL)[f]	2906	2369	1902	2072	2099
$AUC_{0\text{-}inf}$ (nCi·hr/mL)[g]	NC	12917	6429	4506	11440
Clearance (nCi/hr/kg)[h]	NR	NR	65	93	NR
Vdss (mL/kg)[i]	NR	NR	1407	1202	NR
F (%)[j]	--	74	--	100	49

NC: Parameter could not be estimated because the plasma concentration of radioactivity between 0.5 and 8 hours was essentially constant
NR: Oral dose; parameter not reported

[a]Time of maximum plasma concentration
[b]Maximum plasma concentration
[c]Half-life of the terminal elimination phase
[d]Last time point
[e]Concentration of radioactivity at last time point
[f]Area under the plasma concentration versus time curve calculated from 0 to the last time point
[g]Area under the plasma concentration versus time point calculated from 0 to infinity
[h]Total body clearance
[i]Volume of distribution at steady state
[j]Bioavailability
[k]Extrapolated value

(Excerpted from the sponsor's submission)

Urinary Excretion of Radioactivity

Group	Hours	Dose nCi	Urine Vol. (ml)	nCi/mL Urine	% Dose
1	0-8	46328	2.0	8235	17.8
	8-24		3.3	3551	7.7
	Total				25.4
2	0-8	46727	1.7	6730	14.4
	8-24		4.4	6279	13.4
	Total				27.8
3	0-8	49847	1.2	12813	25.7
	8-24		1.2	3136	6.3
	Total				32.0
4	0-8	48590	1.2	11699	24.1
	8-24		1.0	3419	7.0
	Total				31.1
5	0-8	47127	2.6	13013	27.6
	8-24		3.1	3124	6.6
	Total				34.2

(Excerpted from the sponsor's submission)

THU pretreatment resulted in increased plasma concentration of decitabine indicating that THU affected the pharmacokinetics of decitabine by inhibiting the deamination of 5-aza-2'-deoxycytidine to 5-aza-2'-deoxyuridine

6. Pharmacokinetics of decitabine in an IVAP rabbit model.

This study was conducted by the Preclinical Biopharmaceutics Laboratory, Rutgers University, New Jersey for the SuperGen, Inc.

Decitabine was administered by portal vein (PV), upper small intestine (USI), lower small intestine (LSI), colon (IC) or orally to adult female NZW rabbits. Blood samples were taken at different times and results are shown below.

Dose linearity: Dose linearity in AUC was observed at 0.75, 1.5, and 2.5 mg/kg administered iv.

Bioavailability: Bioavailability was site dependent, highest with USI and lowest PO. A technical problem limited the utility of the IC findings.

Animal ID	Route				
	PV	PO	USI	LSI	IC
RU-35		39.12%	72.64%		
RU-41		28.57%			IP dose
RU-44		38.84%			0.22%
RU-45					68.29%
RU-46			64.84%		
RU-48	79.63%			55.05%	
RU-49	58.11%			44.48%	
RU-50	66.02%			51.78%	
RU-52			84.33%		
Mean	**67.92%**	**35.51%**	**73.94%**	**50.44%**	**34.26%**
Std Dev	10.88%	6.01%	9.81%	5.41%	48.13%

(Excerpted from the sponsor's submission)

First pass effect: Direct administration into the portal vein gave 68% bioavailability indicating that hepatic first pass effect resulted in 32% loss (Table above).

Elimination half-life and mean residence time: Elimination half-life (0.72±0.07 hours) and mean residence time (0.93±0.11 hours) were similar regardless of dose or route (and location, e.g., USI) of administration. These data are not captured in this review.

Maximum concentration and time of maximum: there was a technical problem with the IC route limiting utility of this data.

Route	Mean Cmax (ng/mL ±Std Dev)	Mean Tmax (min)
Oral	335±134	0.42±0.14
USI	1218±280	0.083±0.0
LSI	736±95.6	0.14±0.1
IC	2.0 & 611	0.25±0.0

Clearance and volume of distribution: Consistent among routes except PV.

Route of Administration	Clearance (L/hr/kg)	Volume (L/kg)
PV	3.31	3.63
Oral	2.26	2.29
USI	2.23	2.51
LSI	2.23	2.37
IC	2.21	2.16
IV (overall)	2.40 ± 0.20	2.41 ± 0.41

(Excerpted from the sponsor's submission)

2.6.4.4 Distribution:

1. **Plasma and Cerebrospinal Fluid Pharmacokinetics of 5- Aza- 2'-deoxycytidine in Rabbits and Dogs.** Reviewed under Absorption.

2. **Transformation and metabolic effects of 5- aza- 2'- deoxycytidine in mice.** Cihak et al., Biochem Pharmacology 1980; 29:2929-2932

In the liver and other non-lymphatic tissues the utilization of decitabine is limited as compared to spleen where the drug is extensively phosphorylated and incorporated into DNA spleen.

Incorporation of decitabine-^{3}H in H (random bread), DBA/2 and AKR mouse strains

Tissue	Incorporation†		
	H	DBA/2	AKR
Liver	1.00	1.00	1.00
Kidney	1.03	0.97	1.16
Heart	0.33	0.30	0.35
Muscle	0.37	0.42	0.35
Spleen	24.60	22.80	25.90
Thymus	17.20	16.40	14.85

* Groups of five to six female mice (22–26 g body wt) received 5-aza-2'-deoxycytidine-^{3}H (80 μCi/0.02 μmole) i.p. 2 hr before being killed.

(Excerpted from the publication)

3. **Comparison of the in vitro cytotoxicity (L1210) of 5- aza- 2'- deoxycytidine with its therapeutic and toxic effects in mice.** Reviewed under Absorption.

4. **Comparison of the metabolism and inhibitory effects of 5- azacytidine and 5- aza-2'- deoxycytidine in mammalian tissues.** Sorm et al., Rev Roum Biochim 1966; 3:139-147

The phosphorylation of 5-azacytidine and 5-aza-2'-deoxycytidine is studied in this one of the original papers.

Phosphorylation of 5-Azacytidine and 5-Aza-2'-deoxycytidine by Cell-free Extracts of Tissues of Normal and Leukemic AKR Mice

Tissue	Antimetabolite	mμMoles of Nucleotide Formed per 10 mg of Protein			
		Thymus	Spleen	Kidney	Liver
Normal	5-Azacytidine	58	41.2	26.3	21.2
	5-Aza-2'-deoxycytidine	11.1	6.25	0.5	0.1
Leukemic	5-Azacytidine	41.2	44	38	12.3
	5-Aza-2'-deoxycytidine	19	16.9	4.6	7.6

(Excerpted from the publication)

The phosphorylation of decitabine in leukemic tissues is markedly higher than in normal organs.

2.6.4.5 Metabolism:

1. Kinetics of deamination of 5- aza- 2'- deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deazauridine, thymidine or uracil arabinoside. Chabot et al., Biochemical Pharmacology 1983; 32:1327-1328

The interactions of 5-aza-cytidine and other substrates with human liver cytidine deaminase are shown below.

Kinetics of deamination and inhibition of pyrimidine nucleosides by cytidine deaminase from human liver

Substrate	K_m (μM)	V_{max} (μmoles·min^{-1}·mg^{-1})	K_i (μM)		
			3-DU	Thd	Ara-U
Cyd*	12 ± 0.9‡	16.1 ± 3.0	146 ± 20	680 ± 200	1040 ± 300
dCyd	19 ± 4	14.4 ± 2.4	100 ± 20	290 ± 170	930 ± 140
Ara-C	87 ± 10	14.5 ± 3.3	80 ± 10	540 ± 270	480 ± 50
5-Aza-Cyd	216 ± 51	13.3 ± 5.1	170 ± 50	130 ± 40	180 ± 90
5-Aza-dCyd	250 ± 33	15.2 ± 3.8	210 ± 70	290 ± 110	770 ± 300

* Deamination of the substrates was followed spectrophotometrically at various wavelengths. Assays were done at 25° in 20 mM potassium phosphate buffer, pH 7.4.

† Abbreviations: Cyd, cytidine; dCyd, deoxycytidine; ara-C, cytosine arabinoside; 5-aza-Cyd, 5-aza-cytidine; 5-aza-dCyd, 5-aza-2'-deoxycytidine; 3-DU, 3-deazauridine; Thd, thymidine; and ara-U, uracil arabinoside.

‡ Mean ± S.E.; N = 3 or more determinations.

(Excerpted from the publication)

This study demonstrated that Cyd deaminase from human liver has less affinity for 5-aza-dCyd and 5-aza-Cyd than for Ara-C. Two natural substrates, cytidine (Cyd) and deoxycytidine (dCyd) showed the lowest Km for the enzyme Cyd deaminase.

2. Transformation of 5- aza- 2'- deoxycytidine- 3H and its incorporation in different systems of rapidly proliferating cells. Reviewed under Distribution

3. Transformation and metabolic effects of 5- aza- 2'- deoxycytidine in mice. Reviewed under Distribution

4. Kinetics of phosphorylation of 5- aza- 2'- deoxycytidine by deoxycytidine kinase. Momparler and Derse, Biochemical Pharmacology 1979; 28:1443-1444

The interaction of 5-aza-2'-deoxycytidine (5-Aza-CdR) and its triphosphate form with deoxycytidine (CdR) kinase are shown below.

Substrate	Addition	Concn (μM)	Nucleotide formed (nmole)	Inhibition (%)
CdR	None		0.95	0
	dCTP	5	0.81	15
	dCTP	10	0.68	28
	dCTP	20	0.53	44
	5-AZA-dCTP	20	0.83	13
	5-AZA-dCTP	40	0.67	29
	5-AZA-dCTP	80	0.50	47
5-AZA-CdR	None		0.98	0
	dCTP	5	0.96	2
	dCTP	10	0.67	32
	dCTP	20	0.40	59
	5-AZA-dCTP	20	0.74	25
	5-AZA-dCTP	40	0.57	42
	5-AZA-dCTP	80	0.37	62

* The standard reaction mixture contained 100 mM imidazole–HCl, pH 6.8, 0.05 μCi of 20 μM [^{14}C]CdR or [6–^{14}C]5AZA-CdR, as indicated, and the indicated concentrations of dCTP or 5-AZA-dCTP. The reaction mixture was incubated for 5 min at 37° in the presence of 0.3 unit CdR kinase.

(Excerpted from the publication)

5-AZA-dCTP was an inhibitor of CdR kinase when either CdR or AZA-CdR was used as the substrate.

5. **Kinetic Interaction of 5- Aza- 2'- deoxycytidine- 5'- Monophosphate and Its 5'- Triphosphate with Deoxycytidylate Deaminase.** Momparler et al., Molecular Pharmacology 1984; 25:436-440

The interactions of 5-aza-2'-deoxycytidine triphosphate (5-AZA-dCTP) and deoxycytidine triphosphate (dCTP) with deoxycytidine monophosphate deaminase (dCMP deaminase) are studied in this paper to understand the important role played by this enzyme.

Activation of the deamination of dCMP by dCTP or 5-AZA-dCTP

The reaction mixture contained 100 μM dCMP and dCMP deaminase (0.06 μg).

Addition	Concentration	dCMP deaminated
	μM	*nmoles/min*
None		<1
dCTP	0.2	27
dCTP	0.5	113
dCTP	1.0	123
5-AZA-dCTP	0.2	33
5-AZA-dCTP	0.5	103
5-AZA-dCTP	1.0	130

(Excerpted from the publication)

At equimolar concentrations, 5-AZA-dCTP was as effective as dCTP in activating the deamination of the natural substrate, dCMP. Both dCTP and AZA-dCTP at 0.2µM produced >20-fold activation of the deamination of dCMP.

6. **Kinetics of 5- aza- 2'- Deoxycytidine Phosphorylation in Mouse Spleen and L1210 Leukemic Cell Extracts.** Vesely and Cihak, Neoplasma 1980; 27:121-127

Kinetic parameters for the phosphorylation of deoxycytidine (dCyd) and 5-aza-2'-deoxycytidine (AzadCyd) in cell free extracts from L1210 and mouse spleen are shown below. The phosphorylation was measured using increasing substrate concentrations (in case of Ki measurements 0.075 and 0.112 mM) and 6 mM ATP (or 40 times in excess of the two substrate concentrations).

Substrate	Inhibitor	L1210			Spleen		
		K_m ($M \times 10^{-5}$)	K_i (M)	V_{max} (nmol)	K_m ($M \times 10^{-5}$)	K_i (M)	V_{max} (nmol)
dCyd	—	2.7	—	5.8	2.5	—	3.8
AzadCyd	—	6.4	—	5.8	2.9	—	2.9
dCyd	AzadCyd	—	6.5×10^{-4}	—	—	1.2×10^{-3}	—
AzadCyd	dCyd	—	8.7×10^{-6}	—	—	5.8×10^{-6}	—

(Excerpted from the publication)

Apparently deoxycytidine kinase from the spleen extract had higher affinity for the analogue than the enzyme from L1210 cells.

7. **Bioavailability study of [3H] 5- Aza- 2'- Deoxycytidine in mice and the effect of Tetrahydrouridine.** Reviewed under Absorption

2.6.4.6 Excretion

1. **Transformation and metabolic effects of 5- aza- 2'- deoxycytidine in mice.** Reviewed under Distribution

2. **Comparison of the in vitro cytotoxicity (L1210) of 5- aza- 2'- deoxycytidine with its therapeutic and toxic effects in mice.** Reviewed under Absorption

3. **Comparison of the metabolism and inhibitory effects of 5- azacytidine and 5- aza- 2'- deoxycytidine in mammalian tissues.** Reviewed under Distribution

2.6.4.7. Pharmacokinetic drug interactions

No sponsor initiated studies were conducted

1. Preclinical Toxicology of 5- aza- 2'- deoxycytidine in Mice and Dogs.

In this report the preclinical toxicology of 5-aza-2'-deoxycytidine in mice and dogs has been examined. This report was written by Richard Momparler, Ph.D., Research professor in Pharmacology, University of Montreal in 1988.

2. Chemotherapy of L1210 and L1210/ ARA- C leukemia with 5- aza- 2'- deoxycytidine and 3- deazauridine. Momparler and Momparler, Cancer Chemother Pharmacol 1989; 25:51-54

3-Deazauridine (3-DU) was effective against L1210/ARA-C cells. These cells are deficient in deoxycytidine kinase and were completely resistant to the antileukemic effects of 5-aza-2'-deoxycytidine (decitabine). The sequential administration of decitabine followed by 3-DU was effective in mice with both L1210 and L1210/ARA-C leukemic cells as shown below.

Effect of individual or sequential IV infusion of 5-Aza-dCyd and 3-DU on the survival of CD2F1 mice with L1210 and L1210/ARA-C leukemia.

