CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-088

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Clinical Pharmacology Review

NDA	22-088
Submission Date:	5 October 2006
Brand Name:	Torisel™
Generic Name:	temsirolimus
Formulation:	25 mg/mL [redacted] injection
OCP Reviewer:	Julie M. Bullock, Pharm.D.
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OCP Division:	Division of Clinical Pharmacology V
ORM Division:	Division of Drug Oncology Products
Sponsor:	Wyeth
Submission Type; Code:	Original NDA; 000
Dosing regimen:	25 mg IV over 30-60 mins once weekly
Indication:	Treatment of advanced renal cell carcinoma

OCP Briefing held on March 7, 2007 attended by: Julie Bullock, Yaning Wang, Brian Booth, Atik Rahman, Larry Lesko, Dennis Bashaw, Mehul Mehta, Suresh Doddapaneni, Kelli Reynolds, Qi Liu, Derek Zhang, Hao Zhu, Chris Tornoe, Atul Bahttaram, Roshni Ramchandani, Christine Garnett, Leslie Kenna, Ting Ong, Amna Ibrahim, Ginni Kwitkowski, and Assad Noory.

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1 EXECUTIVE SUMMARY

Temsirolimus is an inhibitor of the human kinase mammalian target of rapamycin (mTOR). The current submission is the original NDA for temsirolimus for the treatment of advanced renal cell carcinoma.

To support the approval in advanced renal cell carcinoma, the sponsor conducted one phase 2 and one phase 3 study. Patients in the phase 2 study were randomized to receive 25 mg, 75 mg or 250 mg of temsirolimus IV once weekly. Objective response rate was the primary endpoint and ranged from 5 to 8%. These results indicate that higher doses do not provide further efficacy benefits and therefore the 25 mg/weekly dose was chosen for evaluation in the phase 3 trial.

In the phase 3 study patients were randomly assigned to receive temsirolimus alone, temsirolimus plus interferon, or interferon alone. Preliminary results indicated that the median overall survival rate in the temsirolimus alone arm (25 mg IV once weekly) was 10.9 months. The combination arm and the interferon alone arm had median overall survival rates of 8.4 and 7.3 months respectively.

Based on the results from the drug-drug interaction studies with rifampin and ketoconazole, labeling changes were made to reflect dose adjustments for concomitant administration of CYP3A4 inhibitors and inducers in the Dosage and Administration section. Our recommendations included a dose decrease of 50% for co-administration with potent inhibitors such as ketoconazole. The sponsor proposed a dose increase of 50% when concomitantly administered with potent CYP3A4 inducers, the agency agrees, so this statement was further clarified in the Dosage and Administration section.

The sponsor has two studies currently ongoing, a hepatic study and a thorough QT study. The completion of both studies are to be phase 4 commitments.

1.1 RECOMMENDATIONS

The Office of Clinical Pharmacology/Division of Clinical Pharmacology 5 has reviewed the information contained in NDA 22-088. This NDA is considered acceptable from a clinical pharmacology perspective.

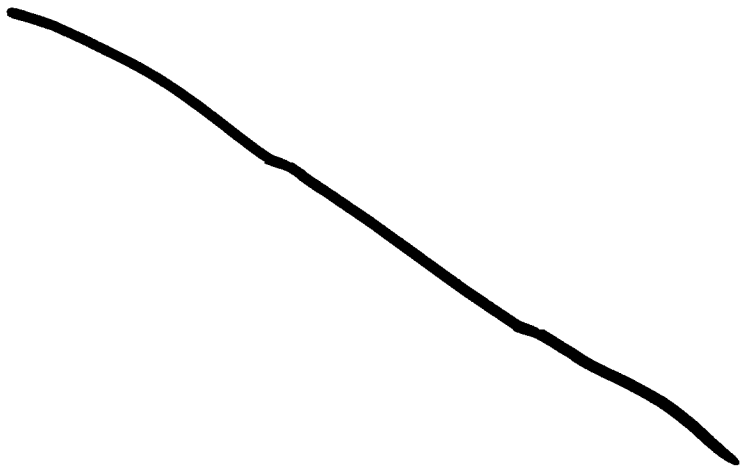
Phase IV commitments

1. Submit the completed report for the thorough QT prolongation evaluation and datasets for study entitled "A single-dose, single-blind, placebo and moxifloxacin controlled 2-period, randomized, crossover, 3rd-period sequential study of the effects of temsirolimus on cardiac repolarization in healthy subjects"
2. Submit the completed report and datasets for the hepatic impairment study (NCI study 6813 (3066K1-152-US)).

Labeling Recommendations

Please refer to Section 3 - Detailed Labeling Recommendations

1. The following should be added under the Dosage and Administration section:



Comments:

1. Since the in vitro studies suggest that temsirolimus is a P-glycoprotein substrate you may wish to consider conducting an in vivo study with a P-glycoprotein inhibitor.
2. Since the in vitro studies suggest that temsirolimus is a P-glycoprotein inhibitor you may wish to consider conducting an in vivo study with digoxin.

Signatures:

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1.2 CLINICAL PHARMACOLOGY SUMMARY

Temsirolimus, and its major metabolite sirolimus, block activity of the human kinase mammalian target of rapamycin (mTOR) and is being developed for intravenous use in the treatment of advanced renal cell carcinoma (RCC).

The applicant has conducted several phase 1 studies in healthy volunteers and patients with solid tumors and advanced renal cell carcinoma to evaluate the safety and pharmacokinetics of temsirolimus and its metabolite sirolimus. The oral bioavailability of temsirolimus is poor, therefore temsirolimus is administered intravenously. The Tmax of temsirolimus typically occurs at the end of infusion, and following administration the concentrations of temsirolimus decreased in a poly exponential manner with a half-life of approximately 20 hours after a 25 mg IV dose. The pharmacokinetics of temsirolimus are less than dose proportional with increasing doses, however, sirolimus exposures increased proportionally with dose. There are no significant differences between the pharmacokinetics in healthy volunteers and patients. After administration of radio-labeled temsirolimus, 78% of the total radioactivity was eliminated in the feces. The percentage un-changed drug excreted in the feces is unknown. The primary pathway of elimination of temsirolimus is NADPH-dependent via CYP3A4. Sirolimus was the active metabolite found in blood and plasma and in-vitro studies indicate that temsirolimus and sirolimus exhibit comparable degrees of biologic activity. Drug-drug interaction studies indicate an 56% reduction in sirolimus exposure (AUC), and no effect on temsirolimus exposure when administered with rifampin. Coadministration of IV temsirolimus with ketoconazole did not effect the exposure of temsirolimus but increased the exposure (AUC) of sirolimus by 3.1-fold. In-vitro, temsirolimus was an inhibitor of CYP2D6 and 3A4, however desipramine concentrations were not effected by co-administration with temsirolimus in-vivo, therefore no inhibition of CYP3A4 of expected.

Results from one phase 1 study in healthy volunteers and one phase 2 study in patients with renal cell carcinoma were conducted to support dose selection and dose-response. The phase 1 study in healthy male subjects investigated the biochemical activity of temsirolimus based on a biomarker (p-S6 ribosomal protein activation status). A dose-response relationship was demonstrated for the dose range of 1 - 25 mg, and these results were used to support dose selection for Phase 2. Data from the phase 2 study indicate no-dose dependence for objective response rate, time to tumor progression, and progression-free survival over the exposure range between 25 mg and 250 mg suggesting that higher exposures under higher doses do not afford additional survival or response benefits as compared with the 25-mg dose. A pooled exposure/safety analysis was done using logistic regression and significant relationships between drug exposure and various adverse events were seen.

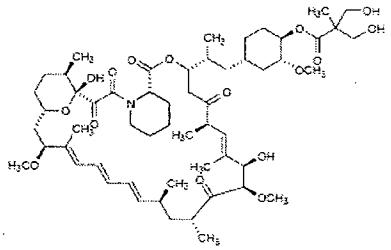
2 QUESTION BASED REVIEW

2.1 GENERAL ATTRIBUTES

2.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?

Physico-chemical properties

1. Structural formula:



2. Established name: temsirolimus
3. Molecular Weight: 1030.30
4. Molecular Formula: $C_{56}H_{87}NO_{16}$
5. Chemical Name: Rapamycin, 42-[3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate

2.1.2 What are the proposed mechanisms of action and therapeutic indications?

Temsirolimus is an inhibitor of the mammalian target of rapamycin (mTOR), an enzyme that regulates cell growth and proliferation. It exerts its effect on cell proliferation by inhibiting mTOR-dependent protein translation induced by growth factor stimulation of cells. Inhibition of mTOR prevents progression from G1 to S phase of the cell cycle.

2.1.3 What are the proposed dosage and route of administration?

The recommended dosing regimen of single-agent temsirolimus for the treatment of advanced RCC is 25 mg IV once weekly.

2.2 GENERAL CLINICAL PHARMACOLOGY

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

Five studies in healthy volunteers and nine studies in cancer patients were completed to support the clinical pharmacology and biopharmaceutics portion of the NDA. Below in Table 1 is the list of studies in healthy volunteers and Table 2 contains the studies in patients with cancer.

TABLE 1. Studies supporting the clinical pharmacology and biopharmaceutics of temsirolimus in healthy volunteers

Study Number	Study Description	Number Enrolled	IV Dose Range
3066K1-133-US	Phase 1, open-label, nonrandomized, parallel-group study to evaluate mass balance and metabolic profile of temsirolimus IV and PO routes.	12	25 mg
3066K1-145-US	Phase 1, open-label study to quantify the temsirolimus exposure/response relationship using S6 ribosomal protein in blood.	30	1 to 25 mg
3066K1-148-US	Phase 1, open-label, nonrandomized, 2-period sequential study to evaluate effect of CYP3A4 inhibition on temsirolimus PK.	17	5 mg
3066K1-149-US	Phase 1, open-label, nonrandomized, 2-period sequential study to evaluate effect of temsirolimus on CYP2D6 metabolism.	26	25 mg
3066K1-151-US	Phase 1, open-label, nonrandomized, 2-period sequential study to evaluate effect of CYP3A4 induction on temsirolimus PK.	16	25 mg
3066K1-155-US[a]	Phase 1, single-blind, randomized, 3-period sequential study to evaluate effect of temsirolimus on the QT interval.	60[b]	25 mg

a - Data analysis is ongoing, data was not included in this submission
b - 48 subjects planned, 60 actually enrolled.

TABLE 2. Studies supporting the clinical pharmacology and biopharmaceutics of temsirolimus in patients with cancer.

Study Number	Study Description	Number Enrolled	IV Dose Range
3066K1-100-US	Phase 1, open-label, 2-part dose-escalation study to determine MTD in patients with advanced solid tumors (daily for 5 days every 2 weeks).	63 (pt1) 25 (pt2)	0.75 to 37 mg/m^2
3066K1-101-EU Part 1 and Part 2	Phase 1, open-label 2-part dose-escalation study to determine MTD in patients with advanced solid tumors (once weekly regimen).	24 (pt1) 16 (pt2)	7.5 to 220 mg/m^2
3066K1-103-EU	Phase 1, open-label, dose-escalation study to determine MTD in patients with advanced solid tumors receiving concomitant 5-FU/LV.	28	15 to 75 mg/m^2
3066K1-104-US	Phase 1, open-label, 2-part study to compare bioavailability of IV and PO formulations and to determine MTD of PO formulation in patients with advanced solid tumors.	24	5 to 20 mg
3066K1-124-US	Phase 1, open-label, dose-escalation combination study with IFN to determine MTD in patients with advanced RCC.	71	5 to 25 mg
3066K1-131-JA	Phase 1, open-label, dose-escalation study to determine MTD in Japanese patients with advanced solid tumors.	10	15 to 45 mg/m^2
3066K1-152-US (NCI study 6813)	Phase 1, open-label, nonrandomized, parallel-group study to evaluate the PK and PD of temsirolimus in patients with cancer and varying degrees of hepatic impairment (Interim).	14 ongoing to 66	15 to 25 ongoing to 175 mg
3066K1-200-US	Phase 2, randomized, blinded, parallel-group, dose-ranging study for efficacy, safety, and population PK in patients with advanced RCC.	111	25, 75, 250 mg
3066K1-203-EU Breast cancer	Phase 2, randomized, open-label, parallel-group, dose-ranging study to evaluate efficacy, safety, and population PK in women with advanced or metastatic breast cancer.	109	75, 250 mg

Data from three studies in patients with RCC were conducted to support the efficacy claim in advanced renal cell carcinoma. Descriptions of these studies are below.

Phase 1 study

Study 3066K1-124-US was a open-label study that evaluated the safety, tolerability and maximum tolerated dose (MTD) of temsirolimus in combination with IFN in patients with advanced RCC. IFN was administered subcutaneously three times a week starting with week 1.

Temsirolimus was given once weekly on a non-IFN day starting with the second week of treatment. Seventy-one patients were enrolled in the study and 39 were treated at the MTD. Six dose levels of the combination were evaluated and temsirolimus 15 mg was determined to be the MTD when given in combination with IFN. The temsirolimus 15 mg plus INF 6 MU dose was concluded to be the safest combination for further study. The primary endpoint of this study was safety and tolerability. A secondary objective was to obtain preliminary information on the antitumor activity of temsirolimus + IFN. Intensive PK sampling was obtained in this study.

Phase 2 study

Study 3066K1-200-US was a randomized, blinded, parallel-group, dose-ranging study of temsirolimus in patients with advanced renal cell carcinoma. One hundred eleven patients with previously treated advanced RCC were enrolled and randomly assigned to receive 25, 75 or 250 mg of temsirolimus once weekly. The primary efficacy endpoint was objective response rate (ORR). The secondary efficacy endpoints included OS and PFS. The ORR was 5.6% in the 25-mg dose group, 7.9% in the 75-mg dose group, and 8.1% in the 250-mg dose group. No patient had a complete response in this study. The clinical benefit rate (percentage of patients with complete response, partial response, minor response, or stable disease for at least 24 weeks) was 52.8% in the 25-mg dose group, 55.3% in the 75-mg dose group, and 43.2% in the 250-mg dose group. The median OS for all patients was 15 months and there was no statistically significant differences in the distribution of survival times observed between dose groups. Sparse PK was obtained in this study and a subset of 16 subjects had intensive PK sampling collected before the first and fourth weekly temsirolimus doses.

Pivotal Phase 3 study

Study 3066K1-304-WW is an ongoing, randomized, open-label, active-controlled study comparing temsirolimus alone to temsirolimus + IFN or IFN alone in patients with first-line, poor-prognosis advanced RCC. The study is ongoing and 626 patients have been enrolled and randomly assigned to receive one of the following treatments:

- IFN SC 3 times weekly (max dose of up to 18 MU)
- Temsirolimus 25 mg IV once weekly
- Or temsirolimus 15 mg once weekly with IFN SC 3 times weekly (max dose of up to 6 MU)

The primary efficacy endpoint is OS. Preliminary results indicate that the median OS is 7.3 months in the IFN arm, 10.9 months in the temsirolimus 25 mg arm, and 8.4 months in the temsirolimus 15 mg/IFN arm. The temsirolimus 25 mg arm showed a 49% increase in median OS over the IFN arm, and the combination arm a 15% increase over the IFN arm. Secondary efficacy endpoints included PFS, OR, duration of response, clinical benefit rate and time to treatment failure (TTF).

2.2.2 What is the basis for selecting the response endpoints or biomarkers and how are they measured in clinical pharmacology and clinical studies?

Overall survival was the primary efficacy endpoint for the Phase 3 study (304). Objective response rate was the primary efficacy endpoints the Phase 2 study (204). Study 124 obtained preliminary data on the anti-tumor activity as a secondary objective.

TABLE 3. Efficacy endpoints in clinical studies of temsirolimus in RCC patients.

Endpoint	Definition		
	Study 304	Study 200	Study 124
Overall survival (OS)	Date of randomization to date of death, censored at last date known alive. Patients are followed until death.	Date of initial temsirolimus treatment to date of death, censored at last date known alive. Patients in long-term follow up are followed until death.	Date of initial temsirolimus treatment to date of death, censored at last date known alive. There is no formal long-term follow up for survival.
Progression-free survival (PFS)	Date of randomization to the date of progression or death (whichever is earlier), censored at the date of last tumor assessment.	Date of initial temsirolimus treatment to the date of progression or death (whichever is earlier), censored at the date of last tumor assessment.	
Objective response rate (ORR)	Percentage of patients with confirmed complete response (CR) or partial response (PR) based on modified RECIST criteria.	Percentage of patients with CR or PR based on WHO or modified WHO criteria.[a]	Percentage of patients with CR or PR based on modified RECIST criteria.
Clinical benefit rate	Percentage of patients with confirmed CR, PR, or stable disease (SD) for at least 24 weeks based on modified RECIST criteria.	Percentage of patients with CR, PR, minor response (MR), or SD for at least 24 weeks based on WHO or modified WHO criteria.[a,b]	Percentage of patients with CR, unconfirmed partial response (uPR), PR, or SD for at least 24 weeks based on modified RECIST criteria.

Abbreviations: CR = complete response; MR = minor response; ORR = objective response rate; OS = overall survival; PFS = progression-free survival; PR = partial response; RCC = renal cell carcinoma; RECIST = Response Evaluation Criteria in Solid Tumors; SD = stable disease; uPR = unconfirmed partial response; WHO = World Health Organization.

2.2.3 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

The major active metabolite of temsirolimus is sirolimus. Therefore both the plasma and whole blood concentrations of temsirolimus and sirolimus were measured by HPLC/MS/MS [redacted] for all studies. In early development, separate assays were used to measure temsirolimus and sirolimus in whole blood or plasma. Later in development, a combined assay was developed in which both temsirolimus and sirolimus were simultaneously measured. This newer assay was split into 2 methods to quantify 2 differing concentration ranges (low range and high range). For the fundamental PK descriptions of temsirolimus, whole blood was consistently used because temsirolimus and sirolimus exhibited preferential distribution in blood cells, and because frozen stability was limited when stored in plasma.

2.2.4 Exposure-response

2.2.4.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy?

A dose-response relationship was demonstrated for temsirolimus biochemical activity based on a biomarker (p-S6 ribosomal protein activation status in the blood) in healthy male subjects for the dose range of 1 mg to 25 mg single dose. Different results, however, were observed in the two cell types selected, CD3+ and CD19+, with CD3+ showing more prolonged response with larger magnitude (Figure 1). These results were used to support dose selection.