Chemotherapy	Dose (mg/kg)	Survival (days)	ILS (%)	60-day survivors	% wt. change, (day 7)
None	0	7.7± 0.4[a]	0	0/6	
5-AZA-dCyd	12.8	12.1± 1.8	57	0/10	−3
3-DU	186	9.8± 1.4	27	0/10	+1
5-AZA-dCyd→3-DU	12.1→182	28.2± 14.3	266	7/10	−16

$CD2F_1$ mice were given an i.v. injection of 10^4 L1210 and 10^3 L1210/ARA-C cells on day 0. On day 1, the mice were given a 9-h i.v. infusion of 5-AZA-dCyd or 3-DU at the indicated dose or a sequential infusion of 5-AZA-CdR (9 h) followed by 3-DU (9 h)
[a] Mean ± SD

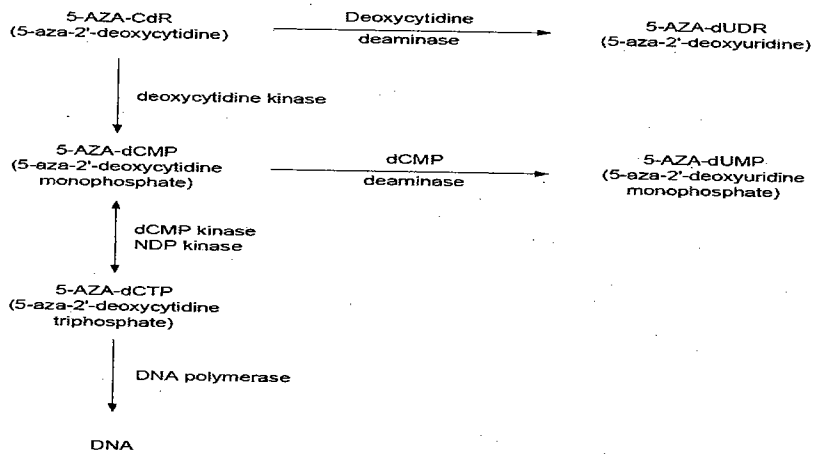
(Excerpted from the publication)

2.6.4.8 Other Pharmacokinetic Studies

No sponsor initiated studies were conducted

2.6.4.9 Discussion and Conclusions

The pharmacokinetic studies of decitabine were carried out in rodents (mice) and non rodents (dogs and rabbits) species. Decitabine undergoes phosphorylation in the presence of kinases and is incorporated into DNA in the presence of DNA polymerase as shown below.



2.6.4.10 Tables and figures to include comparative TK summary

See Pharmacokinetics Tabulated Summary below.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

In mice, $t_{1/2}$ of decitabine increased in the presence of tetrahydrouridine (cytidine deaminase inhibitor). The longer elimination phase of decitabine in dogs compared to rabbits may be due to lower cytidine deaminase activity in dogs (Drug Metab Dispos 1975; 3:309-313). Humans have higher cytidine deaminase activity compared to any other species studied (Leukemia Research 1981; 5:453-462).

Comparative pharmacokinetic values in three animal species (mouse, rabbit and dog) and humans (children and adults) are summarized below.

Mean plasma pharmacokinetic parameters in mouse, rabbit, dog and human

Species	Dose (mg/kg)	Route of Admin.	$t_{1/2\alpha}$ (min)	$T_{1/2\beta}$ (min)	Vd (L/kg)	C_{max} (µg/mL)	CLp (mL/min/kg)	AUC 0→∞ (µ g.min/mL)	Reference
Mouse	10	Iv	-	23	1.14	9.28	37.9	264	Study Report 9189.15
	10 + THU 25 mg/kg	Iv	-	60	1.10	12.8	15.6	640	
	10	Po				1.47		65.1	
	10 + THU 25 mg/kg IV	Po		29		4.56		350	
	10 + THU 25 mg/kg	Po		46		2.4		253	
Rabbit	0.75	Iv		44		0.63		20.4	Rutgers Report
	1.5	Iv		45	2.41	1.08	40.0	34.4	
	2.5	Iv		35		1.8		63.8	
	2.5	Po		41	2.3	0.34	37.7	24.1	
Dog	4.0	Iv	5.5	69	0.96	5.3	8.6	478	Chabot et al 1983
	2.0	Iv	4.1	75	1.27	3.3	8.6	232	
	3.28	Iv	27.7	144	2.1	3.2	10.3	163	
Human (children)	1 mg/kg/h for 40-60 h	Iv	-	12		0.5			Rivard et al 1981
Human (adult)	2.4 I h infusion	Iv	7	35	4.6	0.44 (2 µM)	126	25	Groeningen et al 1986

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: The general toxicity of decitabine has been examined in laboratory animals. Single dose studies (not reviewed) were conducted in the mouse and dog using intravenous route of administration. Repeat-dose toxicity was examined in mice (range finding only), rats, dogs and rabbits.

A multicycle intravenous infusion toxicity study in Sprague Dawley rats followed by 25 day recovery periods. Sprague-Dawley rats (5/sex/group) were dosed with decitabine (0, 1200, 2400, or 3600 µg/kg/day) three times a day via a three-hour IV infusion for three consecutive days with a 25-day recovery period following each dosing cycle. Main study animals were euthanized on Day 85 after 3 cycles of dosing. Two animals (one female in group 3 and one female in group 4) died prior to scheduled termination. There were no test article related effects on body weight gain and food consumption. An anemia (↓ RBC, HGB and/or HCT) was observed in mid and high dose animals after each infusion as compared to control animals. Mean epididymis and testes weights were significantly decreased in group 4 males. Testicular degeneration and decreased spermatozoa within epididymal tubular lumens were observed in treated males.

A multicycle intravenous infusion toxicity study in Beagle dogs followed by 25-day recovery periods. The study was originally designed to dose beagle dogs for three, three-day dosing cycles, each followed by a 25-day recovery period. The toxicity and early deaths (4/6 group 2, 6/6 group 3 and 5/6 group 4) were observed after the first dosing cycle. As a result, the study was terminated early during the first recovery period. Clinical signs observed included soft, mucoid, discolored feces, lethargy, prostration, salivation and cold to touch. Dose-related decreases in white blood cells and platelets were also observed. Test article-associated lesions were apparent in the gastrointestinal tract and visceral lymph nodes.

A multicycle intravenous infusion toxicity study in rabbits. The potential toxicity of decitabine was also determined in New Zealand White rabbits following four dosing cycles. Each dosing cycle had three 3-hour dosing sessions per day for three days for nine dosing sessions per dosing cycle. The dosing cycles were initiated approximately every six weeks. The study was originally designed to dose animals (4/sex/group) at 0, 750, 1500, or 3000 µg/kg/day. However, all the animals at 3000 µg/kg/day died after the first dosing cycle (days 9-13). Three males and one female at 1500 µg/kg/day died after the first cycle (days 13-18). In addition, one male dosed with 750 µg/kg/day decitabine died on day 18 after the first dosing cycle. The morbidity and mortality in these animals was associated with primary lesions in bone marrow (e.g., primarily leukopenia). The

pulmonary lesions were secondary to severe immuno-suppression caused by leukopenia. Baytril® (Enrofloxin) administration prevented additional deaths due to opportunistic infections in 750 and 1500 µg/kg/day groups.

The clinical signs associated with decitabine administration included inappetence, lethargy, hunched posture, and pale mucous membranes. Leukopenia, anemia and thrombocytopenia were observed in treated animals. Pathological alterations included enteropathy of the large intestine, bone marrow hypocellularity, and testicular atrophy in a dose dependent manner.

Genetic toxicology:

Decitabine did not produce mutations in the Ames test in the absence of metabolic activator (S9), but this study was not definitive. Decitabine was mutagenic in the *in vitro* TK mutation test in L5178Y mouse lymphoma cells and *Escherichia coli lac-I* transgene in colonic DNA from decitabine treated mice. Decitabine induced chromosomal rearrangements in larvae of the fruit fly. These studies indicated that decitabine has mutagenic and clastogenic potential. Other studies either indicate that decitabine is not mutagenic under the conditions of the study, or the assay systems are primarily for investigational purposes.

Carcinogenicity: The sponsor did not conduct any study to evaluate the carcinogenic potential of decitabine and no formal carcinogenicity evaluation has been conducted. Several reports of the carcinogenic potential of decitabine are reported in the literature but are not definitive.

Reproductive toxicology: Decitabine has been shown to be teratogenic in both mice and rats. Teratogenic effects were associated with the dose and treatment day. Time dependent skeletal and cranial effects were seen when mice were treated with decitabine (from 0.3 mg/kg) on days 8, 9, 10 or 11. Similar findings were seen in rats (from 0.4 mg/kg). Decitabine has been shown to have marked effects on spermatogenesis and reproductive capacity in mice, including decreased fertility of male mice exposed either prior to mating (from 0.1 mg/kg) or *in utero* (1 mg/kg given on gestation day 10). In the fertility study in mice exposed prior to mating, there was also an increase in preimplantation loss when the male mice were bred to untreated females. In mice exposed *in utero*, there was a decrease in body weight in male and female offspring that persisted for at least 230 days after birth.

Special toxicology: Not studied

2.6.6.2 Single dose toxicity Not reviewed

2.6.6.3 Repeat-dose toxicity

1. Intravenous toxicity (range finding) study with decitabine by dailyadministration (5 days per week) in mice.

Key study findings:

- Intravenous administration of decitabine at 0.25 and 0.5 mg/kg/day for 3 weeks (5 days per week) caused 1/5 and 4/5 mortalities in male mice, respectively.
- Mortality rates at 1.0 and 5.0 mg/kg/day for 2 weeks (5 days/week) were 2/5 and 5/5, respectively.
- Clinical findings consisted of pale discoloration of the kidneys, liver, and salivary glands, reddish discoloration of the testes, and reduced thymus size in a dose-related manner.

2. A multicycle intravenous infusion toxicity study in Sprague Dawley rats followed by 25 day recovery periods.

Key study findings:

- Decitabine infusion caused anemia (↓RBC, HGB and/or HCT) in mid (2.4 mg/kg/day) and high (3.6 mg/kg/day) dose animals relative to control animals.
- A dose-dependent decreased absolute and/or relative testicular/epididymal weight was observed in treated male animals.

Study no.:	FEAW-116
Volume #, and page #:	Electronic module
Conducting laboratory and location:	[redacted]
Date of study initiation:	October 15, 2001.
GLP compliance:	Yes
QA report:	yes (X) no ( )
Drug, lot #, and % purity:	Decitabine, lot # 00C20MA, [redacted] purity.

Methods

Doses:

Group Number	Number of Animals				Test Article	Dosage Level per Infusion (µg/kg)	Dosage Volume per Infusion (mL/kg)	Total Daily Dose (µg/kg)	Dosing Regimen	Necropsy Day
	Main Study		Satellite Animals[a]							
	M	F	M	F						
1	5	5	6	6	Control	0	6	0	~3 hour IV infusion, 3 times a day for 3 days on Days 1-3, Days 29-31, Days 57-59 (males), and Days 58-60 (females)[b]	~ Day 85
2	5	5	6	6	Decitabine	400		1200		
3	5	5	6	6		800		2400		
4	5	5	6	6		1200		3600		

IV = Intravenous; M = Male; F = Female

[a] Satellite animals were used to evaluate plasma concentration; following each infusion cycle, two per sex per group were euthanized and discarded without further evaluation.

[b] Due to the New Year's Day holiday, the third and final dosing cycle for the females was performed on Days 58-60.

(Excerpted from the sponsor's submission)

Species/strain: —:CD® BR Sprague Dawley rats

Number/sex/group or time point (main study): 5

Route, formulation, volume, and infusion rate: IV infusion

Satellite groups used for toxicokinetics: see table above

Age: 8 weeks

Weight: Males 340-380 g, Females 220-245 g

Observations and times:

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Prior to treatment, weekly during treatment, and prior to necropsy.

Food consumption: Weekly

Hematology:

Time Point	Clinical Pathology		
	Main Study Animals		Satellite Animals
	Hematology	Serum Chemistry	Plasma Concentration for Sponsor
Prior to initial treatment, on Days 8, 36, and 64 for the males and Day 65 for the females, and weekly throughout the first recovery phase and prior to necropsy	X	X	
At the end of each 3-day infusion cycle			X
Volume of Whole Blood/ Time Point	0.75 mL	1.1 mL	5.0 mL
Anticoagulant	EDTA	None	EDTA

(Excerpted from the sponsor's submission)

Gross pathology: Day 85
Organ weights: See histopathology inventory for this NDA
Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)
Toxicokinetics: plasma concentration at end of infusion period on 3rd day of cycle, D 4, 32, 60/61

Results

Mortality: 1 F from Gr 3 (D18) and 4 (D5) of unknown reasons
4 other animals for technical reasons

Clinical signs: Open incisions, thinning fur, alopecia, soft or scant feces, urine stains, and opaque or protruding eyes were observed in all groups.
Body weights: No differences as compared to control animals
Food consumption: Normal in all animals
Hematology: Hematology profile relative to control animals

Group	Day	RBC		Platelet*	
		M	F	M	F
3	15		8 % ↓	60 % ↑	116 % ↑
4	15	10 % ↓	16% ↓	100 % ↑.	84 % ↑

* - decreased on days 1, 8, 22, 29, 36, 64, and 85 relative to start values.

Clinical chemistry: Groups 3 & 4 – Serum potassium ↓ (maximum 15% of the control) on different days in M and F.
Gross pathology: No treatment-related affect.
Organ weights: Group 4 – Testes (25% ↓)

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)

Summary of test article-related microscopic findings

Tissue/Lesion	Group Number / Sex / Number Examined	1 M 3	2 M 4[a]	3 M 5	4 M 5
Epididymides					
Reduced Spermatozoa					
	Grade 1	-	1	-	-
	Grade 2	-	2	-	-
	Grade 3	-	1	5	5
Testes					
Germ Cell Depletion					
	Grade 1	-	-	1	2
	Grade 2	-	-	-	3
Spermatid Giant Cell					
	Grade 1	1	3	3	-
	Grade 2	-	-	1	5
Vacuolation					
	Grade 1	1	3	4	1
	Grade 2	-	1	1	4

M = Male; - = No finding; [a] = Animal No. 10 died on Day 65.

(Excerpted from the sponsor's submission)

Toxicokinetics:

	D4 (ng/mL)		D32 (ng/mL)		D60/61 (ng/mL)	
	M	F	M	F	M	F
G2	224	134	115	127	270	186
G3	350	271	257	276	596	365
G4	544	498	384[a]	388[a]	844	742

Mean of N = 2; [a] n = 1

In general, plasma concentrations were higher in males compared to females, increased with dose at all time points, and were higher at D60/61 compared to earlier time points.

3. A multicycle intravenous infusion toxicity study in Beagle dogs followed by 25-day recovery periods.

Key study findings:

- Due to high mortality (4/6 group 2, 6/6 group 3, and 5/6 group 4), the study was terminated on day 15.
- Bone marrow depletion and an enteropathy were the main cause for early mortality/morbidity.

Study no.: FEAW-117
Volume #, and page #: Electronic module
Conducting laboratory and location: ———————

Date of study initiation: September 24, 2001.
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Decitabine, lot # 00C20MA, —— purity

Methods

Doses:

Group Number	Number of Animals		Test Article	Dosage Level per Infusion (µg/kg)	Dosage Volume per Infusion (mL/kg)	Total Daily Dose (µg/kg)	Dosing Regimen	Necropsy Day
	Males	Females						
1	3	3	Control	0	6	0	~3 hour IV infusion, 3 times a day for 3 days on Days 1-3[a]	~ Day 15[a]
2	3	3	Decitabine	400		1200		
3	3	3		800		2400		
4	3	3		1200		3600		

IV = Intravenous
[a] Due to toxicity observed, the study was terminated during the first recovery period.

(Excerpted from the sponsor's submission)

Species/strain: Beagle dogs
Number/sex/group or time point (main study): 3
Route, formulation, volume, and infusion rate: IV infusion
Satellite groups used for toxicokinetics or recovery: No
Age: 6 months
Weight: Males 6.2 – 6.8 kg, Females 5.0 – 5.8 kg

Observations and times:

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Daily

Hematology: Blood samples were collected as shown below.

Time Point	Clinical Pathology		
	Hematology	Serum Chemistry	Plasma for Concentration Analysis
Prior to initial treatment and on Days 8 and 15[a]	X	X	
Prior to initial treatment, on Day 4 (at the end of the 3-day infusion cycle), and on Day 15[b]			X
Volume of Whole Blood/ Time Point	1.3 mL	1.8 mL	5.0 mL
Anticoagulant	EDTA	None	EDTA

[a] Due to toxicity observed, the study was terminated during the first recovery period, therefore, the final scheduled sample collection occurred on Day 15. Samples were collected from animals that were sacrificed moribund.
[b] Final samples were collected prior to sacrifice of moribund animals or on Day 15.

(Excerpted from the sponsor's submission)

Clinical chemistry: Day 8 only
Gross pathology: At necropsy
Organ weights: Not collected
Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)

Results

Mortality:

Group	Sex	Died	Sacrificed	Day
2	M		2	14, 14
	F		2	11, 12
3	M		3	9, 11, 11
	F	1	2	9, 9, 9
4	M*	1	1	7, 7
	F	1	2	6, 7, 7

*Bioanalytical analysis indicated non-detectable level of decitabine for this surviving animal.

Clinical signs: Lethargy, prostration, salivation, thin, pale mucous membranes, rough hair coat, cyanosis, cold to touch, and soft, mucoid, discolored feces in all treated animals.
Body weights: Groups 2-4 – Decreased in a dose-dependent manner.
Food consumption: Decreased in all treated animals
Hematology: Group 4 - ↓ WBC, platelets

Clinical chemistry: Increased serum glucose, decreased serum sodium and calcium were observed in treated animals.

Gross pathology:

Tissue/Lesion / Group Number / Number Examined	1		2		3		4	
	3M	3F	3M	3F	3M	3F	3M	3F
Duodenum								
-Discoloration	-	-	1	1	1	2	-	-
-Foci	-	-	1	-	-	2	2	2
Jejunum								
-Discoloration	-	-	1	-	2	-	2	3
-Foci	-	-	1	2	1	1	-	2
Ileum								
-Discoloration	-	-	-	-	-	1	-	-
-Foci	-	-	-	1	-	1	-	1
Cecum								
-Discoloration	-	-	-	-	-	1	-	-
-Foci	-	-	1	1	1	2	-	1
Colon								
-Foci	-	-	1	2	3	1	-	1
Rectum								
-Discoloration	-	-	-	-	-	1	-	-
-Foci	-	-	-	2	3	1	1	2
Stomach								
-Foci	-	-	2	1	1	-	-	-
Mandibular Lymph Node								
-Discoloration	-	-	1	-	-	-	-	-
Mesenteric Lymph Node								
-Discoloration	-	-	2	1	1	-	1	3
-Foci	-	-	-	-	1	1	-	-
Pancreatic Lymph Node								
-Discoloration	1	-	2	1	3	1	1	1
-Enlarged	-	1	-	-	-	-	1	1
Axillary Lymph Node								
-Discoloration	-	-	1	-	1	-	-	-
-Enlarged	-	-	-	1	1	-	-	-
Hepatic Lymph Node								
-Discoloration	-	-	2	-	1	1	2	2
Iliac Lymph Node								
-Discoloration	-	-	2	1	-	-	1	-
-Enlarged	-	-	-	1	-	-	-	-
Mediastinal Lymph Node								
-Discoloration	-	-	2	2	-	-	-	-
Colic Lymph Node								
-Discoloration	-	-	1	-	-	-	-	-
Ileocecal Lymph Node								
-Discoloration	-	-	-	1	-	-	2	-
Inguinal Lymph Node								
-Discoloration	-	-	-	1	-	-	-	-
Sternal Lymph Node								
-Discoloration	-	-	-	-	-	1	-	-
Thymus								
-Discoloration	-	-	-	-	1	-	1	-
Lung								
-Foci	-	-	1	2	-	1	-	-
Administration Site								
-Foci	1	-	-	-	-	-	-	-
-Nodules	-	-	1	1	1	1	1	1
-Thickened	-	-	-	1	-	-	-	-
Skin-Left Inguinal								
-Thickened	-	-	1	-	-	-	1	-

M = Male, F = Female, - = No Findings.

(Excerpted from the sponsor's submission)

Organ weights: Not done

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)

Tissue/Lesion — Group Number / Number Examined	1		2		3		4	
	3M	3F	3M	3F	3M	3F	3M	3F
Bone Marrow (Sternum)								
-Cellular Depletion	-	-	3	3	3	3	2	3
Stomach								
-Mucosal Necrosis	-	-	-	-	1	-	-	-
-Infarct, Septic	-	-	-	1	-	-	-	-
Duodenum								
-Enteropathy	-	-	2	2	3	3	2	3
-Mucosal Necrosis	-	-	-	1	-	2	-	-
Jejunum								
-Enteropathy	-	-	2	2	2	3	2	2
-Mucosal Necrosis	-	-	-	-	-	-	-	1
Ileum								
-Enteropathy	-	-	-	1	2	3	2	3
-Mucosal Necrosis	-	-	-	1	-	-	-	-
Cecum								
-Enteropathy	-	-	1	1	3	3	2	3
-Mucosal Necrosis	-	-	-	1	-	-	-	1
Colon								
-Enteropathy	-	-	-	1	2	2[a]	2	3
-Mucosal Necrosis	-	-	1	-	-	-	-	1
Rectum								
-Enteropathy	-	-	1	2	3	3	2	3
-Mucosal Necrosis	-	-	-	-	-	-	-	1

M = Male, F = Female, - = No Findings
[a] Only 2 colon sections examined.

(Excerpted from the sponsor's submission)

Toxicokinetics: Only D4 samples were available. Plasma concentrations greater than 100 ng/mL were associated with mortality.

4. A multicycle intravenous infusion toxicity study in rabbits.

Key study findings:

- There were no surviving group 4 animals (3 mg/kg/day) beyond dose cycle 1. All surviving animals were euthanized 5-weeks and 4-5 days after the last dosing cycle.
- Primary toxicity related to decitabine treatment was myelosuppression.

Study no.: FEAW-0126-04-54
Volume #, and page #: Electronic module
Conducting laboratory and location: ———

Date of study initiation: June 18, 2003.
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Decitabine, lot # 02D10TA, — purity

Methods

Doses:

Group Number	Number of Animals		Test Article	Dosage Level per Infusion (µg/kg)	Dose Rate (mL/kg/hr)	Total Daily Dose (µg/kg)	Dosing Regimen	Necropsy Day
	Males	Females						
1	4	4	Vehicle	0	1	0	~3-hour intravenous infusion, 3 times per day, for 3 days for a total of four dosing cycles[b]	5–7 weeks after last dosing cycle
2[a]	4	4	Decitabine	250		750		
3	4	4		500		1500		
4	4	4		1000		3000		

[a] Group 2 actually consisted of 2 males and 5 females, because Animal No. 7 (a female) was mistakenly grouped with the males and Animal No. 5 (a male) was removed from the study before any data was collected. (See Deviations, Appendix 12.)

[b] The length of the recovery time that was needed between consecutive dosing cycles was based on hematology data from the highest dose level.

(Excerpted from the sponsor's submission)

Species/strain: New Zealand White rabbits
Number/sex/group or time point (main study): 4
Route, formulation, volume, and infusion rate: IV, in sterile saline solution, 1 mL/kg/hr,
Satellite groups used for toxicokinetics or recovery: No
Age: 5 months
Weight: M 2.9-3.6 kg, F 2.7-3.6 kg

Observations and times:

Mortality: Twice daily
Clinical signs: 3 times on each dosing day, once daily thereafter
Body weights: Prior to initial treatment and necropsy, weekly during the study
Food consumption: Daily

Hematology: Blood sample collection schedule

Time point	Clinical Pathology		
	Hematology	Serum Chemistry	Blood for Sponsor[a]
Day –6, weekly beginning on Day 8, and prior to necropsy	X		
Day -6, three days after the completion of each dosing cycle, and prior to necropsy		X	
During the first dosing cycle: on Day 1 prior to treatment, and at 30, 60, 120, 180, 190, 210, 230, 250, 270, and 290 minutes after the start of the dosing cycle			X
Volume of Whole Blood/ Time point	1.3 mL	1.8 mL	1.0 mL
Anticoagulant	EDTA	None	EDTA

(Excerpted from the sponsor's submission)

Gross pathology: Approximately 6 weeks after the last dosing cycle. (days 176-177 for group 1 & days 169-170 for groups 2 & 3)

Organ weights See histopathology inventory for NDA 21-790

Histopathology: See histopathology inventory

Adequate Battery: yes (X), no ( )—explain

Peer review: yes ( ), no (X)

Results

Mortality: Group 2 (750 µg/kg/day) – 1M on day 18
Group 3 (1500 µg/kg/day) – 3 M & 1 F on days 13-18
Group 4 (3000µg/kg/day) – All died on days 9-13

Clinical signs: Groups 3 & 4 – Rapid breathing, inappetence, scant or no feces, thin appearance, lethargy, and cold to touch.

Body weights: Decreased in a dose-dependent manner but difficult to compare due to small number of surviving animals.

Food consumption: Low food consumption by treated animals as compared to control animals.

Hematology: Groups 3 and 4 - Red blood cells↓, hemoglobin↓, hematocrit↓, platelet↓, and lymphocytes ↑ as compared to control animals.

Clinical chemistry: Similar among all groups at scheduled termination.

Gross pathology: Discoloration of trachea, heart, lungs, liver, and lymph nodes.

Organ weights: Adrenal gland ↑, spleen ↑, liver ↓, thymus ↓, testes ↓ (no statistical comparison due to low sample size)

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)

Early Deaths

Dose group	2		3		4	
Sex	M	F	M	F	M	F
Animal examined	1		3	1	4	4
Organ/finding						
Adrenal gland, cortical hemorrhage					2	
Brain, hemorrhage			1		3	2
Cecum, lymphoid depletion	1		3	1	4	2
Mineralization	1			1	2	1
Colon, enteropathy					3	2
submucosal hemorrhage						1
Epididymides, hemorrhage					1	
Eye, conjunctivitis			3		2	2
Gallbladder, hemorrhage					2	
Edema				1	2	
Heart, myocardial hemorrhage					2	2
epicard hemorrhage			2			1
mineralization					1	1
Ileum, enteropathy					3	1
Jejunum, enteropathy					3	
Kidneys, tubular casts			2	1	1	
tubular vacuolation				1		1
Liver, hepatocellular necrosis			3		1	2
cytoplasm rarefaction	1				2	1
Kupffer cell hypertrophy	1			1	4	4
chronic inflammation	1		2	1	3	3
Lungs, alveolar hemorrhage			1		2	2
infarct	1		2	1	1	
thrombosis	1		2	1	1	
bacteriosis	1		3		2	2
Mandib. lymph nodes, lymphoid depletion					1	2
sinusoid erythrocyte	1		1	1	3	4
Mesent. lymph node, lymphoid depletion	1		3	1	4	3
sinusoid erythrocyte	1		2		4	4
necrosis					4	4
edema				1	4	2
hemorrhage, supracap					4	3
Ovaries, corpus hemorrhage						1
Bacteriosis						1
Prostate gland, hemorrhage					1	

Dose group	2		3		4	
Sex	M	F	M	F	M	F
Animal examined	1		3	1	4	4
Organ/finding						
Rectum, enteropathy					2	2
Salivary gland, chronic inflammation	1		2	1	2	2
fibroplasia interst	1		2		2	2
acinar cell necrosis	1		1	1	1	
Sciatic nerve, hemorrhage						1
Spleen, lymphoid depletion	1		3	1	4	3
Necrosis					2	1
Sternum-bone marrow, hypocellularity	1		3	1	4	4
Stomach, mucosal hemorrhage			1		3	3
Degeneration parietal cell					2	
Thymus, lymphoid depletion	1		2	1	4	4
edema				1		1
hemorrhage				1		1
Thyroid gland, chronic inflammation					2	
Cyst						2
Trachea, chronic inflammation	1		3		3	3
Hemorrhage					1	1
Urinary bladder, edema					1	
Uterus, hemorrhage						1
Vagina, hemorrhage						1

Scheduled euthanasia

Dose group	1		2		3	
Sex	M	F	M	F	M	F
Animal examined	4	4	1	5	1	3
Organ/finding						
Cecum, mineralization			1		1	
Epididymis, decrease spermatozoa			1		1	
Esophagus, chronic inflammation				3		2
Eye, conjunctivitis	2	3	1	5	1	3
Heart, chron infl myocard					1	
chron infl epicard					1	
Kidneys, chronic inflammation				1	1	1
Lungs, granulomatous inflammation				1		1
hemosiderin				1		1
pleural fibrosis						1
Mammary gland, chronic inflammation		1		4		2
Mandib. lymph nodes, sinusoid erythrocyte			1		1	

Dose group	1		2		3	
Sex	M	F	M	F	M	F
Animal examined	4	4	1	5	1	3
Organ/finding						
Mesen. lymph node, sinusoid erythrocyte		3		2	1	3
Parathyroid glands, cyst						1
Pituitary gland, cyst, pars intermedia	1	2	1	4	1	2
Prostate gland, acinar dilatation					1	
Salivary gland, fibroplasia capsule		1	1	2		1
Chronic inflammation	1		1			
Sciatic nerve, chron inflammation, epineurial				1	1	
Spleen, lymphoid hyperplasia		1		3	1	1
Testes, atrophy			1		1	
Thyroid gland, chronic inflammation	2	2	1	3	1	1
Thymus, ectopic tissue					1	
Trachea, chronic inflammation	2		1		1	

Toxicokinetics:

Day 1 dosing session 1

Group	Dose (µg/kg)	Dose (µg/kg/day)	C_{max} (ng/mL)
2	250	750	69-97
3	500	1500	140-222
4	1000	3000	138-220

Peak plasma concentrations occurred at 120-180 minutes after the end of the infusion.

Histopathology inventory

Study	FEAW-116	FEAW 117	FEAW-0126
Species	Rat	Dog	Rabbit
Adrenals	X*	X	X*
Aorta	X	X	X
Bone Marrow smear			X
Bone (femur)	X	X	X
Brain	X*	X	X*
Cecum	X	X	X
Cervix			
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X*	X	X*
Esophagus	X	X	X
Eye	X	X	X
Fallopian tube			
Gall bladder		X	X
Gross lesions			
Harderian gland			
Heart	X*	X	X*
Ileum	X	X	X
Injection site	X	X	X
Jejunum	X	X	X
Kidneys	X*	X	X*
Lachrymal gland			
Larynx			
Liver	X*	X	X*
Lungs	X*	X	X*
Lymph nodes, cervical			
Lymph nodes mandibular	X	X	X
Lymph nodes, mesenteric	X	X	X
Mammary Gland	X	X	X
Nasal cavity			
Optic nerves			
Ovaries	X*	X	X*
Pancreas	X	X	X
Parathyroid	X*	X	X

Study	FEAW-116	FEAW 117	FEAW-0126
Species	Rat	Dog	Rabbit
Peripheral nerve			
Pharynx			
Pituitary	X*	X	X*
Prostate	X*	X	X*
Rectum	X	X	X
Salivary gland	X*	X	X*
Sciatic nerve	X	X	X
Seminal vesicles	X*		
Skeletal muscle	X	X	X
Skin	X	X	X
Spinal cord	X	X	X
Spleen	X*	X	X*
Sternum		X	X
Stomach	X	X	X
Testes	X*	X	X*
Thymus	X*	X	X*
Thyroid	X*	X	X*
Tongue	X	X	X
Trachea	X	X	X
Urinary bladder	X	X	X
Uterus	X*	X	X*
Vagina	X	X	X
Zymbal gland			

X, histopathology performed
*, organ weight obtained

2.6.6.4 Genetic toxicology

Dr. Shwu-Luan Lee reviewed this section

1. **Evaluation of the mutagenic activity of DAC (PURE) in the Ames *Salmonella*/microsome test.**

Key findings:
- The study is considered exploratory; no firm conclusions can be made.