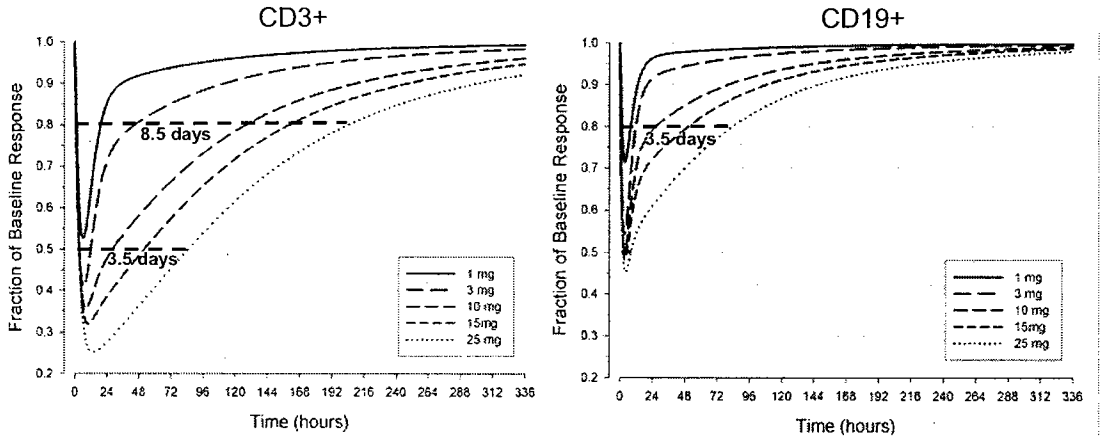


FIGURE 1: Dose-Response for Temsirolimus Biochemical Activity

In the phase 2 dose ranging study in RCC patients, no dose-dependence was observed for objective response rate outcomes among 25 mg, 75 mg and 250 mg (Tables 4 and 5).

TABLE 4. Tumor Response Rates by Protocol Definition in the ITT Population (N, %)

	Treatment Group			Total
Response	**25 mg (n=36)**	**75 mg (n=38)**	**250 mg (n=37)**	**(N=111)**
Complete response	0	0	0	0
Partial response	2 (5.6)	1 (2.6)	3 (8.1)	6 (5.4)
Minor response	5 (13.9)	14 (36.8)	10 (27.0)	29 (26.1)
Stable disease [a]	13 (36.1)	11 (28.9)	9 (24.3)	33 (29.7)
Progressive disease	13 (36.1)	10 (26.3)	11 (29.7)	34 (30.6)
Unknown / no data	3 (8.3)	2 (5.3)	4 (10.8)	9 (8.1)

a: Duration of SD had to be at least 8 weeks ± 1 week.

TABLE 5. Objective and Clinical Benefit Tumor Response Rates by Protocol Definition in the Evaluable Patient Population (N=105)

Treatment Group	**Objective Rate n (%; 95% CI)**	**Clinical Benefit Rate n (%; 95% CI)**
Overall (N=111)	6 (5.4; 2.0–11.4)	68 (61.3; 51.6–70.4)
25 mg (n=36)	2 (5.6; 0.7–18.7)	20 (55.6; 38.1–72.1)
75 mg (n=38)	1 (2.6; 0.1–13.8)	26 (68.4; 51.4–82.5)
250 mg (n=37)	3 (8.1; 1.7–21.9)	22 (59.5; 42.1–75.3)

Similar results were observed for time to tumor progression, progression-free survival and overall survival (Tables 6, 7 and 8).

TABLE 6. Time to Tumor Progression Using the Protocol Definition

	Treatment Group			
Value	**25 mg (n=36)**	**75 mg (n=38)**	**250 mg (n=37)**	**Total (N=111)**
Number (%) of patients whose tumors progressed	33 (92)	35 (92)	34 (92)	102 (92)
Median, months	3.5	3.7	3.6	3.6
95% confidence intervals	1.9–5.1	3.5–6.0	3.4–4.5	3.5–4.1
Progression-free rate at 8 weeks, %	67	74	77	73
Progression-free rate at 24 weeks, %	25	41	23	30

TABLE 7. Progression-Free Survival Information by the Protocol Definition

temsirolimus Treatment Group	**N with Progression or Who Died**	**Median months (95% CI) to progression or death**	**Progression-free survival rate at 8 (24) weeks**
Overall (N=111)	104	3.60 (3.49–4.01)	70.91% (28.85%)
25 mg (N=36)	33	3.54 (1.91–5.13)	66.67% (25.00%)
75 mg (N=38)	36	3.63 (3.49–5.86)	71.05% (39.30%)
250 mg (N=37)	35	3.60 (3.42–4.24)	75.00% (22.22%)

TABLE 8. Patient Survival Information

	Treatment Group			
Value	**25 mg (n=36)**	**75 mg (n=38)**	**250 mg (n=37)**	**Total (N=111)**
Median, months	12.5	11.0	17.3	15.0
95% confidence interval	9.0 – 18.7	8.6 – 18.6	12.0 – 19.3	10.4 – 18.3
Number of deaths, n (%)	24 (67)	25 (66)	23 (62)	72 (65)

Exposure-survival analysis further showed that after correction for survival risk factors, no dose-dependent survival relationship was detected within the exposure range achieved between 25 mg and 250 mg, suggesting that higher exposures under higher doses do not afford additional survival benefit as compared with the 25-mg dose.

2.2.4.2 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for safety?

Various adverse events (AE) were found to be exposure-dependent. A pooled exposure/safety analysis included four studies in healthy subjects and four studies in patients taking various temsirolimus doses (1 mg to 25 mg for healthy subjects and 5 mg to 460 mg for patients). Logistic regression modeling was used to explore the association between PK exposure versus AE occurrence and severity.

In healthy subjects, the Cmax of temsirolimus following single doses was associated with the occurrence and severity of acne and mucositis. Acne, anorexia, pruritis and rash were related to increasing drug exposure in patients.

In patients, a dose-ranging study (25 mg, 75 mg and 250 mg) showed that the occurrence of

nausea was significantly dose-dependent.

An exploratory exposure/safety analysis in the PK subgroup indicated a significant relationship between drug exposure and various AEs, such as thrombocytopenia, pruritus, hyperlipemia, acne, infection, mucositis, macropapular rash/rash, nail disorder, hyperglycemia, anorexia, headache and diarrhea. Despite the exploratory nature of this analysis and the relatively small sample size (N=50), these findings are consistent with the dose/concentration dependent AEs reported in sirolimus product label.

2.2.4.3 Does this drug prolong the QT or QTc interval?

Given the relatively modest number of patients who received QT monitoring in Phase 3 and Phase 1 studies, a potential effect of temsirolimus on the QT/QTc interval cannot be conclusively ruled out. A more definitive analysis regarding QT prolongation will be completed once the results of the ongoing thorough QT study in healthy volunteers are submitted (Study 155).

For the current submission the sponsor provided an analysis of the QT/QTc data from the following studies:

- Phase 3 study in advanced RCC (Study 304)
- Phase 3 study in Mantle Cell Lymphoma (Study 305)
- Phase 1 mass balance study in healthy volunteers (Study 133)
- Phase 1 dose escalation study in healthy volunteers (Study 145)

A summary of the important QT findings from the phase 3 studies is below in Table 9. There were no significant QT findings in the Phase 1 studies. More detail regarding the QT data submitted can be found in Appendix 4.2 - QT Review.

TABLE 9. Incidence of potentially clinically important values in QTcF interval in Study 304 and 305

	INF	TEMSR 25 mg	TEMSR 15mg/IFN	TEMSR 175/25 mg	TEMSR 175/75 mg
First Dose (n)	**8**	**15**	**11**	**26**	**26**
QTcF Interval >450 msec (♂) or >470 msec (♀)		1			3
Increase from baseline 0-30 msec	5	6	6	8	5
3 Months post dose (n)		**5**	**3**	**7**	**8**
QTcF Interval >450 msec (♂) or >470 msec (♀)					2
Increase from baseline 0-30 msec		3	1	3	4
Increase from baseline >30-60 msec					1
Increase from baseline >60 msec		1			
Withdrawal Visit (n)	**4**	**10**	**5**	**10**	**7**
QTcF Interval >450 msec (♂) or >470 msec (♀)				1	1
Increase from baseline 0-30 msec	1	2	1	1	4
Increase from baseline >30-60 msec	1	2	1	1	1
Increase from baseline >60 msec			1		

2.2.4.4 Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

The applicant reported that the 25 mg IV weekly dosing regimen employed in the Phase 2 trials proved to be acceptable based on both efficacy and safety.

2.2.5 Pharmacokinetic characteristics of the drug and its major metabolites

2.2.5.1 What are the single dose and multiple dose PK parameters?

Figure 2 shows the mean concentration time profiles of temsirolimus and sirolimus in whole blood obtained from healthy volunteers following single IV doses of temsirolimus at 1, 3, 10, 15 and 25 mg (study 145). Both temsirolimus and sirolimus exhibit less than dose proportional increases in exposure following increasing doses.

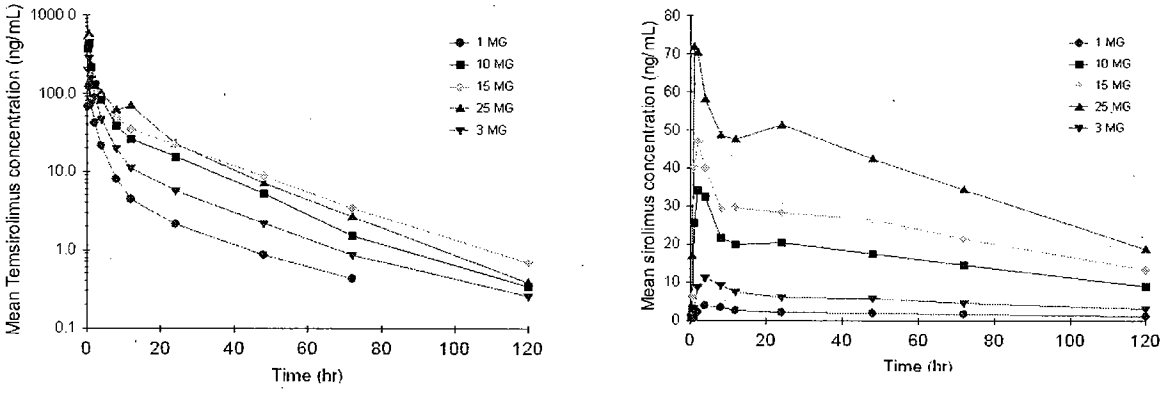


FIGURE 2: Mean temsirolimus (left) and sirolimus (right) concentration vs. time profiles after temsirolimus 1-mg, 3-mg, 10-mg, 15-mg and 25-mg single IV doses in healthy volunteers (Study 145).

After a single temsirolimus dose, concentrations in blood decreased poly-exponentially with an average half-life of 20 hours following a 25-mg dose (see Figure 3). Sirolimus was the major observed metabolite in whole blood and measurable concentrations of sirolimus were detected 15 minutes following the start of the temsirolimus infusion. For sirolimus, mean Cmax values were approximately 10- to 16-fold lower than respective values of temsirolimus. Following a single dose of temsirolimus, concentrations of sirolimus decreased in an apparent monoexponential fashion and the mean half-life of sirolimus is approximately 60 hours after a 25-mg dose.

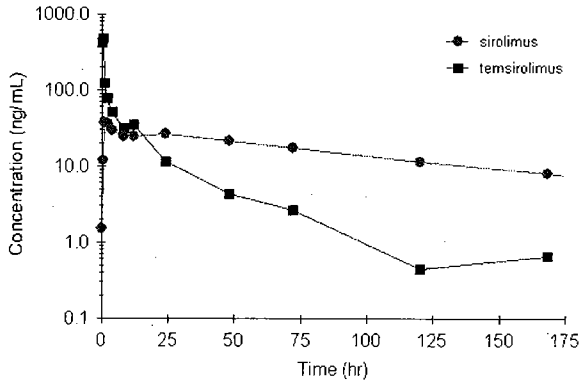


FIGURE 3: Mean temsirolimus and sirolimus concentration vs. time profiles after temsirolimus a 25-mg single IV dose in healthy volunteers (Study 145).

Table 10 summarizes the pooled summary of single and multiple dose PK parameters in patients

with cancer receiving temsirolimus weekly. Values of temsirolimus AUC ratio (SD:MD) were near unity and indicate little or no accumulation with multiple weekly treatments, and may suggest slightly lower values with multiple-dose treatment. Similar results were found for sirolimus, indicating little or no accumulation following weekly treatments with temsirolimus 25 mg IV. The contribution of sirolimus metabolite to total exposure was substantial albeit variable with mean $AUC_{sirolimus}$:$AUC_{temsirolimus}$ values ranging from approximately 2- to 5-fold following the first dose, and 3- to 8-fold following the 3rd dose.

TABLE 10. Pooled summary of single- and multiple-dose pharmacokinetic parameters in whole blood following a 25 mg intravenous temsirolimus dose in patients with cancer.

	Single-dose (N=13)[a]	Multiple-dose (N=11)[b]
Temsirolimus		
Cmax (ng/mL)	585.4 ± 83.1	443.0 ± 109.2
t1/2 (h)	17.3 ± 5.9	NC
AUC (ng·h/mL)	1627 ± 425	1349 ±231.8
CL (L/h)	16.2 ± 3.5	19.0 ± 3.0
Vdss	172.3 ± 39.4	NC
Sirolimus		
Cmax (ng/mL)	55.4 ± 31.8	34.5 ± 19.3
t1/2 (h)	54.6 ± 1.5	NC
AUC (ng·h/mL)	4151 ± 1600	3793 ± 1466
CL/fm (L/h)	6.9 ± 2.6	7.4 ±2.5
Composite		
AUCratio[c]	2.68 ± 1.22	2.98 ±1.47
AUCsum (ng·h/mL)	5778 ±1722	5141 ± 1345

a. For Cmax N=5 and for t1/2 and Vdss N=2.
b. For temsirolimus Cmax N=7, sirolimus Cmax N=3
c. AUC ratio denotes quotient of sirolimus: temsirolimus AUCs
Abbreviation: NC = Not calculated. No subjects provided data.

2.2.5.2 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

To compare the pharmacokinetics between healthy subjects and patients with cancer, a pooled analysis of data with the 25 mg IV dose was conducted. Due to the nonlinear nature of temsirolimus disposition, the analysis was limited to observations in healthy volunteers (n=51) and patients (n=13) with cancer following the 25-mg IV dose.

As seen below in Table 11, in general, the pharmacokinetics seen in healthy volunteers were similar to those in patients. The clearance of temsirolimus and sirolimus was faster in healthy subjects than in patients resulting in higher AUCs for healthy subjects. Variability was similar between the two populations.

TABLE 11. Pooled Summary of Single- and Multiple-Dose Pharmacokinetic Parameters in Whole Blood of Healthy Subjects and Patients Following 25 mg Intravenous Temsirolimus

	Healthy Subjects Single-dose (N=51)	Patients Single-dose (N=13)[a]
Temsirolimus		
Cmax (ng/mL)	592.4 ± 101.9	585.4 ± 83.1
t1/2 (h)	17.7 ± 4.5	17.3 ± 5.9
AUC (ng·h/mL)	2276 ± 340	1627 ± 425
CL (L/h)	11.4 ± 2.4	16.2 ± 3.5
Vdss	189.6 ± 55.2	172.3 ± 39.4
Sirolimus		
Cmax (ng/mL)	57.4 ± 14.3	55.4 ± 31.8
t1/2 (h)	73.3 ± 23.2	54.6 ± 1.5
AUC (ng·h/mL)	5479 ± 1799	4151 ± 1600
CL/fm (L/h)	4.9 ±1.2	6.9 ± 2.6
Composite		
AUCratio[c]	2.44 ± 0.83	2.68 ± 1.22
AUCsum (ng·h/mL)	7755 ± 1874	5778 ±1722

a. For Cmax N=5 and for t1/2 and Vdss N=2.
c. AUC ratio denotes quotient of sirolimus: temsirolimus AUCs
Abbreviation: NC = Not calculated. No subjects provided data.

2.2.5.3 What are the characteristics of drug absorption?

Following a 30-minute IV infusion of 25 mg, the Cmax of temsirolimus was observed by the end of infusion period. Levels of sirolimus were seen immediately (15 mins after infusion) and the median Tmax was 2 hours in healthy volunteers and patients.

2.2.5.4 What are the characteristics of drug distribution?

Protein Binding

The in vitro protein binding of [^{14}C]temsirolimus in male human plasma was determined using erythrocyte partitioning (RPT-62965). Erythrocyte partitioning was used because previous studied have demonstrated that temsirolimus is unstable in plasma from multiple species including humans and is most stable in whole blood of humans. Based on the concentrations observed in clinical IV pharmacokinetic studies concentrations of 10, 100, and 1000 ng/mL of [^{14}C]temsirolimus in erythrocyte suspensions were selected.

The percentages of [^{14}C]temsirolimus bound to proteins at concentrations of 10 and 100 ng/mL when calculated for undiluted human plasma in erythrocyte suspensions were 85.0% and 87.1%, respectively.

The binding of [^{14}C]temsirolimus to proteins in human plasma proteins could not be determined at an erythrocyte suspension concentration of 1000 ng/mL by the erythrocyte partitioning method because the capacity of [^{14}C]temsirolimus to associate with the erythrocytes in suspension was exceeded.

The apparent concentration-dependent partitioning of [^{14}C]temsirolimus in erythrocyte

suspensions suggests that non-linear blood to plasma partitioning may result at higher blood temsirolimus concentrations. Temsirolimus binding to plasma proteins at these concentrations may be different than at lower concentrations.

Blood/Plasma Ratio (C_{rbc}/C_p)

The extent of blood partitioning of temsirolimus was determined in human whole blood (RPT-39803). At a drug concentration of 100 ng/mL and at an incubation time of 30 minutes at 37°C, the blood to plasma concentration ratio (C_{blood}/C_{plasma}) for temsirolimus was 3.4 ± 0.1. The C_{blood}/C_{plasma} ratios of temsirolimus remained unchanged within the drug concentration range of 20 and 100 ng/mL.

The blood to plasma concentration ratios (C_{blood}/C_{plasma}) of sirolimus was 6.3 at drug concentration of 100 ng/mL and at an incubation time of 5 minutes at 37°C. These results indicate that the extent of blood to plasma partitioning for temsirolimus appeared to be less than that for sirolimus.

In-vivo it was shown that with increasing dose the blood-to-plasma ratio for temsirolimus decreased approximately to unity (see Figure 4). This behavior also translated into dose-dependent changes in both Vdss and CL, and resulted in saturation of specific binding in the blood. Data from phase 1 studies in cancer patients indicate that the mean Vdss increases with increasing dose (57 L following 2-mg dose vs. 898 L following a 250-mg dose).