Study no.: [redacted] Project 065115

Volume #, and page #: Module 4, 4.2.3.3.1, Pages 1-26
Conducting laboratory and location: [redacted]

Date of study initiation: October 7, 1991
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: DAC (PURE), 5-aza-2'-deoxycytidine, Batch #: FB1132, **Purity**: not indicated by the sponsor

Methods
Plate incorporation assay.

Strains/species/cell line: *Salmonella typhimurium* strains: TA1535, TA1537, TA1538, TA98, and TA100.

Doses used in definitive study:
Without S9-mix: 3.3, 10, 33.3, 100 and 333 µg/plate.
With S9-mix: 33.3, 100, 333, 1000, 2000 µg/plate.

Basis of dose selection:
The selection of a range of doses was based on a preliminary toxicity test with TA100, with or without S9-mix, in which DAC at concentrations of 0 to 5000 µg/plate was used. The highest concentration selected reduced survival on the non-selective plates. Five mg/plate was used when no toxicity was observed, unless limited by solubility or non-uniformly dispersible in the solvent of choice.

Negative control:
The vehicle of the test article.

Positive controls:

Without metabolic activation (-S9-mix):

Strain	Chemical	Concentration/plate	Solvent
TA1535	sodium azide (SA) (Fluka)	1 µg	Saline
TA1537	9-aminoacridine (9AC) (Janssen Chimica)	60 µg	Saline
TA1538	4-nitro-O-phenylene diamine (4NPD)	10 µg	DMSO
TA98	daunomycine (DM) (Sigma)	4 µg	Saline
TA100	Methylmethanesulfonate (MMS) (Merck)	650 µg	DMSO

With metabolic activation (+S9-mix):

Strain	Chemical	Concentration/plate	Solvent
TA1535, TA1537	2-aminoanthracene (2AA)	5 µg	DMSO
TA1538, TA98, TA100	2-aminoanthracene (2AA) (Sigma)	0.5 µg	DMSO

Solvents for reference substances
Saline = Physiological saline (
DMSO = Dimethylsulphoxide of spectroscopic quality

Table from the sponsor

Incubation and sampling times:
Incubation: at 37°C for 48 h.
The revertant colonies (His^+) were counted automatically with — colony counter or manually.

Results

Study validity

- Tester strain integrity was documented in the report.
- Although historical data were not submitted, according to the sponsor, both negative (vehicle) and positive control data were within the laboratory historical range.
- The mean positive control value (± S9-mix) exhibited at least threefold increase over the respective mean vehicle control value for each tester strain (see table of experiment result, below).
- All tester strain culture titers were equal to or greater than 3×10^8 cells/ml (10^9 cells/mL).

- In the absence of S9-mix, there was a minimum of three nontoxic dose levels (≤ 50% reduction in mean number of revertants/plate relative to the mean vehicle control value) in each tester strain. In the presence of S9-mix, this criterion was not met.
- Cytotoxicity evaluation was not conducted for each strain.
- Negative results were not confirmed.

Study outcome:
Preliminary toxicity and dose range selection study:

TABLE 1 PRELIMINARY TOXICITY DETERMINATION OF THE TEST SUBSTANCE IN TA100

Concentration (µg/plate)	Viable counts/plate (duplicate plates)	
	Without S9-mix	With S9-mix
Control[a)]	395;431	497;411
1.0	359;308	490;409
3.3	448;459	486;406
10.0	412;472	419;438
33.3	455;368	388;388
100	428;353	394;386
333	281;295	406;432
1000	b)	344;335
3330	b)	b)
5000	b)	b)

a) 0.1 ml DMSO
b) extremely small colonies

Table from the sponsor

The following table summarizes the experimental result:

Dose (µg/plate)	Mean number of revertant (His+) colonies/ 3 replicate plates (± S.D.) with different strains of S.typhimurium				
	TA1535	TA1537	TA1538	TA98	TA100
			Without S9-mix		
3.3	10 ; 6[b]	3 ± 1	8 ± 0	23 ± 2	84 ± 13
10.0	7 ± 2	4 ± 2	10 ± 2	22 ± 3	98 ± 8
33.3	5 ± 4	5 ± 6	7 ± 1	15 ± 3[c]	99 ± 16
100	6 ± 3[c]	0 ± 0[c]	3 ± 1[c]	MC	82 ± 10[c]
333	MC	MC	MC	MC	MC
Solvent control[a]	8 ± 3	4 ± 1	9 ± 2	23 ± 3	95 ± 13
Positive control	389 ± 33	554 ± 74	722 ± 55	606 ± 86	1814 ± 50
			With S9-mix		
33.3	12 ± 4	2 ± 1[c]	12 ± 3	23 ± 3[c]	109 ± 9
100	6 ± 2	MC	10 ± 2	MC	88 ± 8[c]
333	3 ± 1[c]	MC	3 ± 2[c]	MC	MC
1000	MC	MC	MC	MC	MC
2000	MC	MC	MC	MC	MC
Solvent control[a]	9 ± 5	3 ± 1	17 ; 16[b]	23 ± 3	92 ± 8
Positive control	575 ± 19	954 ± 82	469 ± 52	521 ± 75	654 ± 227

a) 0.1 ml DMSO
b) One plate infected with other bacteria
c) Bacterial background lawn slightly reduced
MC: Microcolonies

Table from the sponsor

Comment:

- The experimental result indicated that in the case of TA100, the number of revertant colonies was much less than those in the preliminary result at respective dose levels. There was no explanation or comment on this discrepancy by the sponsor.

Conclusion:

This study should be considered exploratory. A cytotoxicity evaluation was not conducted for each of the strains used. In the presence of S9-mix there were less than three non-toxic dose levels for evaluation in all tester strains. The negative findings observed in the absence of S9 should have been confirmed. Historical data are not available in the study report. However, DAC was mutagenic in the L5718Y mouse lymphoma cells (in vitro assay) as well as in transgenic mice. The results of Ames assay serve as reference and are not crucial.

2. **Evaluation of the mutagenic activity of DAC (PURE) in an *in vitro* mammalian cell gene mutation test with L5178Y mouse lymphoma cells.**

Key findings:
- DAC (PURE) was mutagenic in the TK mutation test under the reported experimental conditions.

Study no.: [redacted] Project 121848

Volume #, and page #: Module 4, 4.2.3.3.1, Pages 1-16
Conducting laboratory and location: [redacted]

Date of study initiation: April 25, 1994
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: DAC (PURE), 5-aza-2'-deoxycytidine, Batch #: FB1279AA, Purity: not indicated by the sponsor

Methods
In vitro TK mutation test in L5178Y mouse lymphoma cells

Strains/species/cell line: L5178Y mouse lymphoma cells

Doses used in definitive study:
Without S9-mix: 10, 100, 1000, 3330, and 5000 µg/ml
With S9-mix: 10, 100, 1000, 3330, and 5000 µg/ml

Basis of dose selection:
The selection of a range of doses used in the final mutagenicity testing was based on a preliminary cytotoxicity test without S9-mix, in which DAC at concentrations of 33 to 5000 µg/plate was used. The highest concentration was determined by the solubility in the culture medium. Concentrations exceeding 5 mg/ml were not tested.

Negative controls: vehicle

Positive controls:

Without metabolic activation (-S9-mix):
Ethylmethanesulphonate (EMS; CAS no. 62-50-0; purity [redacted]
(2 mM) was used. EMS causes direct alkylation of DNA.

With metabolic activation (+S9-mix):
Dimethylnitrosamine (DMN; CAS-no. 62-75-9, purity [redacted]
(0.5 mM) was used. DMN had to be activated by microsomal enzymes present in the S9-mix, resulting in a methyldiazonium ion which could react with cellular DNA.

Solvents for Reference Substances
Hank's balanced salt solution without calcium and magnesium.

Solutions of reference substances were prepared immediately before use.

Table from the sponsor

Incubation and sampling times:
- Exposure to DAC: 3 h.
- Expression period for TFT-resistant mutation: 3 days.
- Mutant selection: TK-deficient mutants formed microcolonies in 10-14 days, which were counted manually.
- Cell survival (cloning efficiency): 10-14 days after plating 3 x 200 cells which have exposed to DAC at various concentrations.
- Mutation frequency: the number of mutant per 10^5 survival cells.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
The study is considered valid, because:
- The cloning efficiency of the solvent controls was ≥ 50%.
- At least three of the five doses of the test substance had an acceptable number of surviving cells (10^6) analyzed for expression of the TK mutation.
- The spontaneous mutant frequency in the untreated or solvent control was < 5 per 10^5 clonable cells (CRC criteria: 20 to 100 per 10^6 surviving cells).
- The mutant frequency of the positive control (ethylmethanesulfonate and dimethylnitrosamine) was at least three times (16-fold for EMS and 11-fold for DMN, respectively) that of the solvent controls.
- The spontaneous mutant frequencies were within historical control data range.

Study outcome:
Cytotoxicity test and dose range selection study:

TABLE 1 CYTOTOXICITY DETERMINATION IN L5178Y MOUSE LYMPHOMA CELLS

Dose (µg/ml)	Cells/ml (x 10^5)[1] After 0 h	After 24 h	After 48 h	Suspension growth Total [3]	% of control
		Without metabolic activation (-S9-mix)			
Solvent control	4.8	5.2	6.3	61	100
33	4.1[2]	4.0	2.9	23	38
100	4.8	2.8	3.9	20	33
333	4.4	2.3	2.7	11	18
1000	4.7	3.1	1.3	7	11
3333	3.4	2.9	1.1	4	7
5000	3.3	1.7	1.3	3	5

Solvent control = F10-medium buffered with 20 mM HEPES
[1] Subculture = 1.6 x 10^5 cells/ml
[2] Subculture = 1.3 x 10^5 cells/ml
[3] Total growth = Cell count after 0 h $\times \frac{\text{(24 h cells/ml)}}{\text{cells subcultured directly after treatment}} \times \frac{\text{(48 h cells/ml)}}{\text{cells subcultured after 24 h}}$

Table from the sponsor

The following table summarizes the mutagenicity testing result:

Appears This Way
On Original

DOSE (µg/ml)	C.E. AT DAY 0 (% OF CONTROL)	C.E. AT DAY 3 (ABSOLUTE %)	MEAN NO. OF MUTANTS PER PLATE	MUTATION FREQUENCY x 10^5
	Without metabolic activation (-S9-mix)			
Solvent control	100	61	1.3	1.5
10	84	68	10.4	10.2
100	69	67	11.1	11.1
1000	31	49	13.6	18.6
3330	8	23	1.9	5.6
5000	1	<1	0	-
2 mM EMS	105	74	26.4	23.8
	With metabolic activation (+S9-mix)			
Solvent control	100	74	1.9	1.7
10	75	67	5.8	5.8
100	70	71	9.1	8.5
1000	60	46	12.7	18.6
3330	6	2	1.0	-
5000	2	0	0.2	-
0.5 mM DMN	46	45	12.2	18.1

C.E. = Cloning Efficiency
Solvent control = F10-medium buffered with 20 mM HEPES
EMS = Ethylmethanesulphonate
DMN = Dimethylnitrosamine

Table from the sponsor

Conclusion:
DAC (PURE) was mutagenic in the *in vitro* TK mutation test in L5178Y mouse lymphoma cells, in the absence and presence of S9-mix.

3. **Mutagenicity of 5-aza-deoxycytidine is mediated by the mammalian DNA methyltransferase.** Jackson-Grusby et al., Proc. Natl. Acad. Sci. USA, 94: 4681-4685 (1997).

Key findings:

- DAC was mutagenic *in vivo* under the experimental condition.
- 5-Aza-deoxycytidine (DAC) induced hypomethylation of *lac I* transgene in mice.
- The mutation at CpG dinucleotides (C:G→G:C transversion, C:G→A:T transversion and C:G→T:A transition) in the transgene in colonic DNA was possibly mediated by the DNA methyltransferase.

Volume #, and page #: Module 4, 4.2.3.3.1, Journal Article
Conducting laboratory and location: Whitehead Institute for Biomedical Research and Department of Biology, MIT, Cambridge, MA.
Date of study initiation: publication in April 1997
GLP compliance: Not reported
QA reports: Not reported
Drug, lot #, and % purity: DAC (PURE), 5-aza-2'-deoxycytidine, Batch #: not reported, Purity: not reported

Methods:

Strains/species/cell line:

- Mice (both sex): *lac I* transgenic (see below).
- Substrain of 129/Sv: *Dnmt*^{s}. *Dnmt*^{s} mice are *lac I* transgenic strain mice with loss-of-function *Dnmt*^{s} allele to attenuate the levels of DNA methyltransferase.
- Substrain of C57BL/6: *Big Blue* (*lac I* transgenic mice) strain harboring a tandemly repeated transgene comprising an *E. coli lac I* gene in a λ bacteriophage shuttle vector.

Doses used in definitive study:
Subcutaneous injection, 5 µg (in 2 µl)/5 gm body weight weekly x 14 weeks.

Experimental design:

- Methylation analysis: The wild type and heterozygous transgenic *Dnmt*^{s} offspring (homozygous mutants were embryonic lethal phenotype) were treated with DAC at 5 µg/5 gm body weight weekly or untreated for 14 weeks. The effect of DAC on the endogenous mutagenic and repair mechanism in the intestinal cell was analyzed. Colonic genomic DNA was cleaved with the methylation-sensitive enzyme *Hpa* II along with *EcoR* I and then subjected to Southern blot analysis with a *lac I* probe. *Eco*RI cuts within the flanking λ sequences in which the *lac I* gene was packaged. The extent of the *lac I* transgene methylation after treatment of DAC was compared to that of complete methylation (DAC-untreated DNA digested with *EcoR* I) and unmethylated state (DNA digested with *EcoR* I and *Msp* I (*Hpa* II isoschizomer which is not inhibited by CpG methylation)).
- Spectrum of DAC-induced mutation: Phage-harbored *lac I* transgene from DAC-treated and untreated (to assess spontaneous mutation) *Big Blue* mice was isolated for sequence analysis. Inactivating mutations in the *lac I* transgene are scored as blue plaques in a background of colorless plaques after in vitro packaging of genomic DNA and plating on indicator bacteria. The extent of spontaneous transitions at CpG dinucleotides and the class of mutation was determined.