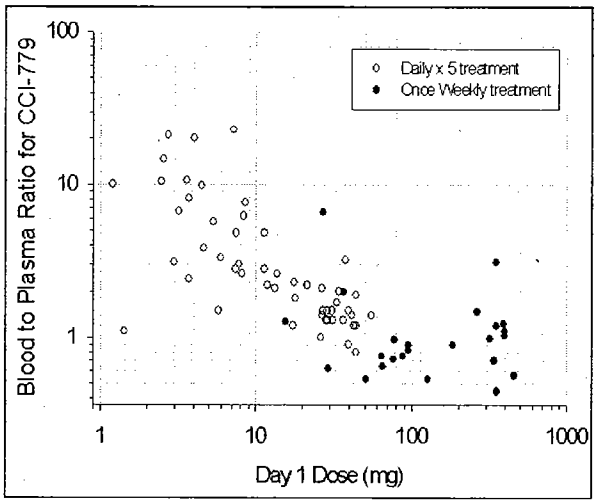


FIGURE 4: Temsirolimus Blood-to-Plasma ratio versus dose (Studies 100 and 101).

2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination?

Six healthy male subjects received a single IV dose of 25-mg [^{14}C]-temsirolimus containing 50 µCi of total radioactivity (Study 133). The 78.1% of total radioactivity was recovered in the feces after 14 days and only 4.5 % was recovered in the urine.

2.2.5.6 What are the characteristics of drug metabolism?

The human whole blood, plasma, urine and fecal samples from the ADME study (Study 133) in

healthy volunteers were analyzed for metabolic characterization (RPT-62848). Sirolimus, the active metabolite, was identified as the major species contributing to circulating total radioactivity, with minor contributions by the inactive metabolites; [redacted] seco-temsirolimus, hydroxy-temsirolimus (M10), and desmethyl temsirolimus.

The percentage of unchanged temsirolimus and sirolimus in the urine and feces was not characterized due to the polar material which was could not be analyzed by LC/MS.

In whole blood, the temsirolimus/sirolimus peak represented at least 84% of the radioactivity in all samples analyzed. The only other metabolite that was observed in all patients was [redacted] representing on average approximately [redacted] of the radioactivity at each time point. Seco-temsirolimus was present but represented less than 5% of the radioactivity.

The biotransformation pathway of temsirolimus is shown below in Figure 5. CYP3A4 is responsible for the metabolism of temsirolimus and sirolimus.

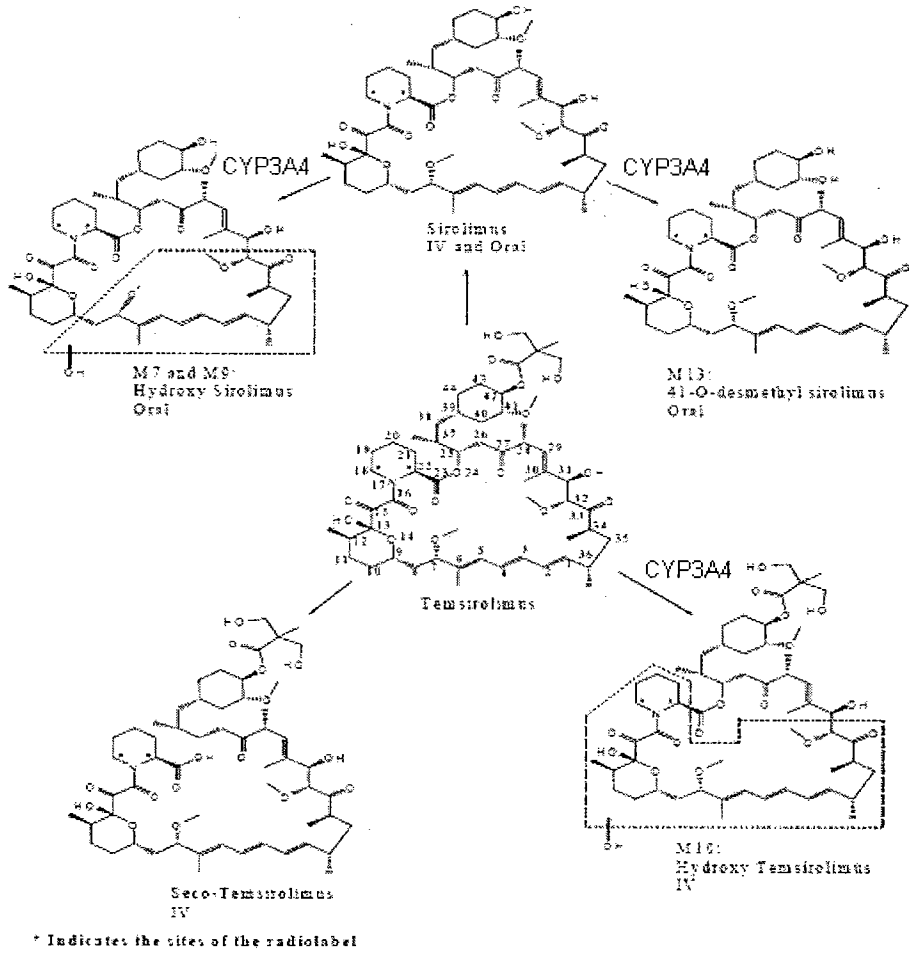


FIGURE 5: [^{14}C]-Temsirolimus-Related Compounds Detected in Human Blood Following Intravenous Administration

2.2.5.7 What are the characteristics of drug excretion?

Route of Elimination

Fecal excretion is the major route of elimination of temsirolimus. Over 14-days post a 25-mg [^{14}C]-temsirolimus IV dose in the human ADME study the majority (78.1 ± 7.38 %) of the total radioactivity was recovered in the feces and only 4.55% was recovered in the urine.

Clearance

The mean (SD) clearance of temsirolimus from whole blood following the 25-mg IV dose was 11.4 (2.4) L/h in healthy subjects and 16.2 (3.5) L/h in patients. Inter-patient variability at a fixed dose was low. Across temsirolimus studies and varying IV doses, clearance was dose-dependent, increasing with dose presumably due to the saturable distribution in blood and tissues.

Half-life

Temsirolimus half-life among healthy subjects and cancer patients was comparable with mean (SD) values of 17.7 (4.5) h in healthy subjects, and 17.3 (5.9) h in cancer patients. For sirolimus, the mean (SD) half-life was 73.3 (23.2) h for healthy subjects and 54.6 (1.5) h for cancer patients. The slightly longer half-life seen in healthy subjects may reflect the more extensive terminal phase sampling schedules attainable in healthy subjects as compared to cancer patients, or the higher hematocrit relative to cancer patients.

2.2.5.8 Based on PK parameters, what is the degree of linearity or non-linearity based in the dose-concentration relationship?

In study 145 single IV doses of temsirolimus ranging from 1 to 25 mg via 30-minute infusion were evaluated in healthy subjects. The pharmacokinetic results indicate that the Cmax and AUC of temsirolimus increased with increasing dose in a less than proportional manner (see Figure 6). This behavior was apparent in the other various clinical studies that have been performed with temsirolimus.

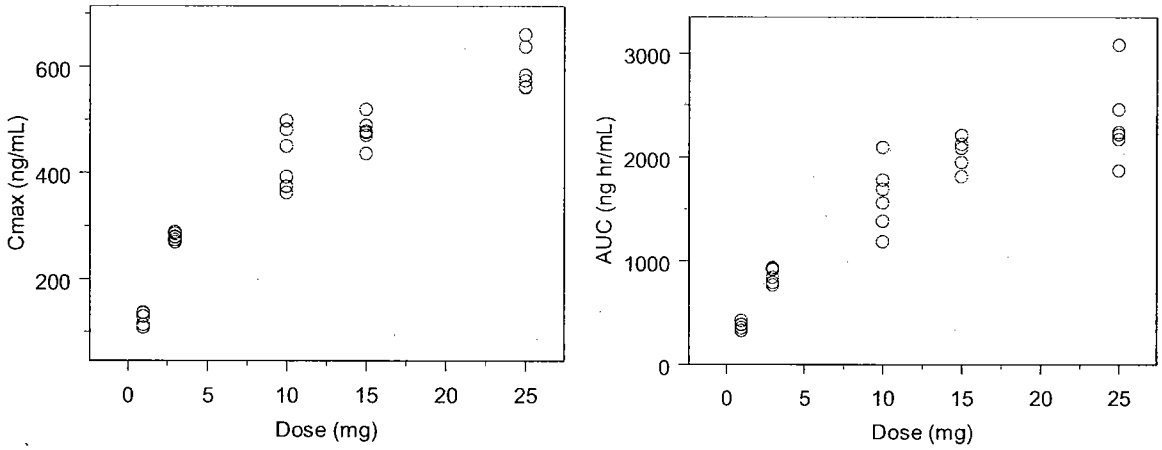


FIGURE 6: Temsirolimus Cmax and AUC in whole blood versus dose in healthy subjects.

The results for sirolimus indicate that the Cmax and AUC increase proportionally with dose (see Figure 7). This is consistent with previous findings for sirolimus (see Rapamune® label or Kofi Kumi, Ph.D. review of NDA 21-083 for Sirolimus (rapamycin) oral solution)

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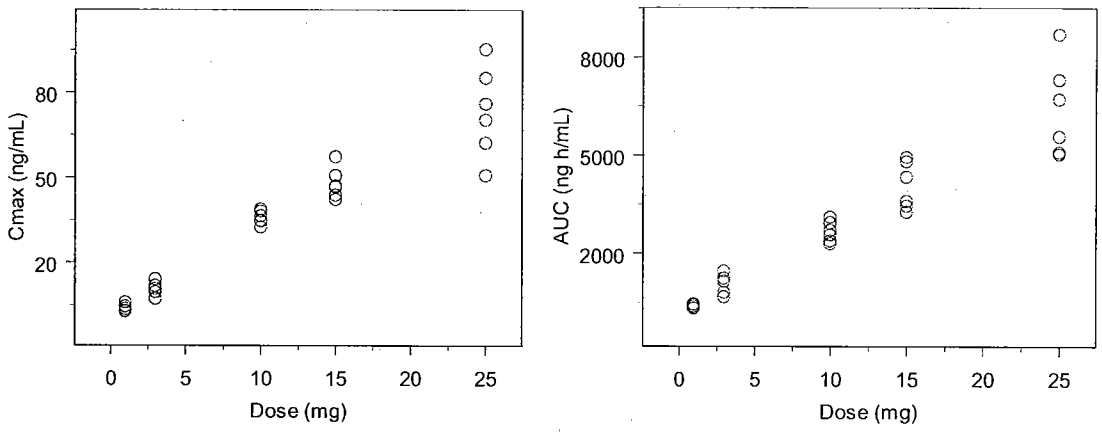


FIGURE 7: Sirolimus Cmax and AUC in whole blood versus dose in healthy subjects.

2.2.5.9 How do the PK parameters change with time following chronic dosing?

No accumulation of temsirolimus or sirolimus is seen after multiple doses. Following multiple weekly doses of temsirolimus exposure is either similar to or less than those seen following the first dose (see Table 12).

TABLE 12: Single and multiple dose (mean (CV%)) pharmacokinetic parameters of temsirolimus and sirolimus in patients with cancer (Study 101).

Dose (mg/m^2)	N	Cmax (ng/mL)	tmax (h)	t1/2 (h)	AUCt (ng.h/mL)	AUC (ng.h/mL)	Cl (L/h)	Vss (L)
Temsirolimus								
Week 1								
34	3	2200	0.24	22.3	3203	3221	19.059	241.50
		(36.3)	(4.7)	(27.2)	(25.2)	(25.1)	(14.1)	(6.5)
45	4	1368	0.58	17.3	3367	3414	27.016	384.62
		(18.2)	(60.4)	(6.9)	(42.0)	(42.2)	(27.2)	(15.7)
Week 4								
34	2	1410	0.38	17.7	2421	2460	26.360	440.14
		(23.1)	(47.1)	(1.6)	(20.9)	(20.5)	(21.6)	(31.1)
45	3	1457	0.33	17.0	2570	2601	32.196	420.35
		(24.8)	(43.3)	(9.5)	(17.5)	(17.9)	(5.5)	(6.7)
Sirolimus								
Cycle 1								
34	3	125	0.83	69.2	7676	8753	7.01	622
		(51.9)	(34.7)	(7.2)	(35.0)	(25.2)	(14.2)	(29.3)
45	4	126	2.26	60.8	9748	11740	7.77	697
		(19.6)	(111.4)	(7.1)	(40.3)	(36.3)	(27.4)	(17.7)
Subsequent Cycle								
34	2	126	3.50	40.5	7919	8468	7.67	498
		(19.1)	(101.0)	(1.8)	(22.9)	(22.3)	(21.3)	(25.5)
45	3	126	10.33	50.8	6977	9161	9.21	704
		(13.6)	(121.6)	(16.1)	(36.6)	(20.2)	(12.4)	(26.2)

Results from Study 200 show the trough concentrations at the end of treatment 4 were lower than the values from treatment week 1 (see Figure 8).

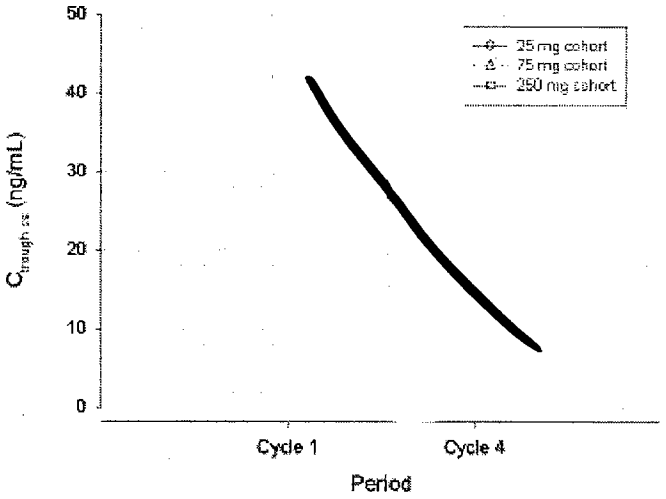


FIGURE 8: Individual patient trough concentrations of sirolimus in whole blood following various intravenous temsirolimus doses (Study 200).

The lower or comparable exposures after multiple doses may occur as a result of saturation of peripheral tissue specific binding which leads to increased amounts of free or unbound drug that were available for distribution and elimination.

2.2.5.10 What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

Variability between patients with cancer ranged between 20-40% for pharmacokinetic measures of exposure. For healthy volunteers the CV% for Cmax and AUC ranged from 2-20%. The increase in variability seen in patients may include practice variability across the large number of clinical sites including; concomitant medications, variability in dosing and sampling times and increased variability in subjects underlying disease status and hematocrit levels compared to healthy volunteers.

2.3 INTRINSIC FACTORS

2.3.1 What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

A population PK analysis (POP1) performed by the sponsor identified body surface area as a significant factor to influence temsirolimus' clearance and hematocrit as a significant factor to influence sirolimus' volume of distribution. Despite the identified intrinsic factors for PK, the efficacy and safety responses were not found to be influenced.

Another population PK analysis (POP2) identified race as a significant factor to influence temsirolimus' clearance using a more complex model. Several limitations, however, made the results from POP2 less reliable.

2.3.2 Based upon what is known about exposure-response relationships and their variability and the groups studied, healthy volunteers vs. patients vs. specific populations, what dosage regimen adjustments, if any, are recommended for each of these groups? If dosage regimen adjustments are not based upon exposure-response relationships, describe the alternative basis for the recommendation.

No dose adjustment is recommended.

2.3.2.1 Pediatric patients

There were no pediatric studies included in the current submission.

2.3.2.2 Renal impairment

Given that <4% of a dose of temsirolimus is eliminated renally, adjustments for renal impairment do not appear necessary.

2.3.2.3 Hepatic impairment

A study investigating the effect of hepatic impairment on the PK of temsirolimus and its metabolites is ongoing (Study 152). The following dose escalation schema is being used:

Group Liver Function	Group A Normal	Group B Mild	Group C Moderate	Group D Severe	Group E Liver Transplant
Level –1	15 mg	15 mg	15 mg	15 mg	15 mg
Starting Level 1	25 mg (6 patients)	25 mg (6 patients)	25 mg (6 patients)	25 mg (6 patients)	25 mg (6 patients)
Level 2	175 mg (6-12 patients)	175 mg (6-12 patients)	75 mg (3 patients)	75 mg (3 patients)	75 mg (3 patients)
Level 3			175 mg (6-12 patients)	125 mg (3 patients)	125 mg (3 patients)
Level 4				175 mg (6-12 patients)	175 mg (6-12 patients)

Preliminary data from 14 subjects was submitted with the NDA and can be found below in Table 13 and 14.

The preliminary data indicate that temsirolimus Cmax increases as the level of hepatic impairment increases at both the 15 and 25 mg dose. The same is seen for AUC. No observable trends can be discerned from the sirolimus results. Since only a few patients with hepatic impairment have data available it is hard to conclude at this time what the proper dose reduction should be for patients with varying degrees of hepatic impairment.

TABLE 13: Preliminary pharmacokinetic parameters of temsirolimus in patients with hepatic impairment following treatment with IV temsirolimus (study 152)

Dose (mg)	Day	Class	N	Cmax (ng/mL)	tmax (h)	t1/2 (h)	AUC (ng·h/mL)	CL (L/h)	Vdss (L)
25	1	Normal	4	562 (36)	0.5 (0.5, 0.5)	21 (22)	2408 (12)	10.5 (13)	237 (16)
		B1-Mild	2	401 (63)	0.75 (0.5, 1)	24.6 (36)	2546 (17)	10 (17)	231 (13)
		B2-Mild	2	1005 (54)	0.5 (0.5, 0.5)_	23 (38)	3504 (35)	7.6 (35)	141 (25)
15	1	B1-Mild	3	320 (0.7)	0.5 (0.5, 0.5)	26 (32)	1965 (19)	7.8 (17)	184 (15)
		B2-Mild	1	477	0.50	19.1	1625	9.23	162
		Moderate	1	304	0.50	28.0	3162	4.74	153
		Severe	1	640	0.50	28.2	2359	6.36	149
25	8	Normal	4	431 (15)	0.5 (0.5, 0.5)	23 (35)	1937 (12)	13.0 (11)	367 (45)
		B1-Mild	2	659 (41)	0.5 (0.5, 0.5)_	21 (19)	2129 (34)	12.5 (34)	276 (34)
15	8	B1-Mild	2	386 (5)	0.5 (0.5, 0.5)	22.6 (11)	2454 (33)	6.4 (33)	228 (15)
		B2-Mild	1	365	0.50	20.8	1285	11.7	235
		Severe	1	688	0.50	29.5	2256	6.65	147

TABLE 14: Preliminary pharmacokinetic parameters of sirolimus in patients with hepatic impairment following treatment with IV temsirolimus (study 152)

Dose (mg)	Day	Class	N	Cmax (ng/mL)	tmax (h)	t1/2 (h)	AUC (ng·h/mL)	CL (L/h)	Vdss (L)
25	1	Normal	4	63 (44)	1 (1, 24)	63 (12)	9177 (37)	4.5 (37)	440 (37)
		B1-Mild	2	84 (15)	13 (2, 24)	92.8 (12)	12157 (14)	2.1 (14)	288 (6.4)
		B2-Mild	2	64 (3.0)	36.5 (1, 72)	75 (48)	9260 (62)	3.3 (62)	338 (11)
15	1	B1-Mild	3	22 (18)	6 (2, 24)	80 (6.5)	3054 (8.8)	4.9 (8.7)	608 (6.4)
		B2-Mild	1	31.5	2.0	72.7	2996	5.01	551
		Moderate	1	21.1	24	80.3	2866	5.23	621
		Severe	1	33.3	1.0	67.0	2583	5.81	575
25	8	Normal	4	84 (34)	1 (1, 1)	63 (22)	6876 (38)	4.1 (39)	373 (25)
		B1-Mild	2	105 (37)	24 (24, 24)	76 (7.2)	14413 (37)	1.8 (37)	215 (42)
15	8	B1-Mild	2	41 (16)	4 (6, 2)	107 (43)	5823 (26)	2.7 (26)	403 (13)
		B2-Mild	1	26.2	2.0	60.8	2795	5.37	518
		Severe	1	48.8	2.0	52.4	3147	4.77	376

2.3.2.4 What pregnancy and lactation use information is there in the application?

No data regarding the excretion of temsirolimus and its metabolites in the milk of humans of animals was provided. Embryo-fetal reproductive toxicity studies showed uterine and embryo-fetal toxicities in rats and rabbits at sub-therapeutic exposures and therefore temsirolimus is pregnancy category [redacted]

2.4 EXTRINSIC FACTORS

2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or -response and what is the impact of any differences in exposure on response?

There were no specific studies or analyses designed to evaluate the effects of factors such as herbal products, diet, smoking or alcohol use on the PK or PD of temsirolimus.

2.4.2 Drug-drug interactions

2.4.2.1 Is there an in vitro basis to suspect in vivo drug-drug interactions?

Since CYP3A4 is the major CYP isozyme responsible for the metabolism of temsirolimus and sirolimus, inhibitors and inducers of CYP3A4 could affect the pharmacokinetics of temsirolimus and sirolimus.

In vitro studies also suggest that temsirolimus is an inhibitor of CYP2D6 and 3A4 and therefore may influence the PK of substrates metabolized by those isozymes.

2.4.2.2 Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?

Temsirolimus undergoes NADPH-dependent biotransformation in human liver microsomes to a large number of drug-derived products(Report GTR-32279). The major metabolites are:

- demethylation to form various desmethyl temsirolimus metabolites (M2, M12 and M14) and desmethyl rapamycin metabolites(M1/M1', M11 and M13).
- Hydroxylation to produce several hydroxy temsirolimus metabolites (M6, M8 and M10) and hydroxy rapamycin metabolites (M5, M7 and M9).
- Low levels of dihydroxy or hydroxy-demethyl temsirolimus or rapamycin metabolites were also detected.

Seco-temsirolimus (M4), rapamycin and seco-rapamycin (M3) were formed from temsirolimus by a non-NADPH-dependent process.

The cytochrome (CYP) P450 isozyme(s) involved in the biotransformation of temsirolimus was initially investigated using microsomes prepared from human lymphoblastoid cells containing different cDNA-expressed CYP isozymes. Microsomes expressing CYP3A4 metabolized temsirolimus to products apparently similar to those characterized in human liver microsomes. Ketoconazole, a potent inhibitor of CYP3A4, markedly inhibited the NADPH-dependent metabolism of temsirolimus in human liver microsomes.

CYP450 isozymes responsible for temsirolimus metabolism in human liver microsomes were further investigated (Report GTR-36765) using chemical inhibitors specific for various CYP450 isozymes(see Table 15). In this study the metabolite names do not nessearily co-inside with the

identifiers above. For clarification; in Table 15 M3 and M6 are the demethyl metabolites, and M1, M2, M4 and M5 are hydroxylated metabolites.

TABLE 15. Inhibition of metabolism of Temsirolimus (25 μM) by CYP450 isozyme specific inhibitors in human liver microsomes.

Inhibitor	Inhibitor Concentration (μM)	CYP450 Isozyme Inhibited	Percent Control Activity for CCI-779 Metabolite Formation[a]					
			M1	M2	M3	M4	M5	M6
Furafylline[b]	10	CYP1A2	100	100	100	98.4	121	99.6
	50		97.5	101	100	97.8	100	107
7,8-Benzoflavone	1	CYP1A2	112	118	102	ND[c]	115	111
	10		110	112	88.1	ND	131	105
Sulfaphenazole	10	CYP2C9	98.7	107	92.2	104	77.8	99.0
	100		93.5	103	100	103	93.5	105
S-Mephenytoin	10	CYP2C19	108	111	104	112	114	110
	100		118	118	98.2	120	116	112
Quinidine	1	CYP2D6	103	97.1	89.4	101	103	102
	10		104	104	106	105	106	108
Diethyldithio-carbamate[b]	10	CYP2E1	77.9	88.5	103	92.8	96.9	101
	100		38.3	58.9	74.3	84.4	169	85.6
Ketoconazole	1	CYP3A4	19.3	24.3	28.9	26.8	129	59.2
	5		0	0.9	0	0	111	0
Troleandomycin[b]	10	CYP3A4	32.2	39.6	44.0	55.2	133	67.5
	50		24.3	31.1	46.9	52.8	141	59.5

a: Values are expressed as percent activity remaining relative to control incubations using the equation described in Section 3.4, n=2 for all incubations.
b: Indicates inhibitor is a mechanism-based inhibitor.
c: Value could not be determined because7,8-Benzoflavone peak interfered with detection of metabolite M4.

In conclusion, CYP3A4 is the major CYP450 isozyme responsible for the formation of five of the six NADPH dependant temsirolimus metabolites analyzed. The formation of M5 could not be conclusively associated with any of the CYP's examined.

2.4.2.3 Is the drug an inhibitor and/or an inducer of CYP enzymes?

In-vitro induction

The potential for temsirolimus to induce CYP3A4 was evaluated in a reporter gene assay using HepG2 cells transfected with the CYP3A4 promoter/enhancer and pregnane X receptor (PXR) plasmid DNA (Report RPT-58912). Temsirolimus did not induce CYP3A4 at a concentration of 20 μM (20,000 ng/mL), which is twice as high as the mean clinical Cmax, 10 μM (10513 ng/mL). The positive control, 10 μM Rifampicin, showed a 44 fold induction in the same system. Based on the FDA Drug-drug interaction guidance if temsirolimus is not an inducer of CYP3A4 then it can be concluded that it is not an inducer of 2C8, 2C9, or 2C19.

Based on the current label for Rapamune® sirolimus is not an inducer of CYP450 enzymes.

In-vitro inhibition

The potential for temsirolimus to act as a direct-acting reversible inhibitor of CYP enzymes was investigated using human liver microsomes (Report RPT-39412). Temsirolimus inhibited

CYP2C9, CYP2D6, and CYP3A4/5 activity, with values for the concentration resulting in 50% inhibition (IC50) of 14 μM (estimated), 2.2 μM, and 5.5 μM, respectively. Little or no inhibition of CYP1A2 or CYP2C19 activity was observed at a temsirolimus concentration of 12.5 μM, the highest drug concentration examined.

The inhibition constant (Ki) values for the inhibition of CYP3A4/5 (testosterone 6α-hydroxylation), CYP2D6 (bufuralol 1'-hydroxylation), CYP2C9 (diclofenac 4'-hydroxylation), and CYP2C8 (taxol 6α-hydroxylation) catalytic activity by temsirolimus were determined using human liver microsomes pooled from 6 subjects (Report RPT-45792). Using the average maximum blood concentration of 585 ng/mL (approximately 0.6 μM) after IV infusion of temsirolimus at the highest clinical dose of 25 mg, the ratios of Cmax/Ki were calculated and are listed below in Table 16.

TABLE 16. Ki values of Temsirolimus and Sirolimus for CYP Isoforms.

Isoform tested	Temsirolimus K_i (μM)	Sirolimus K_i (μM)	Temsirolimus C_{max}/K_i ratio [a]	Sirolimus C_{max}/K_i ratio [a]
CYP3A4/5	3.1	2	0.19	0.031
CYP2D6	1.5	5	0.38	0.013
CYP2C9	14	20	≤0.04	≤0.04
CYP2C8	27		≤0.04	

a. C_{max} follows 25-mg IV temsirolimus dose. K_i values assume C_{max} of 592 ng/mL and 57.4 ng/mL, for temsirolimus and sirolimus, respectively.

Temsirolimus may inhibit the metabolic clearance of drugs that are substrates for CYP3A4/5 or CYP2D6, but not for CYP2C9 or CYP2C8. Given the rank order of Ki values, the sponsor chose to conduct a CYP2D6 inhibition study to anticipate effects for CYP3A4/5.

In-vivo evaluation of inhibition

To investigate the inhibition potential of temsirolimus, a drug-drug interaction study with desipramine (a CYP2D6 substrate) was completed (Study 149). Twenty-six healthy adult subjects received the following two treatments under fasted conditions:

- a single 50-mg oral dose of desipramine on study days 1 and 15 (administration on Day 15 occurred at the start of the temsirolimus infusion).
- a single 25-mg IV dose of temsirolimus via 30-minute infusion on study day 15. Pretreatment with IV diphenhydramine (25 mg) was administered approximately 30 minutes before the start of temsirolimus infusion.

According to the FDA drug-drug interactions guidance a single dose of the inhibitor (temsirolimus) is adequate to produce appropriate inhibition, and desipramine is an appropriate substrate to use for CYP2D6.

Least squares geometric mean ratios between test and reference treatments, and their 90% CIs for Cmax, AUCT, and AUC were determined. The results are summarized below in Table 17. All subjects were genotyped prior to study entry and only one subject was considered a poor metabolizer by 2D6.

All subjects were included in the PK analysis. After excluding the poor metabolizer (Cmax 0.76

ng/mL; AUC 29.6 ng hr/mL) the intersubject variability for AUC decreased from 166 to 85%.

TABLE 17. Statistical analysis of desipramine Cmax, AUC and AUC_T with or without temsirolimus.

	Geometric Mean		LSGM Ratio	Lower	Upper
	Reference	Test	(% of Reference)	90% CI	90% CI
Desipramine					
Cmax (ng/mL)	17.60	15.07	87	81	93
AUC_T (ng hr/mL)	435	388	91	85	98
AUC (ng hr/mL)	570	528	96	85	109

Reference: single 50 mg oral dose of desipramine alone
Test: single 50-mg oral dose of desipramine coadministered with a single 25-mg IV dose of temsirolimus
Abbreviations: CI = confidence interval; LSGM = least squares geometric mean

The statistical analysis concludes that the 90% CI for the LSGM ratios of Cmax, AUC_T and AUC of desipramine were within the range of 80-125%. This suggests that temsirolimus does not inhibit CYP2D6 metabolism in humans. Based on the I/Ki values from in-vitro studies, if temsirolimus did not produce a clinically meaningful inhibition of 2D6 (I/Ki = 0.38) it can be extrapolated that temsirolimus would not inhibit CYP3A4/5 (I/Ki = 0.19) in-vivo.

2.4.2.4 Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?

The ability of temsirolimus to act as a substrate or inhibitor of P-glycoprotein (P-gp) was evaluated in vitro using Caco-2 cells (Report RPT-49956). Incubations were performed for 2 hours at 37°C with temsirolimus administered in the apical (A→B) or basolateral (B→A) compartment and results and are listed below in Table 18.

TABLE 18. Permeability Coefficient (P_{app}) of temsirolimus in modified Caco-2 monolayers.

temsirolimus (uM)	P_{app} (A to B), x 10^{-6} (cm/sec)	P_{app} (B to A), x 10^{-6} (cm/sec)	(B to A) / (A to B) Ratio
5	0.064 ± 0.003	5.08 ± 0.16	79
10	0.098 ± 0.005	5.76 ± 0.65	59
20	0.473 ± 0.009	5.58 ± 0.31	12
50	0.808 ± 0.031	1.99 ± 0.17	2
100	1.59 ± 0.13	0.63 ± 0.07	0.4

Verapamil (100 μM) a P-gp inhibitor, increased the amount of temsirolimus that permeated across the monolayers in the A→B direction by approximately four fold (8.7 ± 1.6 versus 2.1 ± 0.2 pmol/cm^2), while having little effect on the amount permeating in the B→A direction. The net flux ratio of temsirolimus in the presence of verapamil was 7.7 which is significantly reduced compared to the net flux ratio of temsirolimus alone (36). This suggests that temsirolimus is a P-gp substrate.

Inhibition of P-glycoprotein activity by temsirolimus was done using digoxin (a P-gp substrate) and the positive control verapamil (a P-gp inhibitor). Incubations were performed for 2 hours at 37°C with digoxin (5 μM) in the absence or presence of temsirolimus (10 or 100 μM) or verapamil (100 μM, n=3). Verapamil and temsirolimus were administered in the apical compartment. The results are below in Table 19 and suggest that temsirolimus may have the potential to alter the transport of agents that are P-gp substrates.

TABLE 19. Effect of temsirolimus on the permeability of digoxin across modified Caco-2 monolayers.

Treatment	P_{app} (A to B), x 10^{-6} (cm/sec)	P_{app} (B to A), x 10^{-6} (cm/sec)	P_{app} (B to A) / P_{app}(A to B) Ratio
digoxin	1.51 ± 0.16	103.00 ± 5.03	68
digoxin + temsirolimus (10 µM)	20.2 ± 2.06 *	59.4 ± 3.84	2.9
digoxin + temsirolimus (100 µM)	31.6 ± 0.86 *	55.1 ± 9.41	1.7
digoxin + verapamil (100 µM)	38.7 ± 4.36 *	81.5 ± 22.1	2.1

*. Denotes statistically significant difference ($p<0.05$) vs. digoxin in the absence of inhibitor.

2.4.2.5 Are there other metabolic/transporter pathways that may be important?

None have been identified.

2.4.2.6 Does the label specify co-administration of another drug and, if so, has the interaction potential between these drugs been evaluated?

It is recommended that an antihistamine, such as diphenhydramine, be administered before the temsirolimus infusion to avoid hypersensitivity reactions with the administration of temsirolimus. Early in the phase 1 program, a decision was made to pre-treat cancer patients and healthy subjects receiving temsirolimus IV with diphenhydramine. The interaction potential between these agents has not been formally studied.

2.4.2.7 Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

Co-administration of Temsirolimus with CYP3A4 Inhibitors

The effect of ketoconazole, a potent inhibitor of CYP3A4 on the PK of temsirolimus was investigated in Study 148. Seventeen subjects received the following treatments:

- A single 5-mg IV dose of temsirolimus via 30-minute infusion on study days 1 and 15 after an overnight fast.
- 400-mg oral dose (2 x 200-mg tablets) of ketoconazole with 240 mL water on study days 15 through 21,

Pretreatment with IV diphenhydramine (25 mg) was administered approximately 30 minutes before start of temsirolimus infusion on Days 1 and 15. On day 15, the ketoconazole dose was administered approximately 2 hours before the start of the temsirolimus infusion.

According to the FDA drug-drug interaction guidance, the study design and choice of CYP3A4 inhibitor are appropriate.

The 90% confidence intervals (CI) for LSGM ratios of Cmax, AUC_T, and AUC of temsirolimus were within the range of 80 to 125%. For sirolimus, respective LSGM ratio values of Cmax, AUC_T, and AUC were higher than the upper confidence limit of 125%, and yielded values of 223, 251, and 310%. The mean pharmacokinetic parameters can be found in Appendix 1 - Study 148 and the results of the statistical analysis is below in Table 20.

TABLE 20. Statistical analysis of 5-mg IV temsirolimus Cmax and AUC following treatment with or without ketoconazole 400 mg.

	LSGM Ratio (% of Reference)	Lower 90% CI	Upper 90% CI
Temsirolimus			
Ln (Cmax)	93.53	87.87	99.55
Ln (AUCT)	109.41	103.41	115.76
Ln(AUC)	110.19	105.22	115.4
Sirolimus			
Ln(Cmax)	223.69	197.52	253.32
Ln(AUCT)	251.57	232.29	272.44
Ln(AUC)	309.6	277.49	345.43

Reference: single dose temsirolimus IV 5 mg alone
Test: single dose temsirolimus IV 5 mg + ketoconazole oral 200 mg QD
Abbreviations: Ln = logarithmic normal; LSMG = least squares geometric mean.

Coadministration of IV temsirolimus with ketoconazole, a potent CYP3A4 inhibitor, increased the exposure of sirolimus by 2.2-fold and 3.1-fold for Cmax and AUC respectively. There was no significant difference in the AUC and Cmax of temsirolimus when administered with ketoconazole. The results of this study indicate that CYP3A4 inhibitors should be used with caution in patients taking temsirolimus. If the use of a strong inhibitor is unavoidable the dose of temsirolimus should be decreased by half to 12.5 mg weekly when administered with the inhibitor, and the temsirolimus dose should return to 25 mg once treatment with the inhibitor has ceased.

Co-administration of Temsirolimus with CYP3A4 Inducers

The effect of rifampin, a potent inducer of CYP3A4 on the PK of temsirolimus was investigated in Study 151. Thirty-two healthy adult subjects received the following treatments:

- Single oral (30 mg) or IV (25 mg) dose of temsirolimus on Days 1 and 21
- 600 mg rifampin on Days 16 - 27

According to the FDA drug-drug interaction guidance, the study design and choice of CYP3A4 inducer are appropriate.

Least squares geometric mean ratios between test and reference treatments, and their 90% CIs for Cmax, AUC_T, and AUC were determined. The mean pharmacokinetic parameters can be found in Appendix 1 - Study 151 and the results of the statistical analysis is below in Table 21.

TABLE 21. Statistical analysis of 25 mg IV temsirolimus Cmax and AUC following treatment with or without rifampin 600 mg.

	LSGM Ratio (% of Reference)	Lower 90% CI	Upper 90% CI
Temsirolimus			
Ln(Cmax)	107	93	123
Ln(AUCT)	91	87	96
Ln(AUC)	92	87	96
Sirolimus			
Ln(Cmax)	36	31	41
Ln(AUCT)	47	43	52
Ln(AUC)	44	39	49

Reference: single dose temsirolimus IV 25 mg alone
Test: single IV 25-mg dose of temsirolimus + rifampin oral 600 mg QD for 7 days after 6 days of rifampin pretreatment before the temsirolimus dose
Abbreviations: LSGM = least squares geometric mean; CI = confidence interval; Ln = logarithmic normal; Cmax = peak concentration; AUC = area under the concentration time-curve; AUCT = total area under the concentration-time curve evaluated to the last measurable timepoint; QD = once a day.