Results:

Effect of DAC on *lac I* mouse transgene:

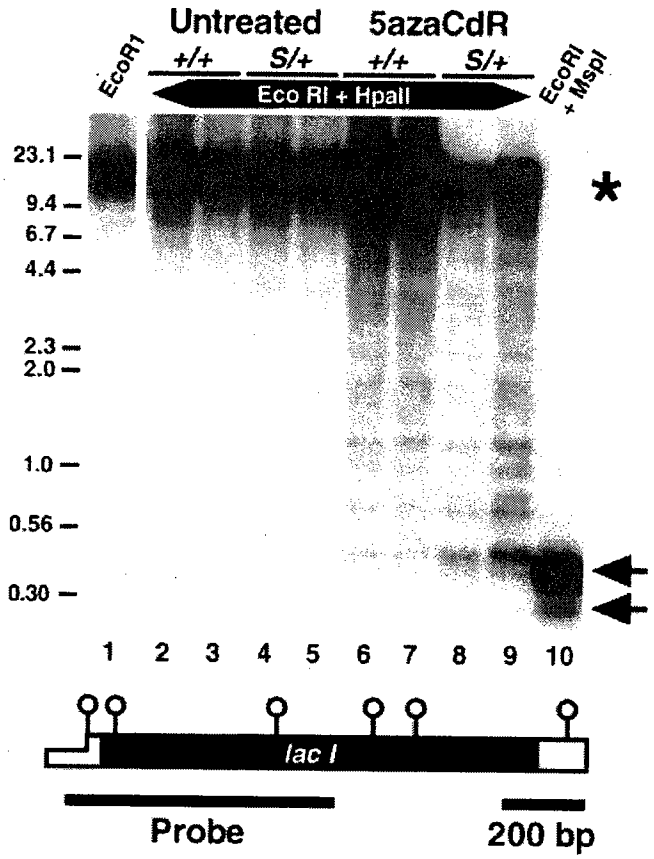


FIG. 1. Southern blot analysis of lac I gene methylation levels. Genomic DNA (5 µg) digested with the indicated enzymes was electrophoresed on an agarose gel and analyzed by Southern blot hybridization. The lac I probe extends from the promoter region (250, thin open box) to nucleotide 601 within the coding sequence (solid box). Position of the six HpaII/MspI sites are shown schematically as open circles over the lac I gene diagram. *, The position of the completely methylated lac I sequences. Arrows indicate the positions of the two complete digest products detected

Figure from the submission

Experiment result:

Methylation analysis:

- There were six *Hpa*II sites within thin the *lac I* gene. *Hpa*II digestion failed when these sites were methylated, since *Hpa*II is methylation-sensitive. However, *Msp*I cuts all the *Hpa*II sites despite the status of methylation at these sites (as shown in lane 10).
- Lane 1 in the figure above represents the result of *Eco*RI digestion of DAC-untreated DNA. This was the fragment of *lac I* gene packaged in the λ phage. When all the *Hpa*II sites were methylated (as in the *Dnmt* wild type +/+ DNA, DAC-untreated), *Eco*RI plus *Hpa*II digestion generated the same fragment as *Eco*RI digestion alone (shown as lanes 2 and 3).
- Lei *et al.*, (Development. 1996, 122:3195-3205) have shown that, although the DNA methyltransferase (*Dmnt*) null mutation was lethal at embryonic stage, the null mutant embryonic stem cells were viable and contained low but stable levels of methyl transferase activity, suggesting the existence of a second DNA methyltransferase in mammalian cells. As shown in lanes 4 and 5, there was extensive methylation on the *lac I* transgene even in the heterozygous *Dmnt* (S/+) mice when compared to lanes 1, 2 and 3.

- DAC treatment (Lanes 6-9) resulted in a range of methylation from complete methylation (star) to near complete demethylation (arrows) in both *Dnmt* wild type (+/+) and *Dnmt* heterozygous (*Dnmt*^{S}/+, alternatively referred to as S/+ in the above figure) mice.
- Introduction of loss-of-function mutated *Dnmt*^{S} allele to the lac *I* transgenic strain was expected to alter methylation status on the *lac I* gene. However as shown in lanes 4-5 and 8-9, introduction of the *Dnmt*^{S} mutation was not as effective as DAC treatment in altering methylation of the *lac I* gene.

Spectrum of DAC-induced mutations:

- A total of 90 independent *lac I* mutations were isolated, among which 39 were from untreated mice (approximately 43%) and 41 from DAC-treated mice.
- Most notable spontaneous mutation at CpG dinucleotides was C:G→T:A (36%). There was no significant difference in the frequency of mutation in the +/+ and S/+ mice.
- Mutations found in DAC-treated mice were mostly at C:G base pair: C:G→G:C transversion, C:G→A:T transversion and C:G→T:A transition. The following table summarized the result:

Table 3. Spontaneous and 5azaCdR-induced mutation spectra

	Spontaneous, *n* (%)		5azaCdR-induced, *n* (%)	
Mutation Class	CpG	non-CpG	CpG	non-CpG
Transitions				
C:G → T:A	14 (36)	3 (8)	11 (22)	1 (2)
T:A → C:G	NA	3 (8)	NA	1 (2)
Transversions				
C:G → A:T	3 (8)	5 (13)	6 (12)	3 (6)
C:G → G:C	0	2 (5)	20 (39)	8 (16)
T:A → G:C	NA	5 (13)	NA	0
T:A → A:T	NA	1 (3)	NA	0
Deletion or insertion	0	3* (8)	0	1* (2)
Total	17 (44)	22 (56)	37 (73)	14 (27)

Mutations identified from Dnmt^S/+ and wild-type mice were combined in this analysis. Spontaneous, n = 39; 5azaCdR-induced, n = 51. NA, not applicable.

*Some of these mutations involved multiple Cs or Gs adjacent to a CpG dinucleotide. Therefore, the exact position of the mutation cannot be determined.

Table from the sponsor

DAC treatment induced a significant increase in the percentage ($p< 0.01$) of mutations at CpG dinucleotides compared to spontaneous mutation.

Conclusion:

DAC is a substrate of DNA methyltransferase functioning by incorporation into DNA and inhibition of methyl transfer. Subcutaneous administration of 1 mg/kg

body weight/week of DAC to mice for 14 weeks induced hypomethylation of *lac I* transgene and mutation at CpG dinucleotides. Thus, DAC was mutagenic *in vivo* under the experimental condition.

4. **Somatic recombination: a major genotoxic effect of two pyrimidine antimetabolitic chemotherapeutic drugs in *Drosophila melanogaster*.** Cunha et al., Mutation Res. 514: 95-103 (2002).

Key Study Findings: Both cytarabine and decitabine showed evidence of recombination in exposed larvae. These data suggest that the antimetabolites induce genomic instability.

Genetic Endpoints: point mutation, deletion, unbalanced half-translocation and mitotic recombination

Description of test system markers: single spots are the result of point mutations, chromosome aberration or mitotic recombination; and twin spots that are due to recombination.
Recessive wing cell markers multiple wing hairs (*mwh*) and flare (flr^3)
Balancer chromosome with large inversions on chromosome 3

Cross: females of $flr^3/In(3LR)TM3, ri\ p^p\ sep\ l(3)89Aa\ Bx^{34e}\ e\ Bd^S$ mated to males*mwh/mwh*

Methods:
Flies were allowed to lay eggs for 8 h, and eggs were allowed to incubate in yeast media. After 3 days, larvae were collected and incubated with various concentrations of test solution (see table below) or water for the remainder of development (approximately 48 h). A positive control was not included in the assay. Dose range finding experiments were conducted but not reported in detail. Concentrations used in the definitive study are shown in the table.

Results: See table from the publication below. Mutational potential (not summarized) of the two compounds was approximately 20% of the genotoxic activity of the two compounds in this assay.

Table 1. Wing spot data obtained after chronic exposure of marker-heterozygous and balancer-heterozygous larvae of *D. melanogaster* with araC and 5-aza-dC.

Controls and compounds	Concentration (mM)	No. of flies (N)	Spots per fly (no. of spots), statistical diagnosis[a]				Total *mwh* clones[c] (n)
			Small single spots (1–2 cells)[b], $m = 2$	Large single spots (>2 cells)[b], $m = 5$	Twin spots, $m = 5$	Total spots, $m = 2$	
Water (historical and pooled controls)							
mwh/flr[3]		135	0.56(75)	0.10(14)	0.03(04)	0.69(93)	45
mwh/TM3		128	0.32(41)	0.03(04)	–[d]	0.35(45)	28
araC							
mwh/flr[3]	0	40	0.43(17)	0.08(03)	0.05(02)	0.55(22)	22
	1	20	0.75(15)i	0.60(12)+	0.15(03)i	1.50(30)+	28
	5	20	0.75(15)i	1.35(27)+	0.75(15)+	2.85(57)+	56
	10	20	0.85(17)+	1.80(36)+	0.90(18)+	3.55(71)+	63
mwh/TM3	0	36	0.33(12)	0.06(02)	–[d]	0.39(14)	14
	1	20	0.30(06)–	0.05(01)–		0.35(07)–	07
	5	20	0.55(11)i	0.10(02)i		0.65(13)i	13
	10	16	0.56(09)i	0.50(08)+		1.06(17)+	17
5-aza-dC							
mwh/flr[3]	0	40	0.50(20)	0.08(03)	0.03(01)	0.60(24)	24
	0.025	20	1.60(32)+	1.10(22)+	0.30(06)+	3.00(60)+	58
	0.05	20	1.85(37)+	2.00(40)+	0.50(10)+	4.35(87)+	72
	0.1	20	1.70(34)+	2.15(43)+	0.35(07)+	4.20(84)+	73
	0.25	20	2.30(46)+	2.75(55)+	0.20(04)+	5.25(105)+	93
mwh/TM3	0	38	0.32(12)	0.00(00)	–[d]	0.32(12)	12
	0.025	20	0.35(07)i	0.20(04)+		0.55(11)+	11
	0.05	20	0.50(10)i	0.20(04)+		0.70(14)+	14
	0.1	20	0.75(15)+	0.80(16)+		1.55(31)+	31
	0.25	20	0.60(12)i	0.75(15)+		1.35(27)+	27

5. **Mutagenicity of 5-azacytidine and related nucleosides in C3H/10T1/2 clone 8 and V79 cells**. Landolph and Jones, Cancer Research 42: 817-823, (1982).

The mutagenic effect of 5-aza-deoxycytidine (DAC) was tested in 10T1/2 mouse embryo fibroblast cell and CHO V79 cells at concentrations of 0.03-1.0 µM and 0.03-10 µM, respectively. Dacitibine did not induce reproducible, concentration-dependent increases in the ouabain resistance mutation frequency in either cell line. Azaguanine resistance mutation frequencies obtained with DAC were not statistically different from the control in V79 cells. The positive control (N-methyl-N'-nitro-N-nitrosoguanidine) worked as expected.

6. **Induction of a step in carcinogenesis that is normally associated with mutagenesis by nonmutagenic concentrations of 5-azacytidine.** Bouck et al., Molecular and Cellular Biology, 4: 1231-1237 (1984).

5-Aza-deoxycytidine induced transformation (anchorage independence) over the dose range tested (0.2 to 2 µM) in baby cells hamster kidney (BHK-21/cl13) cells. Increased resistance to ouabain and 6-thioguanine was not observed, indicating transformation was not accompanied by an increase in mutation in these markers.

7. DNA demethylation and pericentromeric rearrangements of chromosome 1. Ji et al., Mutation Res. 379: 33-41 (1997).

FLEB14 is a cell line derived from Epstein-Barr viral transformed human fetal liver pro-B cells. Cells were exposed to 0.1 or 0.3 μM decitabine for 18 hours, and incubated for 72 hours in media free of the genotoxic agent. Pericentromeric rearrangements observed with decitabine were generally not seen with several other genotoxic compounds. Pericentromeric chromosome rearrangements seen in a mature B-cell line (AHH-1) were mostly simple breaks with genotoxic agents, including 5-azacytidine. The effects of decitabine in this cell line was not reported. It appears that the chromosomal rearrangements are an extension of the hypomethylating activity of decitabine in this cell line.

2.6.6.5 Carcinogenicity

No sponsor initiated studies were conducted. Relevant literature references provided by the sponsor are reviewed below.

1. Carcinogenicity and haemoglobin synthesis induction by cytidine analogues. Carr et al., Br J Cancer 1988; 57:395-402

5-Azacytidine administration for 12 months induced testicular and hepatic tumors in male Fischer rats. Five analogues of cytidine, including 5-deoxyazacytidine tetrahydrouridine (to inhibit the metabolism of DAC) did not appear to be carcinogenic in small experiments as shown below.

Category treatment	*No. rats*		*No. rats with non-testis tumours (%)*	*P*	*No. rats with testis tumours (%)*	*P*	*% rats with any tumour*	*No. rats with Leydig cell hyperplasia*
	Initial	*Evaluable*[a]						
1. Controls	50	49	0		10(20)		20%	6
2. 5-azacytidine 2.5 mg kg^{-1}	100	87	16(18)	<0.01	56(64)	<0.001	72%	11
3. 5-azacytidine 0.25 mg kg^{-1}	10	10	0		2(20)	NS	20%	
4. 5-azacytidine 0.025 mg kg^{-1}	10	10	0		1(10)	NS	10%	
5. 5-deoxyazacytidine + THU	10	10	0		0		0	2
6. 5-fluorodeoxycytidine + THU	10	10	0		1(10)	NS	10%	2
7. 5-fluorocytidine + THU	10	10	0		0		0	2
8. 5,6-dihydro-5-azacytidine	10	9	1(11)	0.12	2(22)	NS	33%	1
9. 6-azacytidine	15	12	0		2(17)	NS	17%	9
10. 5-azacytidine + cytidine	10	5	0		2(40)	NS	40%	
11. 5-azacytidine to 5 pregnant rats (offspring: 13 male, 9 female)	22	22	3(14)	0.03	3/13(23)	NS	27%	3/13
12. 5-azacytidine to weanlings	10	9	1(11)	NS	2(22)	NS	33%	6
13. PH/DEN→5-azacytidine	10	8	5(63) (8/8 hyperplastic liver nodules)	<0.001	1(13)	NS	75%	
14. THU (controls)	10	10	0		3(30)	NS	30%	5

PH, Partial hepatectomy; DEN, diethylnitrosamine 30 mg kg^{-1} i.p. 18 h after PH; THU, tetrahydrouridine; NS, not significantly different from control values; *P* using Fisher's exact test; [a]Evaluable, no. of rats surviving till end of experiment.

(Excerpted from the publication)

There was no clear relationship between DNA methylation and carcinogenic activity as shown below.

Analogue	*Inhibition of methylation*[a]	*Carcinogenic activity*[b]	*Mouse minor Hb synthesis*[c]	*Human foetal Hb synthesis*
6-azacytidine	−	−	−	ND
5-azacytidine	++	+++	+	++[d]
5-aza-2′-deoxyazacytidine	+++	−	+	ND
5-fluorocytidine	ND	−	ND	ND
5-fluorodeoxycytidine	++	−	ND	ND
5,6-dihydro-5-azacytidine	−	±	+	+[e]

[a]*In vitro* data (abstracted from Jones & Taylor, 1980, Table I); [b]This paper, Table II; [c]This paper, Table IV; [d]Ley *et al.* (1982); Charache *et al.* (1983); [e]Carr *et al.* (1987); ND Not done.