Co-administration of temsirolimus with rifampin lowered the exposure of sirolimus by 65% and 56% for Cmax and AUC respectively compared to when temsirolimus was administered alone. The exposures of temsirolimus following IV infusion were unchanged when rifampin was co-administered, while following PO administration the Cmax decreased by 41% and AUC decreased by 29%. Similar decreases were seen with sirolimus following PO administration of temsirolimus and rifampin (60% decrease in Cmax and AUC).

The sponsor suggests increasing the dose 50% up to 50 mg weekly in the presence of CYP3A4 inducers, this appears to be a reasonable recommendation based on the data available, and that doses of up to 250 mg are currently being given in Phase 2 trials

2.5 GENERAL BIOPHARMACEUTICS

2.5.1 Based on BCS principles, in what class is this drug and formulation? What solubility, permeability and dissolution data support this classification?

Not applicable

2.5.2 What is the composition of the to-be-marketed formulation?

Temsirolimus IV was supplied as a concentrate for injection provided in sterile glass ampoules or 25 mg [redacted] vials for the clinical trials.

The dosage form that was developed to support the early clinical trials (100, 101, 102, 103, 104, 124, 200, 201, 203) consisted of a solution of temsirolimus 25 mg/mL in dehydrated alcohol with citric acid [redacted] The drug concentrate was packaged in [redacted] glass ampules. A diluent was co-packaged with the drug concentrate and consisted of an aqueous solution of polysorbate 80, [redacted] polyethylene glycol [redacted] and water for injection.

For late stage clinical development (studies 145, 148, 149, 151, 124, 131, 200, 304) a modified drug product formula was developed. The modified product again consisted of a two-vial system of drug concentrate and diluent. The formula for the drug concentrate contained temsirolimus 25mg/mL, *dl*-alpha tocopherol [redacted] dehydrated alcohol and propylene glycol.

The clinical formulation will be a two-component dosage form is supplied to permit preparation of the intravenous admixture, packaged in [redacted] glass vials. Temsirolimus [redacted] Injection contains a mixture of propylene glycol, dehydrated alcohol (39.5% w/v), *dl, α*-tocopherol (0.075%, [redacted] and a small quantity of anhydrous citric acid (0.0025% [redacted]). The diluent consists of a mixture of polysorbate 80 (40% w/v), dehydrated alcohol (19.9% w/v) in polyethylene glycol 400.

2.5.3 What moieties should be assessed in bioequivalence studies?

Temsirolimus and the metabolite sirolimus should be assessed in human whole blood.

2.5.4 What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

Temsirolimus is administered intravenously therefore an evaluation of food effect is not necessary.

2.5.5 Has the applicant developed an appropriate dissolution method and specification that will assure in vivo performance and quality of the product?

Not applicable.

2.6 ANALYTICAL SECTION

2.6.1 Were relevant metabolite concentrations measured in the clinical pharmacology and biopharmaceutics studies?

[redacted] assayed whole blood and plasma samples from temsirolimus IV studies for concentrations of temsirolimus and sirolimus. In early development, separate assays were used to measure temsirolimus and sirolimus in whole blood or plasma. Later in development, a combined assay was developed in which both temsirolimus and sirolimus were simultaneously measured. This newer assay was split into 2 methods to quantify 2 differing concentration ranges (low range and high range).

2.6.2 Were the analytical procedures used to determine drug concentrations in this NDA acceptable?

Plasma and whole blood samples were assayed for temsirolimus and sirolimus in whole blood and plasma, by a validated HPLC/MS/MS method. [redacted] were used as internal standards.

The accuracy and precision of the analytical methods were < 15%. The freeze thaw stability in whole blood was 3 cycles, and long term stability at -20°C was 3 years. Short term stability was 24 hours at room temperature for whole blood. The long term stability of plasma samples was limited therefore the majority of the pharmacokinetic results are limited to whole-blood which is appropriate because temsirolimus and sirolimus exhibited preferential distribution in blood cells.

Please see appendix 4.3 for the bioanalytical methods that were used at each of the analytical sites, the quantitative range of the assays and the accuracy (% bias) and the precision (% coefficient of variation) of the assay quality control (QC) samples included for each study for temsirolimus and sirolimus.

3 Page(s) Withheld

_____ Trade Secret / Confidential

✓ Draft Labeling

_____ Deliberative Process

Withheld Track Number: Clin Pharm/Bio- 1

4 APPENDICES

4.1 INDIVIDUAL STUDY REVIEWS

4.1.1 152-US: Hepatic Impairment Study

This is an ongoing study. The final study report and conclusions will be submitted at a later date.

Title:

Investigators: [redacted]

Study Period: Date first subject enrolled: 29-Nov-2004
Date last subject completed: 09-Dec-2004

Clinical Phase:

Objectives: The primary objective of this ongoing study is to evaluate the safety and tolerability of temsirolimus IV, and to establish the recommended MTD, in patients with varying degrees of hepatic dysfunction in order to provide appropriate dosing recommendations for this population. Secondary objectives include characterizing the PK profile, PD profile, toxicities other than DLT, antitumor efficacy, and to compare the NCI Organ Dysfunction Working Group (ODWG) criteria and the Child-Pugh classification of hepatic dysfunction to reduce interpatient variability in exposure and response.

Summary: This study is currently being completed as part of the National Cancer Institute Cancer Therapy Evaluation Program (NCI/CTEP) in the US following an ongoing cooperative research and development agreement policy (CRADA) between Wyeth and the NCI. The study is planned to enroll patients into 5 cohorts (A: normal; B: mild dysfunction composed of subgroups B1 and B2; C: moderate dysfunction; D: severe dysfunction; and E: liver transplant) according to their hepatic function as described below:

Table 2.2.9.2-1: Hepatic Impairment Categories for Patient Cohorts (Study 3066K1-152-US)

Group Liver Function	Group A Normal	Group B Mild	Group C Moderate	Group D Severe	Group E Liver Transplant
Total Bilirubin	≤ ULN	B1: ≤ ULN B2: >1.0x-1.5x ULN	>1.5x–3x ULN	>3x ULN	Any
AST (SGOT)	≤ ULN	B1: > ULN B2: Any	Any	Any	Any

Abbreviations: AST = Aspartate aminotransferase; SGOT = Serum glutamic oxaloacetic transaminase; ULN = Upper limit of normal.

Dose escalation will be as follows:

Table 2.2.9.3-1: Pharmacokinetic Parameters of Temsirolimus in Patients With Hepatic Impairment Following Treatment With Intravenous Temsirolimus (Study 3066K1-152-US)

Class	Dose µg	Day	Subject	C_{max} ng/mL	t_{max} h	$t_{1/2}$ h	AUC ng·h/mL	AUC_{extrap} %	CL L/h	V_{dss} L
Normal	25000	1	1014	833	0.50	24.6	2722	0.53	9.18	231
			2004	598	0.50	16.2	2471	1.64	10.1	187
			3001	449	0.50	26.9	2441	0.77	10.2	273
			3002	369	0.50	19.3	1998	2.37	12.5	258
		8	1014	474	0.50	28.2	1870	0.70	13.4	316
			2004	392	0.50	16.7	1933	1.41	12.9	237
			3001	500	0.50	33.2	2261	2.59	11.1	304
			3002	358	0.50	16.6	1685	4.72	14.8	613
B1-Mild	15000	1	2001	319	0.50	16.5	1790	1.32	8.38	151
			2002	319	0.50	32.1	2401	1.61	6.25	199
			3003	323	0.50	30.3	1705	1.34	8.80	202
		8	2001	.	.	.	.	.	.	.
			2002	400	0.50	24.4	3038	5.14	4.94	203
			3003	373	0.50	20.8	1871	3.62	8.02	253
	25000	1	1003	583	0.50	18.3	2238	1.92	11.2	210
			1004	220	1.0	30.9	2854	1.14	8.76	253
		8	1003	465	0.50	18.2	1617	1.55	15.5	344
			1004	854	0.50	23.9	2642	0.45	9.46	208
B2-Mild	15000	1	2003	477	0.50	19.1	1625	1.95	9.23	162
		8	2003	365	0.50	20.8	1285	2.71	11.7	235
	25000	1	1001	621	0.50	29.1	4382	1.07	5.71	166
			1002	1390	0.50	16.7	2626	0.81	9.52	116
Moderate	15000	1	1009	304	0.50	28.0	3162	8.34	4.74	153
Severe	15000	1	1006	640	0.50	28.2	2359	0.74	6.36	149
		8	1006	688	0.50	29.5	2256	0.75	6.65	147

Table 2.2.9.3-2: Pharmacokinetic Parameters of Sirolimus in Patients With Hepatic Impairment Following Treatment With Intravenous Temsirolimus (Study 3066K1-152-US)

Class	Dose µg	Day	Subject	C_{max} ng/mL	t_{max} h	$t_{1/2}$ h	AUC ng·h/mL	AUC_{extrap} %	CL/f_m L/h	V_{dss}/f_m L
Normal	25000	1	1014	43.9	1.0	69.1	4143	19.80	6.03	631
			2004	35.9	24	51.7	4223	13.00	5.92	523
			3001	95.8	1.0	66.1	8407	18.26	2.97	299
			3002	75.3	1.0	62.1	7938	17.39	3.15	310
		8	1014	72.0	1.0	56.1	4269	13.26	5.86	501
			2004	48.9	1.0	47.3	4956	10.14	5.04	393
			3001	111	1.0	73.0	8704	19.61	2.87	302
			3002	105	1.0	78.1	9576	22.94	2.61	298
B1-Mild	15000	1	2001	23.7	24	78.2	3041	23.74	4.93	582
			2002	17.3	6.0	86.1	3328	31.59	4.51	653
			3003	24.9	2.0	76.3	2793	21.28	5.37	589
		8	2001	.	.	.	.	.	.	.
			2002	36.8	6.0	141	6916	43.91	2.17	442
			3003	46.1	2.0	74.2	4730	23.52	3.17	365
	25000	1	1003	93.2	2.0	101	13593	32.30	1.87	275
			1004	75.3	24	84.6	10922	27.36	2.29	301
		8	1003	134	24	72.2	18209	21.05	1.37	151
			1004	77.3	24	80.0	10617	24.14	2.35	280
B2-Mild	15000	1	2003	31.5	2.0	72.7	2996	21.27	5.01	551
		8	2003	26.2	2.0	60.8	2795	16.59	5.37	518
	25000	1	1001	65.8	72	101	13332	36.72	1.88	311
			1002	63.1	1.0	49.6	5189	10.09	4.82	365
		8	1001	.	.	.	.	.	.	.
			1002	.	.	.	.	.	.	.
Moderate	15000	1	1009	21.1	24	80.3	2866	43.65	5.23	621
Severe	15000	1	1006	33.3	1.0	67.0	2583	17.82	5.81	575
		8	1006	48.8	2.0	52.4	3147	11.41	4.77	376

4.1.2 203-EU: Phase 2 single-agent study in Advanced Breast Cancer

Title: Randomized, 2-dose level, open-label, phase 2 study of intravenous temsirolimus administered once weekly to patients with locally advanced or metastatic breast cancer.

Investigators: This was a multicenter trial with 14 sites in Europe.

Study Period: Date first subject enrolled: Oct-2000
Date last subject completed: Apr-2003

Clinical Phase: 2

Objectives: The PK objective of this study was to evaluate the population PK parameters of temsirolimus following treatment with 2 dose levels of temsirolimus when administered to patients with advanced or metastatic breast cancer. A supplementary objective was to evaluate a possible PD relationship with clinical response.

Design: This was a randomized, 2 dose-level, open-label, multicenter trial of temsirolimus in adult female patients with locally advanced or metastatic breast cancer. Eligible patients were randomized in a 1:1 ratio to receive 75 mg or 250 mg of IV temsirolimus once weekly for 6 months or until evidence of disease progression was observed. The number of patients planned was 50 in each of the 2 study arms. Overall, 109 were enrolled and 106 patients were treated.

Formulation: The following batches of temsirolimus were used in this trial:

Table 6.4.2-1: Study Medication (Temsirolimus) Batch Numbers

Strength/Dosage	Batch Number
Temsirolimus, 25 mg/1mL/ampoule	2000B0277, 2000B0349, 2000B0383, 2000B0432,2001B0020
Temsirolimus, 125 mg/5 mL/ampoule	1999B0168, 1999B0178, 1999B0184, 2000B0348, 2000B0389, 2001B0153

Pharmacokinetics: Whole blood for measurement of temsirolimus and sirolimus concentrations was collected during cycles 1 and 4 in 79 patients. In a subset of 11 patients, samples were collected at 0, 0.5, 1, 2, 6, 24, 72, 96, and 168 hours after start of infusion during weeks 1 and 4. In the remaining 68 patients, samples were collected at 0 hours during weeks 1 and 4, and at one other time during week 4.

Pharmacokinetic Analysis: Concentration data for temsirolimus were analyzed using a 3-compartment population PK model with zero-order input for temsirolimus, and a 2-compartment model with first-order input for sirolimus. Modeling was performed using NONMEM.7 Covariates for demography and clinical laboratory parameters were examined as explanatory factors for exposure variability. Covariates included WT, BSA, AGE, total bilirubin (TBIL), creatinine (CREA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and HCT.

Safety: All patients in this study reported treatment-emergent adverse events (TEAEs), and all patients experienced at least 1 drug-related TEAE. The most common TEAEs were mucositis (71%), rash/maculopapular rash (53%), nausea (51%), asthenia (45%), and anorexia (41%). These were also the most common drug-related TEAEs. The most common grade 1 and 2 adverse events were mucositis (69%), nausea (51%), rash (51%), and asthenia (42%). Grade 3 or 4 TEAEs were reported for 77 patients (73%) and drug related grade 3 or 4 TEAEs for 58

patients (55%). The most common NCI grade 3 or 4 TEAEs were mucositis (9%); leukopenia (8%); dyspnea and gamma glutamyl transpeptidase (GGT) increased (8% each), asthenia and hyperglycemia (7% each); and infection, pain, depression, and somnolence (6% each). Mucositis (9%) and leukopenia (7%) were also the most common drug-related grade 3 or 4 event, along with hyperglycemia (7%), GGT increased (6%), and somnolence (6%).

Assay Method: Temsirolimus and sirolimus were measured simultaneously in whole blood and plasma using a validated liquid chromatography–tandem mass spectroscopy (LC/MS/MS) technique.

Results: Blood samples from 79 patients were obtained, of which 247 observations from 74 patients were incorporated in the temsirolimus final model; 328 observations from 73 patients were included for sirolimus. The final model for temsirolimus included an effect for dose on clearance and on central volume of distribution, and a weighting factor for inter-compartmental clearance. The final model for sirolimus incorporated an effect for dose on central volume of distribution, and for HCT on inter-compartmental clearance.

Factors affecting exposure variability were limited to dose number and weight for temsirolimus, and dose number and hematocrit for sirolimus. Patient age and other observed clinical laboratory parameters did not contribute significantly to exposure variability.

Concentrations at the end of the treatment week following the 75 mg dose ranged from 1.7 to 4.9 ng/mL for temsirolimus (a 2.9-fold range), and from 9.3 to 14 ng/mL for sirolimus (a 1.5-fold range). Following the 250 mg dose, end-of-cycle concentrations were approximately 1.5-fold to 1.9-fold higher for temsirolimus (less than proportional) and 3.4-fold higher for sirolimus (proportional) compared to the 75 mg dose. In general, higher body weight appeared to be associated with higher temsirolimus concentrations by the end of the cycle.

4.1.3 200-US: Phase 2 single-agent study in Advanced RCC

Title: A randomized, double-blind, phase 2 study of intravenous temsirolimus administered weekly to patients with advanced renal cell carcinoma

Investigators: This was a multicenter trial with six sites in the United States.

Study Period: Date first subject enrolled: 11-Apr-2000
Date last subject completed: 13-Jan-2005

Clinical Phase: 2

Objectives: The PK objective of this study was to evaluate the population PK parameters of temsirolimus following treatment with 3 dose levels of temsirolimus when administered to patients with advanced RCC. A supplementary objective was to evaluate possible PD relationships with safety and clinical response.

Design: This was a randomized, double-blind, multicenter, outpatient trial of temsirolimus in patients with advanced RCC. Eligible patients were randomized in a 1:1:1 ratio to receive 25 mg, 75 mg, or 250 mg of temsirolimus IV once weekly until evidence of disease progression. One hundred eleven (111) patients were randomly assigned to the 3 study treatment arms; 110 patients were treated. These patients had histologically confirmed advanced RCC and had received prior therapy for advanced disease, or had not received previous treatment for advanced disease but were not appropriate candidates to receive high-dose interleukin-2 (IL-2) therapy.

Formulation: The following formulations of temsirolimus were used in this study:

Table 6.4.2-1: Study Medication (Temsirolimus) Batch Numbers

Strength/Dosage	Formulation Stock Number	Batch Number	Location of Manufacturer
25mg/1mL/Ampoule	0930997H	1999B0160	Gosport
25mg/1mL/Ampoule	0930997H	1999B0102	Gosport
25mg/1mL/Ampoule	0930997H	2000B0225	Gosport
25mg/1mL/Ampoule	0930997H	2000B0209	Gosport
25mg/1mL/Ampoule	0930997H	2000B0301	Gosport
25mg/1mL/Ampoule	0930997H	2000B0349	Gosport
25mg/1mL/Ampoule	0930997H	2000B0383	Gosport
125mg/5mL/Ampoule	0930998H	1999B0103	Gosport
125mg/5mL/Ampoule	0930998H	1999B0178	Gosport
125mg/5mL/Ampoule	0930998H	1999B0184	Gosport
125mg/5mL/Ampoule	0930998H	2000B0348	Gosport
Diluent/Vial 9.1mL	0930986J	1999B0148	Gosport
Diluent/Vial 9.1mL	0930986J	2000B0226	Gosport
Diluent/Vial 9.1mL	0930986J	2000B0414	Gosport
Diluent/Vial 45.5mL	0930987J	1999B0055	Gosport
Diluent/Vial 45.5mL	0930987J	2000B0210	Gosport
Diluent/Vial 45.5mL	0930987J	2000B0346	Gosport
Diluent/Vial 45.5mL	0930987J	2001B0146	Gosport

Pharmacokinetics: Whole blood for measurement of temsirolimus and sirolimus concentrations was collected during cycles 1 and 4 in 90 patients. In a subset of 16 patients, samples were collected before the first and fourth weekly temsirolimus doses, then at 0.5 hours (end of infusion), 1, 2, 6, 24, 72, 96, and 168 hours after infusion. Samples for plasma were also drawn before the first and fourth doses, and at 0.5 and 6 hours. In the remaining 74 patients, samples were collected before and immediately after the fourth weekly dose, and at 1 other time during week 4.