(Excerpted from the publication)

2. **The demethylating agent 5-aza-2'-deoxycytidine is a multipotent carcinogen in rats.** Berger MR. Proc. AACR 1997, vol. 38: page 599, abstract # 4023

5-Aza-2'-deoxycytidine was administered i.p. to SD male and female rats at 0 (control), 0.06 (group 2), 0.3 (group 3), and 6.0 (group 4) mg/kg. Rats of group 2 and 3 were dosed five times weekly for 86 weeks. High dose animals received three injections every 12 weeks for 86 weeks. Malignant tumors were seen in males and females respectively: control, 44 and 30%, group 2, 36 and 44%; group 3, 68 and 74%; and group 4, 94 and 74%. Histologic examination reveled the hematopoietic system, skeleton, nervous tissue, skin, and mammary gland to be target organs of toxicity. According to the authors of this abstract, DAC is a multipotent carcinogen in rats.

3. **5-Aza-2'- deoxycytidine is chemopreventive in a 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone-induced primary mouse lung tumor model.** Lantry et al., Carcinogenesis 1999; 20:343-346

Five week old C3AF1 hybrid male mice were treated with 1 mg/kg decitabine three times per week for 12 weeks and then at 0.5 mg/kg for the remaining 12 weeks. Lung tumors were induced with two consecutive weekly doses of 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone starting 1 week after initial treatment with decitabine. The animals were killed at week 24 and results are shown below.

Group	Treatment	NNK (mg/mouse)[b]	Surviving/initial mice[c]	Body wt (g/mouse)[d]	Incidence of tumors (%)[e]	Lung tumor multiplicity[f]
1	5-aza-dC	3.9	13/16	36.8 ± 2.0	77	1.38 ± 0.96*
2	5-aza-dC	None	13/16	36.3 ± 2.2	23	0.23 ± 0.44
3	PBS	3.9	8/10	37.3 ± 2.6	100	2.38 ± 1.06
4	PBS	None	9/10	35.3 ± 5.4	0	0

[a]Treatment with 5-aza-dC began 1 week prior to initiation with NNK (week −1) and continued for 24 weeks (+23). NNK was given in two i.p. doses at week 0 and week +1.
[b]Total NNK dose (100 mg/kg) per mouse: week 1, 1.9 mg; week 2, 2.0 mg; total, 3.9 mg.
[c]Three mice each were lost due to inherent toxicity at MTD in groups 1 and 2. One mouse was lost in group 4 due to unknown reasons. Two mice were killed prior to the end of the bioassay in group 3 and are not included in the final statistics.
[d]Mean ± SD (at week + 23).
[e]Percent of mice with lung tumors.
[f]Mean ± SD group 1.
*Statistically different from group 3 ($P < 0.05$, Student's t-test).

(Excerpted from the publication)

Treatment with decitabine resulted in 23% reduction in lung tumor incidence induced by NNK in this tumor model.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

5-aza-2'-deoxycytidine induces alterations in murine spermatogenesis and pregnancy outcome.

Key study findings: Treatment of males resulted in ↑ preimplantation loss, ↓ fertility rate, and abnormal histology in testes, in the absence of observable toxicity to other tissues

Study no.: Kelly TL, Li E, and Trasler JM
Volume # and page #: J Andrology 24: 822-30
Conducting laboratory and location: McGill University, Dept Pediatrics
Date of study initiation: Publication date 2003
GLP compliance: no
QA reports: yes ( ) no (x) Peer reviewed publication
Drug, lot #, and % purity: 5-aza-2'deoxycytidine purchased from Sigma,

Methods

Species/strain[a]: male *Dnmt1*$^{+/+}$; Doses: 0.05, 0.1, or 0.15 mg/kg
Species/strain[a]: male Dnmt$^{c/+}$; Dose 0.1 mg/kg
Schedule: 3x/wk for 7 weeks
Number/sex/group: 8 or 12 (saline), 9 (*Dnmt*$^{c/+}$); 12 (*Dnmt1*$^{+/+}$)
Age: 7 weeks
Route, formulation, volume: IP; saline, volume not specified
Drug, purity: 5-aza-2'-deoxycytidine, purchased from Sigma

[a] *Dnmt1*$^{+/+}$ is wild type mouse; *Dnmt1*$^{c/+}$ is heterozygous in the DNA methyltransferase gene. Mouse with this mutation are phenotypically normal but have half the DNMT1 levels as wild-type mice.

Study design:
Mating: each male mated for 6 days with 2 untreated CD1 females at the end of 7 weeks
Parameters: sperm counts; gross DNA methylation of CCGG sites in sperm; corpora lutea on d19 of pregnancy (determined by vaginal plug); # implantations; resorptions; live fetuses; fetal weight; gross malformations

Observations and times:

Clinical signs: not specified
Body weights: not specified
Hematology: WBC at end of treatment

Clinical chemistry: hemoglobin at end of treatment
Organ weights: testes, epididymides, seminal vesicles
Histopathology: testes

Results:

Body weight, "behavior", hemoglobin, WBC; no effect
Organ weight:
Seminal vesicle: unremarkable
Testes: ↓ vs saline control 35 and 55% (0.1 and 0.15 mg/kg in *Dnmt1*$^{+/+}$) and 15% (0.1 mg/kg in *Dnmt1*$^{c/+}$)

Testicular histology:

Strain	Dose (mg/kg)	Observations	Sperm counts (per g testes wt)
Dnmt1$^{+/+}$	0.05	Unremarkable	Unremarkable
	0.1	Disordered germ cell associations, vacuolizations, multinucleate cells	↓ 33%
	0.15	Germ cell populations often absent, sloughing of germ cells, vacuolization	↓ 66%
Dnmt1$^{c/+}$	0	Higher baseline abnormalities than control *Dnmt*$^{+/+}$	---
	0.1	Similar to control *Dnmt1*$^{c/+}$	Similar to control *Dnmt1*$^{c/+}$

Effects on mating:

	Dnmt1$^{+/+}$			*Dnmt1*$^{c/+}$	
	0.05 mg/kg	0.1 mg/kg	0.15 mg/kg	0	0.1 mg/kg
Sperm positive	No effect	No effect	No effect		No effect
Pregnancy rate	No effect	↓67%	None pregnant		No effect
# corpora lutea	No effect	No effect	No effect		No effect
Preimplantation loss	Shown as similar to control (6%)	27%		8%	27%
Postimplantation loss	No effect	No effect	No effect		No effect
Resorptions	No effect	No effect	No effect		No effect
Live fetuses	No effect	No effect	No effect		No effect
Litter size	No effect	No effect	No effect		No effect
Sex ratio	No effect	No effect	No effect		No effect
Fetal wt	No effect	No effect	No effect		No effect
Placental wt	No effect	No effect	No effect		No effect

Genomic DNA methylation: ↓ 29% in 0.15 mg/kg group

Conclusion: The effects observed in *Dnmt1*$^{+/+}$ mice dosed with 5-aza-CdR may be due to cytotoxicity and hypomethylation, whereas the more mild effects see in *Dnmt1*$^{c/+}$ dosed with 5-aza-CdR may be due primarily to hypomethylation.

Embryofetal development

Reviewed by S. Leigh Verbois, Ph.D.

1. Teratogenic effects of the demethylating agent 5-aza 2'deoxycytidine in the Swiss Webster mouse

Key study findings:

- Decitabine was a developmental toxin in the absence of overt maternal toxicity in mice.

Study no.: Branch, S; Francis, BM; Brownie, CF; Chernoff, N.
Volume # and page #: Toxicology 112: 37-43 (1996).
Conducting laboratory and location: North Carolina State University, Dept of Tox
Date of study initiation: Publication date 1996
GLP compliance: no
QA reports: yes ( ) no (x) Peer reviewed publication
Drug, lot #, and % purity: Purchased from Sigma

Methods

Doses: 0, 0.3, or 1 mg/kg
Species/strain: Swiss Webster CD1 Mice
Number/sex/group: 18-19 mice were bred/group, see table in results for average litter
Route, formulation, volume, and infusion rate: IP, sterile saline, 0.2 mL, bolus infusion.
Satellite groups used for toxicokinetics: not included
Study design: Dams were administered a single injection of decitabine on day 8, 9, 10, or 11 of gestation. Mice were killed on day 17 of gestation. Two control and treated dams (1 mg/kg, GD 10) were allowed to give birth for evaluation of postnatal viability; these animals were sacrificed on day 23.
Parameters and endpoints evaluated: Gravid uterine and litter weights and gross malformations were recorded. Detailed skeletal examinations were conducted. All cranial, hyoid, sternebrae, pelvic, pectoral girdle, forelimb and hind limb bones were examined for absence, irregular shapes and lack of or reduction in ossification. The axial skeleton was examined for degree of linearity of spine, any irregularities in number and anatomical feature of the individual vertebral bones and ribs. Distal limb anatomy was examined including metacarpal, metatarsal, carpal, tarsal and phalangeal bones.

Results

Mortality/Clinical Signs (dams): none observed
Postnatal functional deficits did not impact pup viability.

Body weight (dams): no effect of decitabine on maternal body weight.

Food consumption (dams): not reported.

Terminal and necroscopic evaluations:

Effect of d-AZA on fetal survival and weight

Gestation day	Dose (mg/kg)	No. mice bred	No. pregnant	Average maternal wt. gain (GD 7–17 (g))	Average no. live fetuses	Average no. dead Fetuses	Average fetal wt. (g)
GD 8	0	19	15	3.11 ± 0.54	8.8 ± 0.8	0.1 ± 0.1	1.50 ± 0.03
	0.3	19	12	3.70 ± 0.38	9.2 ± 0.6	0.4 ± 0.1	0.91 ± 0.04[b]
	1.0	19	16	3.59 ± 0.33	9.9 ± 0.4	0.9 ± 0.4	0.96 ± 0.03[b]
GD 9	0	19	14	3.47 ± 0.47	10.1 ± 0.79	1.4 ± 0.7	1.07 ± 0.04
	0.3	19	12	3.40 ± 0.45	8.4 ± 0.6	0.7 ± 0.3	0.99 ± 0.03
	1.0	19	16	3.92 ± 0.56	7.8 ± 0.7[a]	2.0 ± 0.4	0.80 ± 0.03[b]
GD 10	0	18	13	3.19 ± 0.30	9.2 ± 0.7	0.4 ± 0.2	1.11 ± 0.04
	0.3	19	16	3.54 ± 0.28	10.2 ± 0.5	0.9 ± 0.3	0.96 ± 0.02[a]
	1.0	19	12	3.41 ± 0.44	8.5 ± 0.8	0.8 ± 0.4	0.82 ± 0.03[b]
GD 11	0	18	13	4.46 ± 0.04	8.7 ± 0.9	0.2 ± 0.1	1.30 ± 0.13
	0.3	18	11	3.71 ± 0.34	8.8 ± 1.1	0.8 ± 0.3	0.96 ± 0.03[b]
	1.0	18	12	3.8 ± 0.7	10.0 ± 0.54	0.3 ± 0.1	0.88 ± 0.03[b]

Averages are mean ± S.E.
[a]Significantly different from controls of same treatment day ($P < 0.05$).
[b]Significantly different from controls of same treatment day ($P < 0.01$).

(Excerpted from Toxicology (1996) 112: 37-43)

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Offspring (malformations, variations, etc.):

Axial Skeletal and Cranial defects following decitabine administration:

Gestation day	Dose (mg/kg)	Fused ribs	Supernumerary ribs	Vertebral defects*	Tail	Cleft pala
GD 8	0	0	32.0 ± 9.2	0	0	0
	0.3	4.4 ± 2.3	52.1 ± 9.3[b]	0	0	1.2 ± 1.2
	1.0	88.7 ± 6.5[b]	89.9 ± 3.0[b]	76.0 ± 6.0[b]	0	0
GD 9	0	0	31.5 ± 8.1	0.8 ± 0.8	0	0
	0.3	0	38.2 ± 7.0	2.3 ± 2.3	0	11.4 ± 6.3
	1.0	7.8 ± 6.3	30.8 ± 7.2	69.2 ± 9.1[b]	1.4 ± 1.0	80.2 ± 8.2
GD 10	0	0	26.3 ± 8.5	0	0	0.6 ± 0.6
	0.3	0	43.6 ± 6.1	0.6 ± 0.6	0	0
	1.0	0	53.9 ± 8.5[b]	13.7 ± 8.6[b]	64.4 ± 11.3[b]	17.5 ± 7.9
GD 11	0	0	15.9 ± 6.1	0.8 ± 0.8	0	0
	0.3	0	18.0 ± 4.5	0.8 ± 0.8	3.5 ± 2.7	0
	1.0	0	6.5 ± 3.2	0	7.6 ± 3.7	0.8 ± 0.8

Averages are mean ± S.E.
[a]Significantly different from control of same treatment day ($P < 0.05$).
[b]Significantly different from control of same treatment day ($P < 0.01$).
*Exclusive of tail defects.

Excerpted from Toxicology (1996) 112: 37-43

Appendicular skeletal defects following decitabine administration in mice:

Gestation day	Dose (mg/kg)	Femoral	Tibial	Fibular	Pelvic	Forelimb digit	Hindlimb digit
GD 8	0	0	0	0	0	0	0
	0.3	0	0	0	8.3 ± 8.3	2.4 ± 2.4	0
	1.0	0	0	0	0	0.7 ± 0.7	0.7 ± 0.7
GD 9	0	0	0	0	0	0.7 ± 0.7	0.7 ± 0.7
	0.3	0	0	0	0	0	0
	1.0	0	0.8 ± 0.8	0	0.8 ± 0.8	0.8 ± 0.8	0.8 ± 0.8
GD 10	0	0	0	0	0	0	0
	0.3	0	0	0	0	0	0
	1.0	32.8 ± 7.6[a]	84.1 ± 8.1[b]	81.3 ± 8.4[b]	37.5 ± 9.4[b]	3.4 ± 1.9	6.2 ± 2.5[a]
GD 11	0	0	0	0	0	0	0
	0.3	0	0	0	0	0	0
	1.0	0	0	0	0	20.8 ± 7.7[a]	40.5 ± 10.9[b]

Averages are mean ± S.E.
[a]Significantly different from control of same treatment day ($P < 0.05$).
[b]Significantly different from control of same treatment day ($P < 0.01$).

Excerpted from Toxicology (1996) 112: 37-43

Tail defects are described in the text of the article as offset, fused and abnormally shaped coccygeal vertebrae.

Conclusions:

- In dams treated with 1 mg/kg decitibine (unless noted otherwise) on GD 8, 9, 10, 11 and sacrificed on day 17, fetal defects included:
  - Gestational day 8-supernumerary ribs (≥0.3 mg/kg), fused vertebrae and ribs
  - Gestational day 9- cleft palate and vertebral variations

- Gestational day 10- hind-limb defects ("including phocomelia (absent or reduced femurs), meromelia (specifically absent fibulae), unossified and reduced fibulae, reduced pelvic bones, and reduction and curvature of the tibiae"), tail defects
- Gestational day 11- digital defects of the fore- and hindlimbs, described as brachydactyly, oligodactyly, polydactyly and syndactyly, stronger in hindlimb.
- Day 23 mice attained normal weight and internal organs appeared normal.

2. Cell death and cell cycle perturbation in the developmental toxicity of the demethylating agent 5-Aza-2'-Deoxycytidine

Key study findings:
- Decitabine was a developmental toxin in the absence of overt maternal toxicity in mice.