Pharmacokinetic Analysis: Concentration data were analyzed using a 3-compartment population PK model with zero-order input for temsirolimus, and a 2-compartment model with first-order input for sirolimus and the nonlinear mixed-effects modeling application NONMEM. Covariates for demography and clinical laboratory parameters were examined as explanatory factors for exposure variability, and included age (AGE), weight (WT), body surface area (BSA), serum albumin concentration (ALB), hematocrit (HCT), and renal cancer survival risk factors (IRSK).

Whole blood samples from patients undergoing full PK sample collection were analyzed using compartmental analysis and NCA. Parameters obtained were then used to develop a population PK model that considered data from all patients who submitted to blood sample collection. Covariates for demography parameters, IRSK, HCT, and ALB were examined as explanatory factors for PK variability.

Safety: Safety assessments included monitoring of adverse events (AEs), clinical laboratory evaluations (hematology, serum chemistries, and urinalysis), electrocardiograms, physical examinations, and vital signs.

The most common (reported for ≥30% of patients) treatment-emergent adverse events (TEAEs) were rash/maculopapular rash (76%) and pruritus (33%), mucositis (71%; includes stomatitis), asthenia (ie, fatigue; 55%), nausea (47%), anorexia (42%), diarrhea (38%), cough increased (36%) and dyspnea (35%), infection (35%) and fever (30%), acne (35%), anemia (34%),

vomiting (33%), and pain (33%).

Assay Method: Temsirolimus and sirolimus concentrations were analyzed using a validated liquid chromatography/tandem mass spectroscopy (LC/MS/MS) procedure.

Results:

The results from the non-compartmental analysis of the intensively sampled patients are below:

ST 3-C: Temsirolimus Pharmacokinetic Parameters in Whole Blood From Fully Sampled Patients With Renal Cancer Receiving Once-Weekly IV Temsirolimus Treatment

Patient No.	Dose Group (mg)	C_{max}[1] (ng/mL)	t_{max}[1] (h)	$t_{1/2}$ lambda1 (h)	$t_{1/2}$ lambda2 (h)	AUC (ng.h/mL)	CL_c (L/h)	Q (L/h)	V_c (L)	V_p (L)	Vd_{ss} (L)
614	25										
618	25										
620	25										
624	25										
	Mean	595	0.51	0.221	12.8	1581	16.1	73.7	20.6	212	232
	SD	102	0.01	0.207	1.09	270	2.5	61.7	19.3	21	36
611	75										
613	75										
617	75										
621	75										
623	75										
625	75										
	Mean	876	0.50	0.509	13.5	1860	41.6	72.8	89.7	475	565
	SD	316	0.01	0.788	1.53	374	8.0	27.5	136	121	181
610	250										
615	250										
616	250										
619	250										
622	250										
627	250										
	Mean	2827	0.50	0.518	12.5	2704	98.0	78.5	151	747	897
	SD	871	0.01	0.441	2.46	719	25.5	16.2	151	191	316

1: Observed following Week 1 of treatment

ST 3-D: Sirolimus Pharmacokinetic Parameters in Whole Blood From Fully Sampled Patients With Renal Cancer Receiving Once-Weekly IV Temsirolimus Treatment

Patient No.	Dose Group (mg)	C_{max}[1] (ng/mL)	t_{max}[1] (h)	AUC (ng.h/mL)	$t_{1/2}$ (h)	CL/f_m (L/h)	Vd_{ss}/f_m (L)	AUC ratio[2]	AUC sum[2] (ng.h/mL)
614	25								
618	25								
620	25								
624	25								
	Mean	65.9	1.02	3808	48.8	7.87	527	2.84	5,862
	SD	35.0	0.03	2221	7.9	3.02	226	1.75	2,341
611	75								
613	75								
617	75								
621	75								
623	75								
625	75								
	Mean	157	1.79	11014	57.1	7.35	545	5.34	11,560
	SD	40.7	2.01	3584	15.7	2.08	125	1.29	1,861
610	250								
615	250								
616	250								
619	250								
622	250								
627	250								
	Mean	266	1.77	13292	40.4	20.1	1043	5.24	15,995
	SD	93.3	2.16	3692	4.7	5.67	214	2.18	3,590

1: Observed following Week 1 of treatment
2: AUC's of sirolimus and temsirolimus not corrected for differences in molecular weight

4.1.4 124-US: Dose-escalation with Interferon-alpha.

Title: A phase 1 study of the safety, tolerability, and antitumor activity of escalating doses of intravenous temsirolimus given in combination with escalating doses of interferon-alfa to patients with advanced renal cancer.

Investigators: This was a multicenter trial. The study was conducted at 8 investigational sites in the US (5 sites) and Europe (3 sites).

Study Period: Date first subject enrolled: Feb-2002
Date last subject completed: Oct-2005

Clinical Phase: 1

Objectives: The primary objective was to determine the safety, tolerability, and MTD of temsirolimus given IV once weekly in combination with IFN administered subcutaneously 3 times weekly in patients with advanced RCC in whom treatment with IFN was appropriate. A secondary objective was to obtain preliminary information on the antitumor activity of temsirolimus when administered to this population.

Design: This was a multicenter, outpatient, open-label, ascending-dose, single-arm study of temsirolimus given IV once weekly, with ascending dose IFN administered subcutaneously 3 times weekly. During the first week, IFN was administered alone. Six dose levels of temsirolimus were tested in combination with 6 or 9 million units (MU) IFN, as displayed below:

Table 2.2.6.2-1: Dosing in Study 3066K1-124-US

Week	1	2	3	4	5	6	7
Temsirolimus IV (mg)	0	5	10	15	25	15	20
IFN SC 3x/wk (MU)	6	6	6	6	6	9	6

Abbreviations: IFN = interferon alfa; MU = million units; SC = subcutaneous; 3x/wk = 3 times per week administration.

Once appropriate doses of each agent were determined two expanded cohorts were added to treat patients (up to 40 additional patients in the US and EU) at or below the MTD to obtain further safety information.

Formulation: The following formulations of temsirolimus were used in the study:

Table 6.3.4-1: Temsirolimus Batch Numbers

Drug Product	Strength	Dosage Form	Batch Number
CCI 1.1mL Ampoule	25mg/mL	[redacted] injection	2000B0432, 2001B0020
CCI 1.1mL/ 5mL Vial	25mg/mL	[redacted] injection	2003B0016, 2003B0077, 2004B0050
Diluent/ 9.1mL/ 1.1mL Ampoule	Diluent	Diluent	2001B0006
Diluent/2 mL Vial	Diluent	Diluent	2001B0232, 2004B0049

Pharmacokinetics: PK for INF-α were taken on Week 1 at 0, 0.5, 1, 2, 4, 6, 8, and 24 hours post INF-α injection and on weeks 3, 4 or 5 at 0, 0.5, 1, 2, 4, 6, 8, and 24 hours post INF-α injection. Samples for temsirolimus and sirolimus were taken before the INF-α injection, and at predose, 0.5, 2, 8, 24, 84, and 168 hours post temsirolimus dose.

Pharmacokinetic Analysis: The whole blood concentration data for temsirolimus and sirolimus were analyzed for each patient in both study periods using a non-compartmental method.

Safety: Dose-limiting toxicities, including grade 3 stomatitis, grade 3 fatigue, and grade 3 nausea/vomiting, dehydration, and syncope, were reported with temsirolimus 25 mg/(IFN-α) 6 MU, temsirolimus 15 mg/IFN-α 9 MU, and temsirolimus 20 mg/IFN-α 6 MU. Following a safety review of all available data, the temsirolimus 15 mg/IFN-α 6 MU dose was established as the MTD.

Temsirolimus had an acceptable safety profile when used in combination with IFN-α. The most frequently reported types of related TEAEs reported in the study were body as a whole, digestive, metabolic, or hematologic in nature with the most frequent drug-related TEAEs being asthenia (69%); mucositis (68%); anorexia (46%), diarrhea (45%); nausea and anemia (42% each); rash/maculopapular (39%), leukopenia (37%), thrombocytopenia (35%), hyperlipemia (32%), and hypophosphatemia (25%).

Assay Method: Temsirolimus and sirolimus concentrations were measured simultaneously using a validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) combination method with internal standard [redacted]

INF-α 2b concentrations were measured using an ELISA technique that was developed and performed by [redacted]

IFN-α 2a concentrations in serum were validated using an ELISA technique. An issue of limited long-term storage stability occurred during interim analysis of independently prepared quality control samples of IFN-α 2a. During this assessment, adequate storage stability at –70 °C was demonstrated through 109 days, but stability was not maintained for subsequent time points. Since the duration of study sample storage exceeded this period, no PK parameters were calculated for subjects receiving IFN-α 2a.

Results: Seventy-one patients were enrolled in the study and all patients received at least done dose of IFN-α and 69 received at least 1 cycle of INF-α and temsirolimus. Whole blood sample concentrations were available from 31 subjects for temsirolimus and sirolimus, 22 subjects for IFN-α 2b during week 1, and 17 subjects for IFN-α 2b during week 3-to-end. Because of assay stability there was no results for the 10 patients who received INF-α 2a.

The summary PK parameters of temsirolimus and sirolimus are below for the temsirolimus 15 mg/INF-α 6 MU dose group. The pharmacokinetic characteristics of temsirolimus were similar to those results from previous studies.

Table 8.1.1-1: Summary Statistical Findings of Temsirolimus Pharmacokinetic Parameters in Whole Blood When Administered in Combination with IFN-α 2b

Treatment: TEMSR 15 mg/IFN 6 MU (Week 3-to-end)

Mean±SD [N]	Temsirolimus	Sirolimus
C_{max} (pg/mL)	407±130 [20]	60.8±19.5 [20]
t_{max} (hr)[a]	0.53 [20]	2.00 [20]
$t_{1/2}$ (hr)	16.2±8.51 [18]	68.8±20.1 [19]
AUC (pg·h/mL)	1506±422 [18]	5159±3262[19]
CL/f_m (L/h)	10.7±3.07 [18]	4.99±5.19 [19]
V_{dss}/f_m (L)	157±60.0 [18]	497±695 [19]

Abbreviations: IFN = interferon-α; TEMSR = temsirolimus;
V_{dss} = steady-state volume of distribution
a: Values for t_{max} are median
f_m for temsirolimus = 1

Below are the PK findings from the temsirolimus 15mg/INF-α 6 MU dose group. During week 1 INF-α was given alone and from Week-3 to end the two drugs were given in combination. As seen below, when given concomitantly with temsirolimus the exposure of INF-α increased by approximately 2-fold.

Table 8.1.3-1: Summary Statistical Findings of IFN-α 2b Pharmacokinetic Parameters in Serum When Administered in Combination with Temsirolimus

Treatment: TEMSR 15 mg/IFN-α 6 MU

Mean±SD [N]	Week 1	Week 3-to-end
C_{max} (pg/mL)	82.6±32.8 [8]	159±82.4 [9]
t_{max} (hr)[a]	7.08±NC [8]	6.78 [9]
$t_{1/2}$ (hr)	NC	14.1±NC [1]
AUC_T (pg·h/mL)	985±645 [8]	1995±1795 [9]
AUC_{SS} (pg·h/mL)	NC	3658±1012 [4]
AUC (pg·h/mL)	NC	NC
CL (L/h)	NC	6.63±1.51 [4]
V_{dss} (L)	NC	91.3±NC [1]

Abbreviations: IFN= interferon-α; NC = not calculated; TEMSR= temsirolimus;
V_{dss} = steady-state volume of distribution
a. Values for t_{max} are median

4.1.5 103-EU: Dose-escalation with 5-FU/Leucovorin

Title: A Phase 1 study of escalating doses of temsirolimus in combination with 5-flurouracil and leucovorin in patients with advanced solid tumors.

Investigators:

[redacted]

Study Period: Date first subject enrolled: 06-Sept-1999

Date last subject completed: 09-March-2001

Clinical Phase: 1

Objectives: The primary objective of this study was to determine the safety, tolerability, and maximum tolerated dose (MTD) of weekly IV injections of temsirolimus, when given in combination with continuous intravenous infusion (CIV) 5-FU/LV in patients with advanced solid tumors. Secondary objectives included determination of pharmacokinetic (PK) parameters of temsirolimus and 5-FU given on this schedule and preliminary assessment of efficacy of this combination.

Design: This study was an open-label, multicenter, phase 1 combination therapy study. In Cycle 1, temsirolimus was administered on day 1 of week 2. For all remaining cycles, patients were given the 3 drugs sequentially (temsirolimus/LV/5-FU) on the 1st day of weeks 1 - 6 of a 7-week treatment cycle in which the 7th week was a rest period.

The starting dose of temsirolimus was 15 mg/m^2 which was escalated based on a modified Fibonacci sequence until MTD was reached. The planned 5-FU dose was 2600 mg/m^2 at every dose administration for most patients. Per a site-specific modification, the first cohort of patients at the [redacted] site was scheduled to receive 5-FU at 2000 mg/m^2 with an increase to 2600 mg/m^2 for subsequent cohorts unless a hematologic toxicity of NCI grade ≥ 3 or a nonhematologic toxicity of NCI grade ≥ 2 was observed in the previous cohort. The LV dose (200 mg/m^2) was the same for all patients.

Formulation: The following formulations were used during the trial:

Table 6.3.2-1: Batch Numbers for CCI-779

Component[a]	Formulation Number	Batch Number	Investigative Sites[b]
CCI-779 (125 mg/5 mL ampules)	0930998H	1999B0056 1999B0034	Netherlands Germany
Diluent for CCI-779 (45.5 mL/vial)	0930987J	1998B0278 1998B0217	Netherlands Germany

a: All supplies manufactured at Wyeth, Gosport, UK.
b: The investigative site(s) (identified by country) at which each batch was used. This information is included to tell which patients received which batches.

Pharmacokinetics: PK samples were only taken during the week 1, 2, and 4 drug administrations of cycle 1; no PK samples were taken during subsequent treatment cycles.

Samples for temsirolimus and sirolimus were taken on Weeks 2 and 4 (days 8 and 22 of cycle) at 0, 0.25, 0.5, 1.5, 3.5, 8, 24, 48, 96, and 168 post start of temsirolimus infusion.

Samples for 5-FU were taken on Week 1 (day 1 of cycle 1) at 1 hr, 1.5, 2, 3, 5.5, 8, and 24 hours post start of the leucovorin infusion (5-FU infusion began at the start of hour 1). On weeks 2 and 4 (days 8 and 22 of Cycle 1) samples for 5-FU were taken at 1.5, 2, 2.5, 3.5, 6, 8 and 24 hours post start of the temsirolimus infusion (5-FU infusion starts at 1.5 hours)

Pharmacokinetic Analysis: Concentrations of temsirolimus, sirolimus, and 5-FU were analyzed using a noncompartmental modeling approach.

Safety: Safety was assessed on the basis of routine laboratory tests, and adverse events. Patients were routinely given physical examinations, including determination of vital signs, weight and performance status; electrocardiograms (ECG) and chest X-rays; complete blood count (CBC)

with differential; fasting blood chemistry tests, electrolyte measurements, and coagulation profile determinations; urinalysis; liver function tests. The most common (incidence ≥ 10%) NCI grade 3 or 4 treatment-emergent AEs (TEAEs) were asthenia, mucositis, hyperglycemia, diarrhea, anemia, hypokalemia, dyspnea, nausea, vomiting, leukopenia, and hypoxia. Other common TEAEs that were generally NCI grade ≤ 2 were epistaxis, rash/maculopapular rash, anorexia, and fever.

Assay Method: The bioanalytical method used to measure temsirolimus and sirolimus concentrations in this study was performed via an LC/MS/MS procedure and was and validated [redacted]

The bioanalytical method used to measure 5-FU in plasma in this study employs a [redacted] chromatography tandem mass spectroscopy [redacted]/MS/MS) procedure. The method was performed and validated at [redacted] It exhibits a linear range from plasma of 1 to 100 ng/mL. Inter- and intra-assay variability of quality control samples measured during validation were < 7%, and biases were < 13%. 5-FU is stable in plasma stored at room temperature for up to 4 hours, following 3 freeze/thaw cycles, in autosampler extracts (24 hours at room temperature), and for at least 11 months when stored in plasma at –20°C.

Results: Pharmacokinetic samples were obtained from 27 patients. The temsirolimus dose was escalated from 15 mg/m^2 to 75 mg/m^2.

Following IV infusion of temsirolimus, peak concentrations and AUC of sirolimus and temsirolimus increased in a dose-related but less than proportional fashion. Little or no accumulation was observed with multiple (weekly) treatments. Following a single dose of temsirolimus, mean t½ ranged from 14 to 18 hours. The mean sirolimus:temsirolimus AUC ratios ranged from approximately 2-fold to 5-fold during week 2, and 3-fold to 8-fold during week 4. As with temsirolimus, the ratio of sirolimus AUCs of week1:week 2 was near unity, indicating little or no accumulation with multiple treatments. Following a single dose of temsirolimus, mean half-life of sirolimus ranged from 46 to 53 hours and exhibited modest variability

The mean clearance of 5-FU observed in the absence (week 1) and presence (weeks 2 and 4) of temsirolimus ranged from 300 to 346 L/h and were not significantly different. Mean steady state concentrations decreased from weeks 1 to 4, however a consistent treatment effect during weeks 2 and 4 was not apparent. The data does not suggest that temsirolimus exerts a consistent clinically relevant effect on exposure to 5-FU.

4.1.6 104-US: BA of PO formulation

This was a 2-phase study. The first phase characterized the absolute bioavailability (BA) of oral (PO) temsirolimus in a crossover design by comparing the PK of temsirolimus given PO versus administered IV. The second phase was a dose escalation study of PO temsirolimus administered once daily for 5 days every 2 weeks. The sponsor only submitted a brief summary of this study and they focused on the PK findings of the IV treatment arm.

Objectives: The primary objectives were to determine the safety, tolerability (and, if possible, identify the MTD), and preliminary PK of temsirolimus (including BA data), given PO once daily for 5 days every 14 days in subjects with advanced solid tumors. The secondary objective was to obtain preliminary information on the antitumor activity of temsirolimus when

administered PO.

Design: Patients with solid tumors or lymphomas received temsirolimus IV doses of 5 to 20 mg and PO doses of 25 to 100 mg as follows:

- Temsirolimus IV was administered in the BA phase as a single IV dose at one-fifth the amount of the PO dose. Treatment was followed by approximately 1 week of rest, followed by a single dose given by the alternate route of administration.
- In the dose-escalation phase, temsirolimus was administered PO under fed conditions, once daily for 5 days, repeated approximately every 14 days.