Study no.: Rogers, JM; Francis, M; Sulik, KK; Alles, AJ; Massaro, EJ; Zucker, RM; Elstein, KH; Rosen, MB and Chernoff, N.
Volume #, and page #: Teratology 50: 332-339
Conducting laboratory and location: EPA- Developmental Toxicology Division, Research Triangle Park, North Carolina
Date of study initiation: publication date 1994
GLP compliance: no
QA reports: yes ( ) no (x) peer reviewed publication
Drug, lot #, and % purity: 5-aza-2'-deoxycytidine from Sigma

Methods

Doses: 0, 0.05, 0.1, 0.2, 0.3, 1, 2 and 3 mg/kg
Species/strain: CD1 mice
Number/sex/group: 4/dose/time period
Route, formulation, volume, and infusion rate: IP, sterile saline, 0.1 mL, bolus

Study design: 4 pregnant females/dose were killed 4, 8, and 24 hours after treatment on gestational day 10 for analysis of cell death patterns using Nile Blue Sulfate (NBS- stained cell corpses). Two fetuses per litter were selected at random and examined. Cell cycle analysis of limb buds with flow cytometry performed on additional fetuses. Additional pregnant animals were sacrificed on gestational day 17 for examination of gross morphology.

Parameters and endpoints evaluated: see study design

Results

Mortality, Clinical signs, body weight (dams): None observed
Food consumption (dams)/Toxicokinetics: Not evaluated

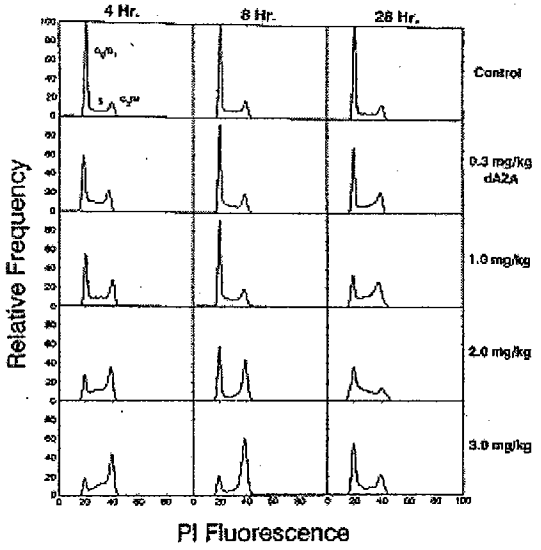
Terminal and necroscopic evaluations including offspring malformations:

Effects of decitabine on mouse fetuses observed at GD17 after maternal treatment on day 10 of gestation:

	Dose (mg/kg)						
	0	0.05	0.1	0.2	0.3	1.0	2.0
Litter data							
No. of litters	37	28	17	21	20	12	13
No. of all resorbed	0	0	0	0	0	1	3
No. of live/litter	10.8	9.8	10.4	10.8	11.7	12.0	8.4*
Percent dead/litter	7.3	8.2	10.8	10.2	7.3	5.2	33.1*
Fetal wt (g)	1.18	1.16	1.15	1.08	1.04	0.83*	0.76*
External defects[1,2]							
Cleft palate	0	0	0	0	14.3*	15.3*	70.6*
Skeletal anomalies[1]							
No. of litters	31	28	17	21	16	11	10
Micromelia							
Forelimb	0	0	0	0	0	13.2*	25.0*
Hindlimb	0	0	0	0	78.6*	88.2*	61.4*
Digit defects							
Forelimb	0	0	0	0	0	13.7*	77.6*
Hindlimb	0	0	0	0	19.8*	75.3*	75.3*
Vertebral centra	0	0	0	0	7.1	19.1*	37.6*
Pelvic girdle	0	0	0	0	7.2	26.5*	31.8*
No. of ossification sites							
Caudal vertebrae	5.1	5.4	4.9	5.3	4.8	3.4*	2.8*
Phalanges							
Forelimb	17.0	18.1	16.7	17.1	16.3	11.2*	7.2*
Hindlimb	16.0	16.5	15.4	16.2	14.8	6.8*	2.8*
Supraoccipital score[3]	1.4	1.4	1.5	1.4	1.6	2.4*	2.7*

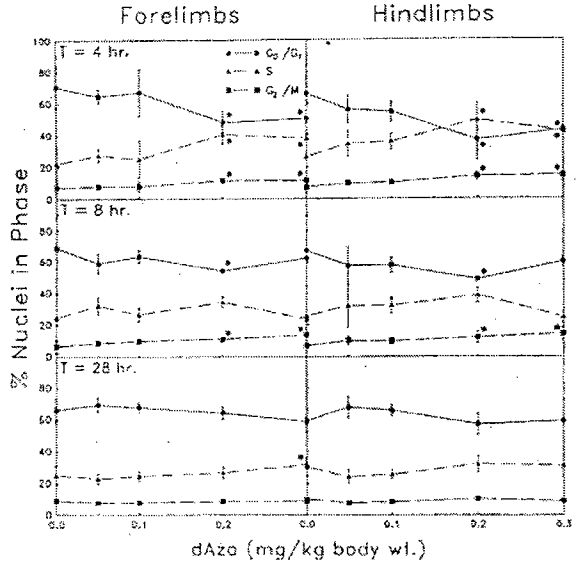
[1]All external and skeletal anomalies are expressed as the mean percent affected per litter.
[2]Micromelia was also evident externally, but the incidence of this effect was assessed only during skeletal examinations.
[3]A score of 1 indicates complete ossification, while 4 indicates no ossification.
*Significantly different from control, $P \leq 0.05$.

Histograms showing the distribution of nuclear propidium iodide fluorescence in the Hindlimb buds following doses of decitabine on day 10 of gestation:



Excerpted from Teratology (1994) 50: 332-339.

Analysis of cell cycle histograms from embryological limb buds from dams treated with decitabine on day 10:



Excerpted from Teratology (1994) 50: 332-339.

Conclusions:

- Significant decreases in live fetuses per litter and increased number of dead or resorbed fetuses were observed at 2 mg/kg. No live fetuses were found at term in pregnant females treated with 3 mg/kg.

- Decitibine decreased fetal weight, induced cleft palate and limb malformations at ≥0.3 mg/kg on day 10 of gestation. Significant increases in micromelia and digit defects occurred at doses ≥ 0.3 mg/kg with a higher incidence in hindlimbs than forelimbs. Significant increases in abnormalities of the vertebral centra and pelvic girdle and decreases in ossification of the supraoccipital bone, distal phalanges, and caudal vertebrae were observed at doses of ≥1 mg/kg.
- Increased levels of cell death in areas of rapid cell proliferation in fetuses exposed to ≥ 0.1 mg/kg. Areas included hindlimb and forelimb buds, facial prominences, visceral arches, somites and neural tube.
- At doses of ≥ 0.3 mg/kg, a dose related increase in the percentage of nuclei in the S and G2/M phases was evident in fore and hindlimbs by 4 hours after maternal dosing and continued through 28 hours after dosing.

3. Differentially expressed genes associated with 5-Aza-2'-Deoxycytidine induced hindlimb defects in the Swiss Webster Mouse

Key study findings:

- Subtraction hybridization, with confirmatory RT-PCR, completed 4, 12 and 24 hours after a 1 mg/kg on gestational day 10 indicated that there were observable down regulation of transcript from a member of the murine B1 family of repetitive sequences at 4 and 12 hours and upregulation of the mouse activin receptor type II gene, and a homolog avian myogenic regulatory protein mRNA and mammalian MyoDat 12 hours post-dose.

Study no.: Branch, S; Francis, BM; Rosen, MB; Brownie, CF; Held, GA; Chernoff, N.
Volume # and page #: Journal of Biochemistry and Molecular Toxicology 12(3): 135-141.
Conducting laboratory and location: North Carolina State University, Dept of Tox
Date of study initiation: Publication date 1998
GLP compliance: no
QA reports: yes ( ) no (x) peer reviewed publication
Drug, lot #, and % purity: 5-AZA-2'-deoxycytidine purchased from Sigma

Methods

Doses: 0, and 1 mg/kg
Species/strain: Swiss Webster CD-1 Mice
Number/sex/group: ≥3 dams/dose/sacrifice period.
Route, formulation, volume, and infusion rate: IP, sterile saline, 0.2 mL, bolus
Study design: Dams were administered decitabine on day 10. Subtraction hybridization experiments were conducted using limb buds collected 4, 12, and 24 hours post-decitabine treatment. Treated and untreated animals were also sacrificed on GD 17 to confirm the induction of characteristic hindlimb defects.

Parameters and endpoints evaluated: RT-PCR, using cyclophilin as the internal standard, was conducted to confirm the differential expression of SB10, SB6 and SB-Lmb, sequences that were identified via subtraction hybridization.

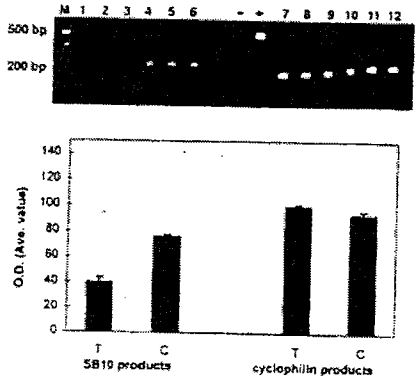
Results:

Mortality (dams)/clinical signs (dams)/Body weight (dams)/Food consumption or TK: not reported

Offspring (malformations, variations, etc.):

- Fetuses from treated dams allowed to proceed to GD17 exhibited hindlimb defects: phocomelia, reduced tibiae and femurs, absent fibulae and pelvic bone reductions.
- Changes in phenotypic expression patterns were evident at the 12 hour hindlimb collection. There was a decreased indentation in the treated limb buds when compared to untreated buds.
- Observable changes in genotypic expression patterns were limited to 1 sequence at 4 hours (SB10-down-regulated), and 3 sequences at 12 hours (SB10-down-regulated, SB6-up-regulated, and SB-lmb-upregulated). SB10 was found to be associated with B1 family of repetitive sequences. SB6 was found to have sequence homology with mouse activin receptor type II gene. SBlmb was found to have sequence homology with avian myogenic regulatory protein mRNA and mammalian MyoD.

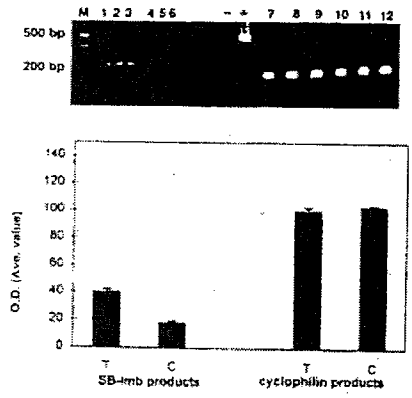
Confirmation of differential expression of SB10 with RT-PCR



Excerpted from J Biochem Molec Tox (1998) 12: 135-141.

Products are shown separated on agarose gel. M, molecular weight marker; lanes 1,2,3-DAC treated sample, lanes 4,5,6-DAC untreated sample (experiment control); (-)-negative control; (+)-positive

Confirmation of differential expression of SB-lmb with RT-PCR:



Excerpted from J Biochem Molec Tox 12: 135-141.

4. 5-AZA-2'-Deoxycytidine-induced dysmorphogenesis in the rat

Key study findings: Decitabine was a developmental toxin in the absence of overt maternal toxicity in the rat.

Study no.: Branch, S; Chernoff, N; Brownie, C; and Francis BM.
Volume # and page #: Teratogenesis, Carcinogenesis, and Mutagenesis 19: 329-338 (1999).
Conducting laboratory and location: Department of Toxicology, North Carolina State University
GLP compliance: no
QA reports: yes ( ) no (x) peer reviewed publication 1999
Drug, lot #, and % purity: 5-AZA-2'-deoxycytidine purchased from Sigma

Methods

Doses: 0, 0.4, 0.6, or 1.0 mg/kg based on maternal weight on GD8.
Species/strain: Sprague-Dawley Rats
Number/sex/group: 4-9
Route, formulation, volume, and infusion rate: IP, sterile saline, 0.2 mL, bolus
Study design: Administered on GD9, 10, 11, or 12. A small group was dosed with 0.125, or 0.25 mg/kg on GD9.
Parameters and endpoints evaluated: Rats were killed on GD20. Dam, gravid uterus, and litter weights were obtained and gross malformations and variations recorded. Detailed skeletal examinations were conducted.

Results

Mortality/clinical signs/body weight (dams): no evidence of maternal toxicity as evidenced by lethality or weight gain during gestation
Food consumption (dams): not reported

Terminal and necroscopic evaluations:

Effects of decitabine on maternal weight change, fetal survival and weight:

GD	Dose (mg/kg)	# Mice bred	# Pregnant	Ave. maternal weight gain (GD 8–20)	Ave. % live fetuses	Ave. fetal weight
Controls	0	7	7	47.35 ± 0.45	97.93 ± 0.11	4.05 ± 0.03
9	0.4	6	6	40.67 ± 0.31	0*	N/A
	0.6	4	4	34.25 ± 0.64	0*	N/A
	1.0	8	8	40.08 ± 4.47	0**	N/A
10	0.4	4	4	32.57 ± 0.45	100.00 ± 0.00	4.10 ± 0.11
	0.6	6	6	41.00 ± 1.07	57.83 ± 1.27*	2.65 ± 0.32*
	1.0	8	8	45.04 ± 5.01	13.33 ± 1.33**	2.30 ± 0.24**
11	0.4	6	6	35.42 ± 0.52	90.27 ± 0.42	4.15 ± 0.06
	0.6	4	4	37.98 ± 0.97	100.00 ± 0.00	3.05 ± 0.17*
	1.0	9	9	37.91 ± 3.92	92.86 ± 0.89	2.83 ± 0.31**
12	0.4	4	4	27.55 ± 0.44	100.00 ± 0.00	3.97 ± 0.11
	0.6	6	6	35.17 ± 0.42	83.33 ± 0.68	3.82 ± 0.07
	1.0	9	9	37.77 ± 6.02	96.72 ± 0.63	2.65 ± 0.12**

Values are averages of the percent of litter affected ± SE.
*Significantly different from controls ($P < 0.05$).
**Significantly different from controls ($P < 0.01$).

Excerpted from Teratogenesis, Carcinogenesis and Mutagenesis (1999) 19: 329-338

Appendicular Skeletal Defects

GD	Dose (mg/kg)	Humerus	Ulna	Radius	Foredigits	Femur	Tibia	Fibula	Hindigits
Controls	0	0	0	0	0	0	0	0	0
10	0.4	0	0	0	2.94 ± 2.94	0	0	0	0
	0.6	0	0	0	9.92 ± 6.67**	0	0	0	11.11 ± 7.86**
	1.0	0	0	0	50.00 ± 8.87*	0	0	0	0
11	0.4	0	0	0	0	0	0	0	0
	0.6	0	0	0	0	0	0	0	0
	1.0	30.59 ± 12.31**	49.92 ± 14.86**	63.32 ± 17.02**	53.34 ± 14.93**	0	0	0	0
12	0.4	0	0	0	16.67 ± 16.67	0	4.17 ± 4.17	0	0
	0.6	0	0	0	28.65 ± 16.00**	0	0	0	0
	1.0	0	11.80 ± 10.22	11.80 ± 10.22	100.00 ± 0.00**	20.97 ± 9.80*	97.22 ± 1.76**	97.22 ± 1.76**	100.00 ± 0.00**

Values are averages of the percent of litter affected ± SE.
*Significantly different from controls ($P < 0.05$).
**Significantly different from controls ($P < 0.01$).

Excerpted from Teratogenesis, Carcinogenesis and Mutagenesis (1999) 19: 329-338.

Axial Skeletal Defects

GD	Dose (mg/kg)	Vertebral	Cervical ribs	Fused ribs	SNR	Missing ribs	Supernumerary presac. vert.	Coccygeal defects
Controls	0	0	0	0	0	0	0	0
10	0.4	45.00 ± 26.30**	0	1.47 ± 1.47	46.07 ± 23.48**	0	25.00 ± 25.00	0
	0.6	63.89 ± 23.73**	0	41.02 ± 19.38**	39.58 ± 22.92**	2.78 ± 2.78	15.00 ± 15.00	0
	1.0	41.67 ± 13.93*	0	66.67 ± 3.43**	0	58.33 ± 3.84**	0	91.67 ± 9.98**
11	0.4	44.25 ± 14.04**	0	0	0	0	0	0
	0.6	96.88 ± 3.12**	3.57 ± 3.57	3.57 ± 3.57	1.56 ± 1.56	27.27 ± 24.34	0	0
	1.0	30.34 ± 8.04*	4.76 ± 2.88	17.56 ± 0.73	0	72.18 ± 0.89**	0	69.71 ± 0.79**
12	0.4	0	0	0	5.56 ± 5.56	0	0	0
	0.6	17.50 ± 12.25*	0	0	7.5 ± 7.5	0	2.00 ± 2.00	0
	1.0	11.94 ± 8.04	0	8.33 ± 5.80	0	1.04 ± 0.27*	0	98.61 ± 1.39**

Values are averages of the percent of litter affected ± SE.
*Significantly different from controls ($P < 0.05$).
**Significantly different from controls ($P < 0.01$).

Excerpted from Teratogenesis, Carcinogenesis and Mutagenesis (1999) 19: 329-338.

Cranial defects

GD	Dose (mg/kg)	Cleft palate	Exophthalmia	Exencephaly
Controls	0	0	0	0
10	0.4	0	0	0
	0.6	3.84 ± 3.84	0	0
	1.0	50.00 ± 8.67*	8.33 ± 8.24	50.00 ± 8.67*
11	0.4	0	0	0
	0.6	3.57 ± 3.57	0	0
	1.0	58.96 ± 11.43**	80.57 ± 4.67**	4.76 ± 3.57
12	0.4	0	0	0
	0.6	0	0	0
	1.0	29.24 ± 10.15*	0	0

Values are averages of the percent of litter affected ± SE.
*Significantly different from controls ($P < 0.05$).
**Significantly different from controls ($P < 0.01$).

(Excerpted from publication)

Conclusions:

- Decitibine treatment did not induce maternal toxicity as measured by weight change during gestation or lethality. Treatment on GD9 resulted in complete resorptions of litters at all doses ≥ 0.4 mg/kg. A significant decrease in fetal survival and decreased fetal weight was observed following 0.6 and 1 mg/kg doses in the GD10 group.
- Axial skeletal defects: Vertebral and rib anomalies, were observed at doses ≥0.4 mg/kg on GD 10 and 11.
- Cranial defects: Exophthalmia (GD 10/11), exencephaly (GD10/11), and cleft palate (GD10/11/12) were observed following 1 mg/kg.
- Appendicular defects: Foredigit defects (syndactyly and oligodactyly) were observed on GD 10, 11, and 12 at doses of ≥0.6 mg/kg with the highest incidence on GD12. Long bone defects (reduction in size and ossification) were noted in the high dose group, with forelimb defects occurring primarily on GD 11 and hindlimb defects on GD12.

Prenatal and postnatal development.

1. 5-Aza-2'-deoxycytidine-induced inhibition of differentiation of spermatogonia into spermatocytes in the mouse.

Key study findings: 5-aza-2-deoxycytidine treatment of neonatal mice resulted in hypomethylation of DNA, inhibited differentiation of germ cells, and affected protein synthesis

Study no.: Raman R and Narayan G
Volume # and page #: Mol Reprod and Develop 42: 284-290 (1995)
Conducting laboratory and location: Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi India
Date of study initiation: Publication date 1995
GLP compliance: no
QA reports: yes ( ) no (x) peer reviewed publication
Drug, lot #, and % purity: 5-aza-2-deoxycytidine; not specified

Methods

Doses: 0, 1 µg/g bw
Species/strain: 5 day old albino mice, Parkes strain, laboratory bred
Number/sex/group: not specified; males
Route, formulation, volume: IP, water, 0.1 mL
Study design:

- 3 injections total at 8 hour intervals
- animals sacrificed at 15, 21, 28 and 30 days of age
- testes processed for chromosome preparation, histological sectioning and ^{35}S-methionine labeling

Results:

- At 1 mg/kg bw, no effect on bw or general physical condition seen; this dose was chosen over 2 and 5 µg (2 and 5 mg/kg)
- Restriction enzyme digestion (MspI, HpaII, and HhaI, the last 2 being methylation sensitive) of DNA from testes of control and treated animals at D 15 (10 days after dosing) showed that DNA from treated animals was hypomethylated compared to controls.
- Cytological analysis indicated treated testis was lacking meiocytes at D 15, 21, and 28, compared to controls, suggesting that DAC blocked germ cell to spermatocyte maturatrion
- Autoradiograms of labeled proteins fractionated by gel electrophoresis indicated the absence or presence of new molecular weight proteins in testes from treated animals relative to control; this difference was not observed between control and treated animals on day 30 (25 d after dosing)

2. Susceptibility to postnatal growth retardation induced by 5- aza- 2'-deoxycytidine in utero: gender specificity and correlation with reduced insulin-like growth factor.

Key study findings: Decitabine exposure decreased body weight and affected reproductive parameters in mice exposed *in utero.*

Study no.: Cisneros FJ, Wilson R, Travlos G, Anderson LM, and Branch S.
Volume # and page #: Life Sciences 72: 2887-2894 (2003)
Conducting laboratory and location: Env Mol Toxicology Dept, NC State University, and NIEHS, RTP, NC; NCI, Frederick MD
Date of study initiation: Publication date 2003
GLP compliance: no
QA reports: yes ( ) no (x) peer reviewed publication
Drug, lot #, and % purity: 5-aza-2-deoxycytidine purchased from Sigma Chemical

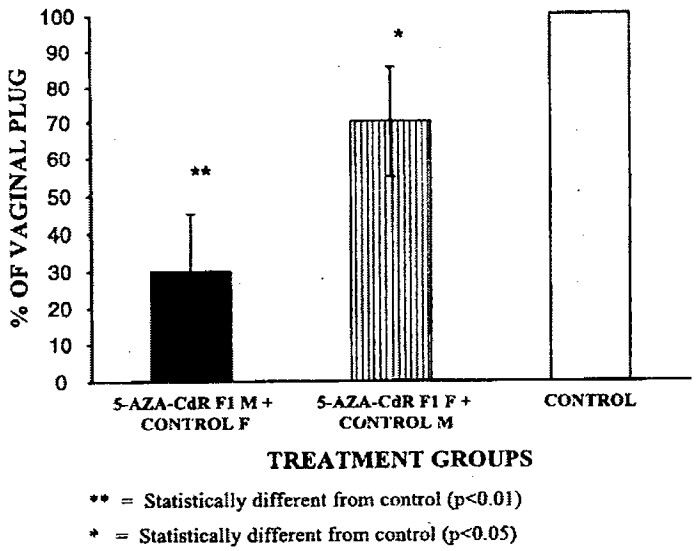
Methods

Doses: 1 mg/kg
Species/strain: CD-1 mice
Number/sex/group: 18 timed pregnant females
Route, formulation, volume: IP, sterile saline, 0.2 mL
Study design:

- Injection at 8 AM on gestation day 10
- Dose level based on average bw on GD 9
- Females allowed to deliver; weaned at D21

Results

- No maternal toxicity
- Decreased bw at weaning (M17%, F 11%, vs control); at 5 mo, males reduced 38%, females decreased but not statistically significant
- A reduction in fertility in males and females exposed to 5-aza CdR *in utero* and mated at 3 mo of age to untreated controls was noted (75 and 30%, respectively); see figure from publication below.



3. 5-AZA-2'Deoxycytidine (5-AZA-CdR): a demethylating agent affecting development and reproductive capacity.

Key study findings: Decitabine exposure decreased body weight in males and females and affected reproductive parameters in male mice exposed *in utero*.

Study no.: Cisneros FJ, and Branch S.
Volume # and page #: J Appl Toxicol 23: 115-120 (2003)
Conducting laboratory and location: Env Mol Toxicology Dept, NC State University
Date of study initiation: Publication date 2003
GLP compliance: no
QA reports: yes ( ) no (x) peer reviewed publication
Drug, lot #, and % purity: 5-aza-2'-deoxycytidine purchased from Sigma Chemical

Methods

Doses: 1 mg/kg
Species/strain: CD-1 mice
Number/sex/group: 16 timed pregnant females
Route, formulation, volume: IP, sterile saline, 0.2 mL
Study design:

- Injection gestation day 10
- Dose level based on average bw on GD 10
- Females allowed to deliver; weaned at D21

Parameters: body weight; reproductive capacity in 3 and 5 month old mice as determined by presence of vaginal plug; pregnancy rate in bred animals (determined on GD 17)

Results:

Average Body weights (g)

Age (days)	Male		Female	
	Control	Treated	Control	Treated
66	43.43	29.19	32.16	23.31
161	49.73	31.04	54.49	26.66
182	57.92	31.4		
230	53.77	30.45		

Reproductive Parameters

Mating	3 month of age		5 months of age	
	Vaginal plug	Pregnancy rate	Vaginal plug	Pregnancy rate
Rx M x C F	5/11	4/11	2/19	0/19
C M x Rx F	10/11	9/11	4/4	3/4
C M x C F	9/10	9/10	5/11	2/11

No treatment effects were seen in testes or epididymis, except for organ weight (possibly reflecting a decrease in spermatid heads per testes), including an evaluation of spermatid heads per mg/testes (not affected due to a decrease in absolute organ weight).

2.6.6.7 Local tolerance: Not studied

2.6.6.8 Special toxicology studies: None

2.6.6.9 Discussion and Conclusions: Decitabine (5-aza-2'-deoxycytidine) is an analog of the natural nucleoside 2'-deoxycytidine. It is phosphorylated to 5-azadeoxycytidine triphosphate, the active moiety. Decitabine inhibits DNA methyltransferases. In the repeat dose toxicity studies, the primary toxicity was hematological with testicular atrophy and decreased epididymal weights. Decitabine has genotoxic potential and may have carcinogenic potential. Decitabine demonstrated teratogenic effects in mice and rats. Similar adverse embryotoxic and teratogenic effects have been reported for other nucleoside analogues, such as cladribine (Teratology 2002; 66:6-18) and gemcitabine (Teratology 1993; 48:65-81).

2.6.7 TOXICOLOGY TABULATED SUMMARY

General Toxicology

Study	Route	MTD		Target organs of toxicity
		mg/kg/day	mg/m^2/day	
Rats: 3 x Daily on days 1-3, 29-31 & 57-59	IV infusion	1.2	7.2	Testes and epididymis,
Dogs: 3 x daily for 3 days	IV infusion	<1.2	<24	Bone marrow
Rabbits: 3 x daily for 3 days every six weeks for 4 cycles of treatment	IV infusion	<0.75	<9	Bone marrow and testes

Genetic Toxicology

Study	Concentration or Dose	Result		
		Positive control	No metabolic (-S9)	Plus metabolic (+S9)
Bacterial reverse mutation assay	33-2000 µg / plate	Yes	Not definitive	Not definitive
In vitro TK mouse lymphoma cells	10-5000 µg/mL	Yes	Positive	Positive
In vivo Lac I transgenic mice	1 mg/kg bw weekly for 14 weeks by sc injection	No	Mutations in the transgene in colonic DNA were mostly C:G→G:C transversion, C:G→A:T transversion and C:G→T:A transition.	
Drosophila melanogaster larvae	0.025-0.25 mM (~6-60 mg/mL)	No	↑ in mitotic recombination	
CH310T1/2 clone 8 mouse embryo fibroblast and V79 cells	0.03-1 µM and 0.03-10 µM (~0.007-2 µg/mL)	Yes	No reproducible increase in ouabain resistance	
BHK-21cl13 cells	0.2-2 µM (~0.05-0.5 µg/mL)	No	No increase in resistance to ouabain or 6-thioguanine	

Reproductive Toxicology

Study	Route	Duration	Dose	Result
Fertility and early embryonic development	IP	3x/wk for 7 weeks	0.05, 0.1, 0.15 mg/kg	From 0.1 mg/kg: ↓ sperm counts and disordered germ cell associations; when mated to untreated females, ↓ pregnancy rate and ↑ preimplantation loss

Study	Route	Duration	Dose	Result
Embryofetal development, mouse	IP	Single injection on gestation day 8, 9, 10, or 11	0.3 or 1.0 mg/kg	No maternal toxicity Gestation Day 8 0.3 mg/kg ↓ avg fetal wt; ↑ axial skeletal defects Gestation Day 9 0.3 mg/kg ↑ cleft palate; 1.0 mg/kg ↓ avg fetal wt; ↑ axial skeletal defects Gestation Day 10 1.0 mg/kg ↑ axial skeletal defects; cranial defects; ↑ appendicular skeletal defects, including forelimb and hindlimb digits Gestation Day 11 1.0 mg/kg ↑ tail defects, appendicular defects of fore- and hindlimb digits
Embryofetal development, mouse	IP	Single dose on GD 10	0.05, 0.1, 0.2, 0.3, 1.0, 2.0, or 3.0 mg/kg	0.3 mg/kg ↑ skeletal and cranial defects including hindlimb; 1.0 mg/kg ↓ fetal wt, # ossification sites; 2 mg/kg ↓ # live/litter; 3 mg/kg no live fetuses
Embryofetal development, rat	IP	Single dose on GD 9, 10, 11, or 12	0.4, 0.6 or 1.0 mg/kg	No maternal toxicity; Gestation Day 9 No live fetuses at D20 in any dose group Gestation Day 10 0.4 mg/kg ↑ axial skeletal defects; 0.6 mg/kg ↓ live fetuses, average fetal wt; ↑ appendicular skeletal defects; 1 mg/kg ↑ cranial defects Gestation Day 11 0.4 mg/kg ↑ axial skeletal defects; 0.6 mg/kg ↓ average fetal wt; 1.0 mg/kg ↑ appendicular skeletal defects; cranial defects Gestation Day 12 0.6 mg/kg ↑ appendicular skeletal defects 1.0 mg/kg ↓ average fetal wt; ↑ axial skeletal defects; cranial defects

Study	Route	Duration	Dose	Result
Prenatal and postnatal development, mouse	IP	Single dose GD 10	1 mg/kg	No maternal toxicity; ↓ bw at weaning for males and females, remaining ↓ for males at 5 mo of age; ↓ fertility when mated to untreated animals at 3 months of age (75 and 30% vs C in males and females, respectively)
Prenatal and postnatal development, mouse	IP	Single dose GD 10	1 mg/kg	No maternal toxicity; ↓ in body weights vs C in both males and females offspring at D66 (first reported time point); ↓ in fertility and pregnancy rate when males exposed *in utero* were bred at 3 or 5 monts of age to unexposed females; these parameters were not altered in females exposed *in utero* vs unexposed animals.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Product is approvable from pharmacology/toxicology point of view. There are no outstanding issues.

Unresolved toxicology issues (if any): None

Recommendations: This NDA is approvable from a pharmacology perspective.

Suggested labeling: Separate review

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS: NONE

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/s/

Anwar Goheer
6/22/05 12:28:16 PM
PHARMACOLOGIST

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