After IV dose, whole blood was collected at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 24, 48, 72, and 120 hours. Plasma was collected at 0.5, 2, 4, 6, 8, and 24 hours, and measured concentrations of temsirolimus and sirolimus were analyzed using NCA. Whole blood and plasma samples were available from 24 patients receiving temsirolimus IV doses ranging from 5 to 20 mg. The sponsors summary PK parameters for temsirolimus and sirolimus are below.

Table 2.2.4.3-1: Summary Pharmacokinetic Parameters of Temsirolimus Following a Single Intravenous Dose in Patients With Cancer (Study 3066K1-104-US)

Dose (mg)		N	C_{max} ng/mL	t_{max} h	$t_{1/2}$ h	AUC ng·h/mL	CL L/h	V_{ss} L	B/P ratio
5	Mean	2	217	0.50	23.9	835	5.99	207	8.0
	%CV		6.2	0.0	3.0	1.4	1.4	4.3	4.2
10	Mean	2	378	0.55	18.1	1190	9.98	245	14.1
	%CV		9.0	64.3	20.7	42.8	55.7	37.7	76.0
15	Mean	12	516	0.43	19.1	1759	8.84	244	17.9
	%CV		21.0	22.7	22.4	21.9	18.4	29.1	44.4
20	Mean	6	551	0.40	18.7	1964	10.5	280	27.0
	%CV		15.0	27.4	15.9	20.7	18.2	19.4	29.8

Table 2.2.4.3-2: Summary Pharmacokinetic Parameters of Sirolimus Following a Single Intravenous Dose of Temsirolimus in Patients With Cancer (Study 3066K1-104-US)

Dose (mg)		N	C_{max} ng/mL	t_{max} h	$t_{1/2}$ h	AUC ng·h/mL	AUC_{inf} ng·h/mL	AUC ratio[a]	B/P ratio
5	Mean	2	22.7	4.00	88.7	1735	2571	2.1	18.7
	%CV		28.4	0.0	38.0	16.0	11.2	14.6	75.3
10	Mean	3	29.1	1.83	45.0	2225	3416	1.7	40.3
	%CV		5.6	41.7	43.7	75.9	62.3	43.5	78.0
15	Mean	12	62.1	1.56	57.1	3815	5575	2.2	35.7
	%CV		34.9	35.4	42.9	54.6	39.1	53.5	57.2
20	Mean	6	70.1	1.33	59.5	4343	6307	2.3	47.4
	%CV		38.8	38.7	29.1	39.9	26.8	48.7	43.0

a. AUC ratio denotes quotient of sirolimus: temsirolimus AUCs

4.1.7 131-JA: Dose escalation in Japanese patients

Title: Phase 1 clinical trial of temsirolimus in advanced solid cancer patients

Investigators: [redacted]

Study Period: Date first subject enrolled: 29-Oct-02
Date last subject completed: 02-Apr-04

Clinical Phase: 1

Objectives: To investigate the safety and tolerance of temsirolimus in patients with advanced solid cancers when it was administered intravenously once a week. Secondary objectives include; to investigate the pharmacokinetics of temsirolimus and to obtain preliminary information on the antitumor efficacy of temsirolimus.

Design: This was a single dose-escalating trial where the temsirolimus dose was gradually increased from the initial dose of 15 mg/m^2. The transition to the next dose level was decided on with the cooperation of the physician responsible for the trial and the trial requester. The levels of temsirolimus and sirolimus in the whole blood were analyzed by non-compartment analysis. Pharmacokinetic parameters were calculated by non-compartmental analysis.

Results: Temsirolimus was administered to 10 patients in this study. Following administration, temsirolimus decreased in a polyexponential manner. Sirolimus was a major metabolite, was observed early (during infusion by 15 minutes) and decreased in an apparent monoexponential fashion. Pharmacokinetic data were available from 7 and 3 patients, respectively, for the 15 and 45 mg/m^2 doses.

Table 2.2.3.3-1: Summary Pharmacokinetic Parameters of Temsirolimus Following Intravenous Administration in Patients of Japanese Origin With Cancer (Study 3066K1-131-JA)

Dose (mg/m^2)	N		C_{max} ng/mL	t_{max} h	$t_{1/2}$ h	AUC ng·h/mL	CL L/h	V_{dz} L
15	7	Mean	1013.9	0.51	14.8	2873	8.48	83.85
		SD	316.3	0.0	0.68	358	1.73	10.91
		%CV	31.2	2.5	4.6	12.4	20.4	13.0
45	3	Mean	1793.3	0.34	13.5	2750	27.19	162.9
		SD	421.6	0.2	1.09	290	6.37	26.67
		%CV	23.5	47.1	8.1	10.5	23.4	16.4

Abbreviations: CV = coefficient of variation; SD = standard deviation

Table 2.2.3.3-2: Summary Pharmacokinetic Parameters of Sirolimus Following Intravenous Administration of Temsirolimus in Patients of Japanese Origin With Cancer (Study 3066K1-131-JA)

Dose (mg/m^2)	N		C_{max} ng/mL	t_{max} h	$t_{1/2}$ h	AUC ng·h/mL	CL/F_m L/h	Vd_z/F_m L	AUC_{inf} ng·h/mL	AUC ratio[a]
15	7	Mean	89.1	7.53	67.0	8168	3.05	189.6	11041	2.94
		SD	40.5	11.3	17.37	2089	0.61	23.46	1935	1.08
		%CV	45.5	150	25.9	25.6	19.9	12.4	17.5	36.9
45	3	Mean	157.3	1.87	59.2	13524	7.11	325.2	16274	4.79
		SD	37.1	1.9	28.94	9763	3.41	103.4	5970	3.03
		%CV	23.6	101	48.9	72.2	47.9	31.8	61.3	63.3

a. AUC ratio denotes quotient of sirolimus: temsirolimus AUCs
Abbreviations: CV = coefficient of variation; SD = standard deviation

4.1.8 101-EU: Dose-Escalation Once weekly

Title: A phase 1 study of safety/tolerability and pharmacokinetics of weekly intravenous administration of CC1-779 in patients with advanced solid tumors

Investigators: [redacted]

Study Period: Date first subject enrolled: Nov-98
Date last subject completed: July-00

Clinical Phase: 1

Objectives: The primary goal of part 1 of the study was to determine the safety, tolerability, and MTD of temsirolimus, given intravenously (IV) once a week, in patients with advanced solid tumors who were not taking anticonvulsant therapy. The primary goal of Part 2 of the study was to determine the safety and tolerability of temsirolimus and to assess the effect of CYP enzyme-inducing concomitant medication on the PK profile of temsirolimus administered by IV infusion once a week to patients with nonoperable and/or recurrent malignant gliomas or brain metastases from other tumors. All patients in part 2 received 220 mg/m^2 of temsirolimus once weekly. The secondary goal of both parts of the study was to obtain preliminary information on the antitumor activity of temsirolimus and to determine the preliminary PK of temsirolimus administered on this schedule.

Design: This was an open-label, 2-part, phase 1 study of temsirolimus given as a 30-minute IV infusion once a week. Part 1 was conducted to establish the MTD of temsirolimus in patients with solid tumors who were not receiving any anticonvulsant therapy. The selected starting dose for Part 2 was 7.5 mg/m2.

Part 2 was done to assess the PK profile of temsirolimus at a dose level of 220 mg/m^2 in patients with nonoperable and/or recurrent malignant gliomas or brain metastases from solid tumors who were receiving anticonvulsant therapy with or without high-dose dexamethasone (12 evaluable patients) or no enzyme-inducing medication (6 evaluable patients).

Formulation: The following formulations of temsirolimus were used during Part 1:

Strength, mg (Ampule)[a]	Formulation Number[b]	Batch Number
25 (1 mL)	0930997H	1998B0275
25 (1 mL)	0930997H	1999B0102
125 (5 mL)	0930998H	1999B0056
125 (5 mL)	0930998H	1999B0034
125 (5 mL)	0930998H	1999B0103

a: Supplied in [redacted] form.
b: Sites of manufacture: Wyeth-Ayerst Laboratories, Gosport, UK

For part 2, the following formulations were used:

Study Medication	Formulation Number	Batch Number	Strength
temsirolimus 25 mg/mL liquid in ampoule	0930997H	1999B0102	24.8 mg/mL
		2000B0383	24.3 mg/mL
		2000B0349	24.2 mg/mL
temsirolimus 125 mg/5 mL liquid in ampoule	0930998H	1999B0056	25.3 mg/mL
		1999B0178	24.33 mg/mL
		2000B0384	24.5 mg/mL
Diluent for temsirolimus / 9.1 mL vial	0930986J	1999B0277	-
		2000B0414	-
		2000B0209	-

Diluent for temsirolimus / 45.5 mL vial	0930987J	1998B0278	-
		2000B0343	-

Pharmacokinetics: Whole blood for determination of temsirolimus and sirolimus was collected at 0, 0.25, 0.5 (end of infusion), 1, 2, 4, 6, 24, 48, 72, 96, and 168 (before the next weekly treatment) hours following the start of infusion during the first and fourth doses. Plasma was also collected to determine the blood-to-plasma ratio.

In Part 2, anticonvulsant trough concentrations were measured in all patients on anticonvulsant medications at baseline and before the fifth administration of study medication. If the patient received less than 4 weekly administrations, the second measurement of anticonvulsant concentration was performed as soon as possible after the last weekly temsirolimus administration.

Pharmacokinetic Analysis: The concentration-versus-time data for temsirolimus and sirolimus in whole blood were analyzed by using a noncompartmental analysis technique.

Safety: Safety was assessed from routine laboratory tests, other procedures, and adverse events (AEs). The most common drug-related treatment-emergent AEs (TEAEs) were mucositis (75%), rash/maculopapular rash (50%), nail disorder (45.8%), and asthenia (45.8%). The most common NCI grade 3 or 4 TEAEs were anemia (20.8%) and hypercholesterolemia (20.8%). One (1) of 24 patients died of acute respiratory distress during the study, probably due to disease progression.

Assay Method: Bioanalytical method validation and analyses were performed by [redacted] The bioanalytical methods for measuring temsirolimus in whole blood and plasma employed a validated, [redacted] extraction procedure, followed by a sensitive and specific tandem mass spectrometry procedure [redacted] The range of quantitation is 0.25 to 100 ng/mL. The bioanalytical method for sirolimus from whole blood also employs a validated, [redacted] extraction procedure, followed by a sensitive and specific tandem mass spectrometry procedure [redacted] The range of quantitation is 0.1 to 100 ng/mL

Results - Part 1: Data from dosages ranging from 7.5 mg/m^2 to 220 mg/m^2 were obtained from 24 patients in Part 1 Following treatment temsirolimus concentrations decreased in a poly exponential manner. Sirolimus, the major metabolite was observed early (during infusion by 15 mins) and decreased in an apparent monoexpoential fashion. The mean terminal half-life for temsirolimus appeared to decrease with increasing dose from 22 hours following the 34 mg/m^2 dose to 13 hours following the 220 mg/m^2 dose. The mean terminal half-life for sirolimus ranged from 61 to 69 hours. The exposure to sirolimus was higher than to temsirolimus, as the mean AUC ration (sirolimus to temsirolimus) ranged from 2.5 to 3.5. The mean accumulation ratio values were less than 1, indicating little or no change in exposure with multiple once-weekly dosing. Over the wide dose range, Cmax in whole blood tended to increase in a less than proportional manner. Dose-related increases in AUC and AUCsum were significantly less than proportional.

Table 8.1A. Mean Pharmacokinetic Parameters of CCI-779 and Sirolimus Following a Single Intravenous Dose in Patients With Cancer

	Dose Group (mg/m^2)	C_{max} (ng/mL)	t_{max} (h)	λ_z (1/h)	$t_{1/2}$ (h)	AUC (ng.h/mL)	CL/f_m[a] (L/h)	Vd_{ss}/f_m[a] (L)	AUCsir/ AUCcci	AUC_{SIRM} (ng.h/mL)
CCI-779										
	34	2200	0.24	0.0330	22.3	3221	19.1	242	.	.
	45	1368	0.58	0.0403	17.3	3414	27.0	385	.	.
	220	11417	0.34	0.0580	13	8001	51.3	127	.	.
Sirolimus										
	34	125	0.83	0.0100	69.2	8753	7.01	622	2.72	11973
	45	126	2.26	0.0114	60.8	11740	7.77	697	3.52	15154
	220	798	0.74	0.0151	60.5	19057	27.2	1732	2.51	27058

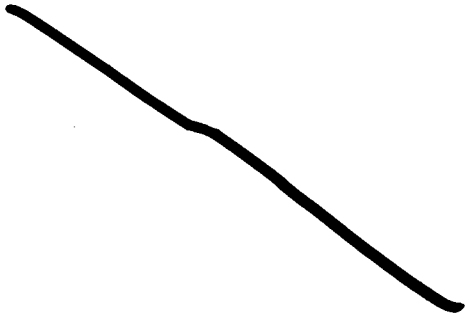
a: f_m = unknown fraction of drug metabolized. The f_m for CCI-779 =1

Results Part 2: Eleven patients on enzyme inducers (carbamazepine or phenytoin) and five patients who were not taking enzyme inducers were enrolled. Data from the 220 mg/m^2 dose in Part 1 were used to compare PK. When coadministered with enzyme inducers the temsirolimus Cmax was significantly decreased by 36% (p=0.02) and Vdss increased by 99% (p=0.05). Exposure to sirolimus during cycle 1 appears more affected by the presence of CYP3A4 inducers than that of parent drug. In this study, sirolimus Cmax decreased by 67% (p<0.001), tmax increased by 204% (p=0.008), and AUC decreased by 43% (p=0.05).

4.1.9 100-US: Dose-Escalation QD x 5 Days every 2 Weeks

Title: A Phase 1 study of the safety, tolerability and pharmacokinetics of intravenous temsirolimus given once daily for 5 days every 2 weeks to subjects with advanced solid tumors

Investigators:



Study Period:

Clinical Phase: 1

Objectives: The primary objective of part I was to determine the safety and tolerability, and if possible identify the MTD of temsirolimus, given IV once daily for 5 days, every 2 weeks, in subjects who had advanced solid tumors and who were not taking anticonvulsants.

The primary objective of part II was to determine the safety and tolerability, and possibly identify the MTD of temsirolimus, given IV once daily for 5 days, every 2 weeks, in subjects with recurrent gliomas or brain metastases from other tumors, and who were receiving anticonvulsants that are known cytochrome P450 inducers. In addition, preliminary PK data were to be obtained

in this group of subjects.

The secondary objectives of this study were to determine the preliminary pharmacokinetics of temsirolimus on this schedule (for part I) and to obtain preliminary information on the antitumor activity of temsirolimus.

Design: This trial was an open-label, 2-part, ascending-dose, single-arm study of temsirolimus given as a 30-minute IV infusion once daily for 5 days, every 2 weeks. Sixty-three subjects were enrolled in Part I. The starting dose for Part I was 0.75 mg/m^2/day and was escalated to 24 mg/m^2/day. Twenty-five subjects enrolled in Part II. The starting dose for part two was 15 mg/m^2 and was escalated to 37 mg/m^2. A treatment cycle was defined as five consecutive days of treatment with temsirolimus followed by at least a nine day rest period. Cycles were repeated every 2 weeks.

Formulation: The following batch numbers were used during the study:

Study Medication	Strength (units)	Batch Number	Source
temsirolimus	25mg/1mL/ Ampule	1998B0214, 1998B0275, 1999B0102, 2000B0225, 2000B0277, 2000B0349, 2000B0383, 2000B0432	Gosport
Diluent for temsirolimus	Vial 9.1mL	1998B0262, 1998B0277, 1999B0148, 2000B0209, 2000B0226, 2000B0414	Gosport

Pharmacokinetics: PK samples were collected on days 1 and 5 of cycle 1 (and cycle 3, if the subject received 3 cycles of treatment) at 0, 0.25, 0.5, 1, 2, 4, and 6 hours, before the day 2, 3, and 4 doses of temsirolimus and on (approximate) days 8, 10, and 12. A PK sample was also collected immediately before the day 1 dose of cycle 2. During cycle 1, additional blood samples were collected immediately after the day 1 and day 5 infusion of temsirolimus, and before the day 1 dose of cycle 2 to evaluate blood to plasma partitioning.

Pharmacokinetic Analysis: Both noncompartmental and compartmental techniques were used for temsirolimus PK parameters. For sirolimus analysis, Non-compartmental analysis only was used. The compartmental analysis used a 2-compartment, open-model with dose administration and elimination from the central compartment.

Safety: Since it was not known if temsirolimus was immunosuppressive when given on an intermittent schedule, the investigators had to be aware of the possibility of opportunistic infections and were vigilant in looking for any indication of infection. In part I only, lymphocyte subsets were followed, and mitogen proliferation assays were performed during the first 3 cycles of therapy. Additional safety measurements were performed: complete blood count with

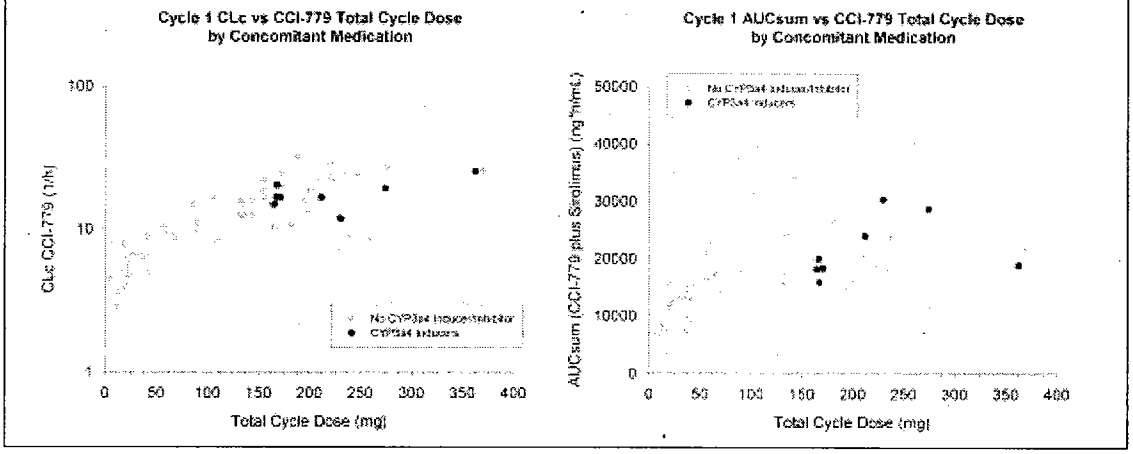
differential, liver tests, coagulation tests, serum electrolytes, serum chemistry, total serum testosterone, follicle stimulating hormone and luteinizing hormone (males), urinalysis, electrocardiogram, physical examinations, and vital signs.

Assay Method: Human samples were frozen and shipped to [redacted] Samples were stored at -80 °C prior to analysis for temsirolimus and sirolimus. A validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was used.

Part I Results: Following treatment temsirolimus concentrations decreased in a polyexponential manner. Dose related increases in exposure were less than proportional. Volume of distribution at steady state was extensive, increased with dose and exhibited values typically exceeding total body weight. At doses below 15 mg/m^2 temsirolimus exhibited preferential partitioning into red blood cells with mean blood-to-plasma ratio values ranging from 3.7 to 10.9, while at doses above 15 mg/m^2 the ratios approached unity suggesting that red blood cell binding was saturated with doses at or greater than 15 mg/m^2. The mean terminal half-life ranged from 13 to 25 hours. Sirolimus was the major metabolite, and was observed during infusion by 15 mins, and decreased in an apparent mono- or bi-exponential manner. Exposure to sirolimus was similar to temsirolimus with the sirolimus-to-temsirolimus AUC ratio ranging from 0.64 to 1.8. There was no drug accumulation among cycles due to the long dosage interval.

Part II Results: Samples were available from 23 subjects who were all on medications with potential for interacting with CYP3A4 (carbamazepine, phenytoin, and/or phenobarbital all are strong CYP3A4 inducers). IV doses ranged from 15 to 37 mg/m^2 and spanned a total of 5 dose escalation cohorts.

Figure 2.2.1.3-2: Temsirolimus Clearance and AUC_{sum} Versus Total Cycle Dose Following Intravenous Temsirolimus Treatment in Patients (Study 3066K1-100-US)



There was no discernable effect of AUC, Clearance or Cmax between patients on the inducers versus patients who were not on inducers. However a definitive conclusion is hard to assess with these small numbers.

Safety Results: Most TEAEs were NCI grade 2 or less for both Part 1 and Part 2. The most common NCI grade 3 and grade 4 TEAEs were in Part 1 were hypophosphatemia and hyperglycemia. The most common TEAEs of NCI grades 3 or 4 in Part 2 were hypophosphatemia, leukopenia, and thrombosis, with the observed laboratory abnormalities

consistent with the TEAE results.

In part I Cmax was significantly positively correlated to AE severity for thrombocytopenia (p=0.0141), weight decrease (p=0.0188), anorexia (p=0.0246), oral moniliasis (p=0.0313), and neuropathy (p=0.0362). The AUC of temsirolimus was positively correlated to SGOT increased (p=0.0119), hypocalcemia (p=0.0300), alkaline phosphatase (p=0.0324), and taste perversion (p=0.0352). The AUC of sirolimus was positively correlated to hypophosphatemia (p=0.003), thrombocytopenia (p=0.0109), myasthenia (p=0.0185), and epistaxis (p=0.0336). The AUCsum was significantly correlated to insomnia (p=0.0071), epistaxis (p=0.0026), hyperlipemia (p=0.0329), and hyperglycemia (p=0.0408).

4.1.10 133-US: ADME study

Title: An open-label, single-dose, nonrandomized study of the mass balance and metabolic disposition of orally and intravenously administered [+14C]-labeled temsirolimus in healthy male subjects.

Investigators: [redacted]

Study Period: Date first subject enrolled: 29-Nov-2004
Date last subject completed: 09-Dec-2004

Clinical Phase: 1

Objectives: The aim of the study was to assess the mass balance, metabolic disposition and identification of metabolites following a single IV or PO administration of radiolabeled temsirolimus in healthy male subjects. In addition the pharmacokinetic profile of total radioactivity and of temsirolimus and sirolimus was characterized.

Design: This was a Phase 1 study conducted at one investigational sight with 12 male subjects who received a single dose of IV (n = 6) or PO (n = 6) temsirolimus. In the subjects who received the IV dose, pretreatment with diphenhydramine 50 mg occurred 30-mins prior to the 30-min IV infusion of 42.5-57.5 μCi [^{14}C]-temsirolimus 25 mg. No pre-treatment was given for patients who received 85-115 μCi [^{14}C]-temsirolimus 30 mg as an oral solution followed by 240 mL of water. Subjects fasted from 10 hours before dose administration until 4 hours after receiving the dose.

Formulation:
^{14}C-temsirolimus concentration for injection, 25 mg/1.0 mL, 2μCi/mg (formulation # 0932028J, batch # 9191015).
^{14}C-temsirolimus oral solution, 30mg/vial, 100 μCi/dose (formulation #0932037J, batch #9201025).

Pharmacokinetics: Blood samples for radioactivity and for temsirolimus and sirolimus concentrations were collected:

1. in whole blood at predose, and at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 120, 168, 216, 264, and 336 hours after dose administration
2. in plasma at predose, and at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 120, 168, 216, 264, and 336 hours after dose administration

Venous blood samples for metabolite analyses in whole blood and plasma were collected at predose and at 0.5, 4, and 24 hours after IV dose administration; or predose, and at 2, 8, and 24 hours after PO dose administration.

Complete urine output was collected for determination of total radioactivity and metabolite assessments beginning on Day 1.

Fecal samples were obtained for determination of total radioactivity and metabolite assessments beginning on Day 1.

If any emesis occurred at any time after IV administration it was to be collected for determination of radioactivity.

Pharmacokinetic Analysis: Whole blood and plasma concentration data for temsirolimus, and total radioactivity profiles were analyzed for each subject in both study treatments using non-compartmental methods.

Safety: Safety was evaluated from reported adverse events, scheduled physical examinations, vital signs, 12-lead ECG's and clinical laboratory test results.

Assay Method:

Whole blood for total radioactivity samples were collected in evacuated EDTA tubes. Samples were frozen and shipped to [redacted]

Whole Blood for Temsirolimus and Sirolimus Determination was collected in evacuated EDTA tubes. Samples were frozen and shipped [redacted] Two validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) combination method analytical methods were used for sample analysis. The high range method, has a quantitation range of 2.50-2500 ng/mL for temsirolimus and 2.50-250 ng/mL for Rapamycin from a 0.200 mL sample. The low range method, has a quantitation range of 0.250-25.0 ng/mL for both temsirolimus and Rapamycin from a 1.00 mL sample. Typically, samples are first analyzed by the high range method, and then re-assayed by the low range method if the results are below the 2.50 ng/mL lower limit of quantitation of the high range method. Samples with expected concentrations below 2.50 ng/mL for both analytes may be initially analyzed by the low range method.

Plasma for Total Radioactivity samples were collected in evacuated EDTA. Samples were frozen and shipped to [redacted]

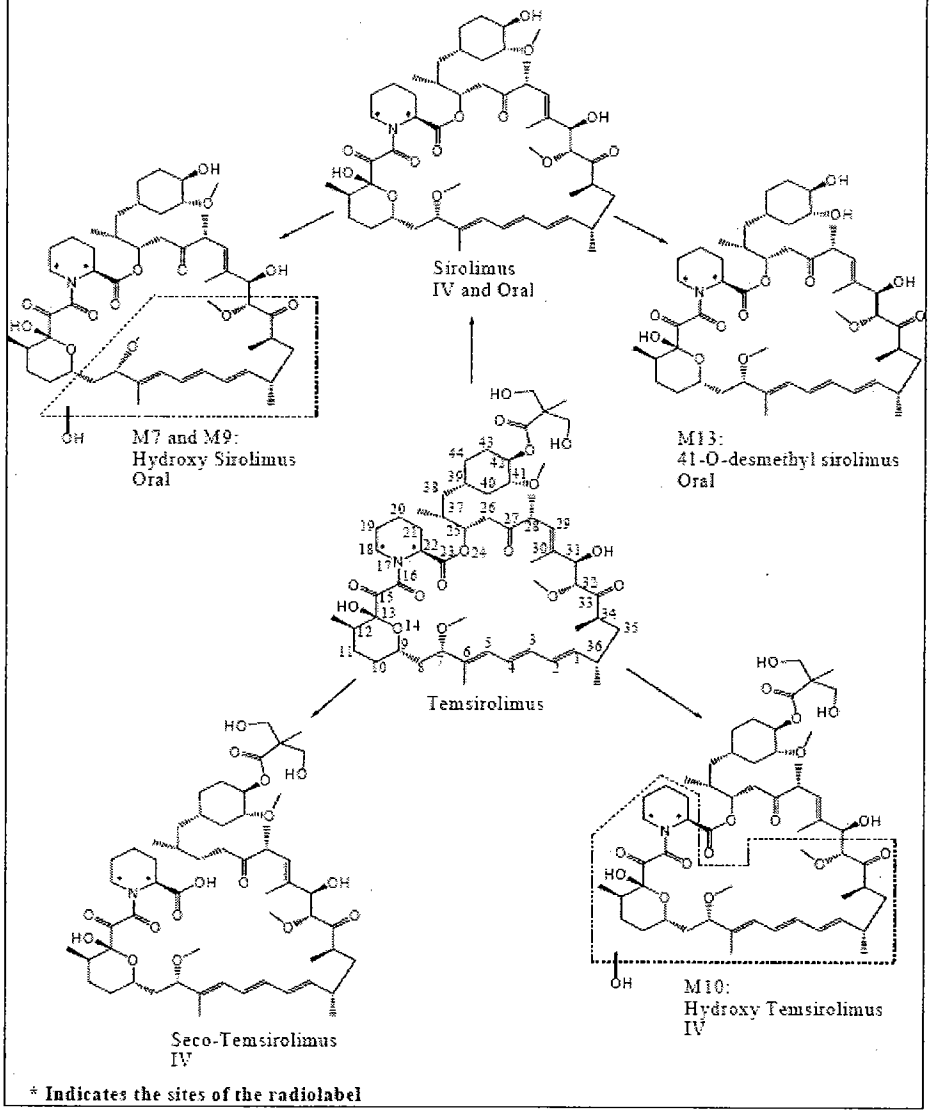
Plasma for Temsirolimus and Sirolimus Determination samples were collected in evacuated EDTA tube and frozen and shipped tc [redacted]

Whole Blood and Plasma for Metabolite Determination samples were collected in evacuated EDTA tubes and were frozen and shipped to Wyeth Research.

Results: In whole blood following IV administration temsirolimus and sirolimus (87% of radioactivity) were the major species contributing to circulating total radioactivity, with minor contributions by [redacted] seco-temsirolimus (6%), and hydroxy-temsirolimus-M10 (6%). Following PO administration temsirolimus and sirolimus (55% of radioactivity) were the major species contributing to circulating total radioactivity and hydroxylated sirolimus metabolites (M7 and M; 8% and 23% respectively) were detected which

were not seen after IV administration. Below are the temsirolimus related compounds detected in human blood following oral and IV administration:

Figure 8.1.4-1: [^{14}C]-Temsirolimus-Related Compounds Detected in Human Blood Following an Intravenous or Oral Administration



* Indicates the sites of the radiolabel

Eighty-two (82%) of the administered radioactive IV dose was recovered, with 4.55% was recovered in the urine and 78.1% was recovered in the feces. After the oral dose, 84.4% of the administered radioactive dose was recovered with 2.12% from the urine and 82.2% from feces. The pharmacokinetic parameters of total radioactivity following IV or PO dose of [^{14}C]-temsirolimus is below in sponsors table 8.1.2.1-1

Table 8.1.2.1-1: Mean (SD) Pharmacokinetic Parameters of Total Radioactivity Following a Single 25-mg IV or 30-mg Oral Dose of [^{14}C]-Temsirolimus

Route Matrix	C_{max} (ng/mL)	t_{max}[a] (hr)	$t_{½}$ (hr)	AUC (ng·h/mL)	CL/f_m (L/h)	V_z/f_m (L)
IV						
Whole Blood	633±106[b]	0.38	25.3±7.42	5251±783[b]	5.11±0.608	167±27.6
Plasma	541±150[b]	0.50	7.47±4.89	758±232[b]	37.0±8.39	221±132.2
Oral						
Whole Blood	160±27.8	1.0	12.1±3.08	1241±361	26.1±7.28	423±151.2
Plasma	30.9±9.26	1.0	3.3±1.35	170±64.8	198±72.3	878±265.5

a: Value for t_{max} is median.
b: Units for C_{max} represented as ng-Eq/mL and for AUC as ng-Eq·h/mL.
Note: Values C_{max} and AUC were transformed from units of nCi/g and nCi·h/g based on specific activity=2.07μCi/mg and density 1=mL/g. Values for CL/f_m and V_z/f_m were transformed from grams to liters

The pharmacokinetic parameters of temsirolimus and sirolimus in plasma and whole blood following IV and PO doses is below in sponsor table 8.1.3.1-1. The pharmacokinetic results from this study were respective of observations from studies in patients with renal cancer.

Table 8.1.3.1-1: Mean (SD) Pharmacokinetic Parameters of Cold Temsirolimus and Cold Sirolimus Following a Single 25-mg IV or 30-mg Oral Dose of [^{14}C]-Temsirolimus

Route Analyte Matrix	C_{max} (ng/mL)	t_{max}[a] (hr)	$t_{½}$ (hr)	AUC (ng·h/mL)	CL/f_m (L/h)	V_z/f_m (L)
IV						
Cold Temsirolimus						
Whole Blood	628±87.7	0.25	20.2±2.71	2452±231	10.9±0.95	206±17.1
Plasma	263±126	0.58	5.74±3.98	153±45.5	186±53.1	932±1323
Cold Sirolimus						
Whole Blood	58.5±12.86	1.5	86.9±25.94	6124±1365	4.51±1.06	550±65.41
Plasma	7.22±5.16	0.50	32.9±53.41	128±205	3881±5401	8961±1769
Oral						
Cold Temsirolimus						
Whole Blood	17.8±4.06	0.75	13.8±4.57	79.8±24.41	410±128	7617±2154
Plasma	0.64±0.19	1.0	NA	NA	NA	NA
Cold Sirolimus						
Whole Blood	101±21.9	1.0	51.1±15.82	1089±399	29.8±7.83	2094±456
Plasma	1.71±0.77	1.0	NA	NA	NA	NA

a: Value for t_{max} is median.

There were no deaths, SAEs, or safety discontinuations in the study. The most frequent treatment emergent adverse events were canker sores (aphthous stomatitis) and thrombocytopenia which occurred in > 50% of the patients.

4.1.11 148-US: Ketoconazole Study

Title: An open-label, nonrandomized, 2-period, sequential, drug interaction study to evaluate the potential pharmacokinetic interaction between multiple doses of ketoconazole and a single dose of temsirolimus administered intravenously as a 30-minute infusion to healthy subjects.

Investigators: [redacted]

Study Period: March 2005 to March 2005

Clinical Phase: 1

Objectives: The primary objective was to evaluate the effects of multiple doses of ketoconazole on the PK profile of a single IV dose of temsirolimus in healthy subjects. The secondary

objective was to assess the safety and tolerability of the coadministration of ketoconazole and temsirolimus.

Design: There were 17 subjects enrolled in the study. Each subject received a single 5-mg IV dose of temsirolimus via 30-minute infusion on study days 1 and 15 after an overnight fast. Pretreatment with IV diphenhydramine (25 mg) was administered approximately 30 minutes before start of temsirolimus infusion. On study days 15 through 21, each subject received a 400-mg oral dose (2 x 200-mg tablets) of ketoconazole with 240 mL water. On day 15, the ketoconazole dose was administered approximately 2 hours before the start of the temsirolimus infusion.

Formulation: Temsirolimus 25 mg/mL (formulation # 0932028J, batch # 2004B0050). Ketoconazole oral 200mg tablets were purchased commercially and supplied by the study site.

Pharmacokinetics: Blood samples were collected for determination of temsirolimus and sirolimus. In period 1, samples were collected on study day 1 before temsirolimus administration (predose) and at 0.5 (immediately before end of infusion), 1, 3, 8, 24, 48, 72, 96, 120, 144, and 168 hours after test article administration. In period 2, blood samples were obtained at the same times listed above after temsirolimus administration.

Pharmacokinetic Analysis: The whole blood concentration data for temsirolimus were analyzed for each subject in both study periods using a noncompartmental method. Least squares geometric mean ratios between test and reference treatments, and their 90% confidence intervals for Cmax, AUCT and AUC were determined using the WinNonlin Enterprise application version 4.110.

Safety: Routine safety and tolerability were evaluated from the results of reported signs and symptoms, scheduled physical examinations, vital sign measurements, ECGs, and clinical laboratory evaluations.

Assay Method: Temsirolimus and sirolimus concentrations were measured simultaneously using a validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) combination method with internal standard. This combination method was split to determine both a low range and a high range of concentrations.

Results: Of the 17 subjects enrolled, 14 subjects received both ketoconazole and temsirolimus. Mean concentrations of temsirolimus in whole blood were near comparable following treatment with concomitant ketoconazole as compared to temsirolimus alone. Exposures to sirolimus, however, were significantly increased with concomitant ketoconazole treatment. The mean PK parameters of temsirolimus and sirolimus with or without ketoconazole administration are below.

	Cmax (ng/mL)	Tmax (hr)	AUCt (hr ng/mL)	AUC (hr ng/mL)	Thalf
Temsirolimus					
without ketoconazole	278.3 (16.1)	0.5 (0.0)	873.4 (11.9)	885.5 (11.7)	22.31 (19.6)
with ketoconazole	262.6 (15.5)	0.5 (0.0)	969.1 (15.0)	991.6 (4.5)	23.67 (17.0)
Sirolimus					
without ketoconazole	13.3 (27.4)	3.3 (37.7)	918.3 (28.3)	1204.7 (30.2)	74.7 (10.8)

with ketoconazole	29.0 (13.6)	3.0 (0.0)	2410.7 (22.0)	2888.9 (29.2)	112.8 (18.1)

Time to maximal concentration was consistently at the end of infusion (0.5 hr) for temsirolimus and at 3 hr for sirolimus metabolite, irrespective of treatment. Mean temsirolimus Vdss was approximately 87 liters and appeared comparable among treatments. Mean clearance of temsirolimus appeared to decrease from 5.72 to 5.14 L/hr (approximately 10%) when temsirolimus was administered together with ketoconazole; however, this decrease did not manifest into a clinically relevant change in temsirolimus exposures. Change in the mean apparent metabolic clearance of sirolimus metabolite was more substantial, decreasing from 4.50 to 1.40 L/hr (approximately 69%) with ketoconazole treatment. Mean temsirolimus half-life was generally unchanged between treatments (from 22.3 to 23.8 hr). Sirolimus half-life increased from 74.7 hr for temsirolimus alone to 113 hr when given with concomitant ketoconazole.

Findings from statistical comparison indicated that the 90% confidence intervals (CI) for least squares geometric mean (LSGM) ratios of Cmax, AUCT, and AUC of temsirolimus were within the range of 80 to 125%. For sirolimus, respective LSGM ratio values of Cmax, AUCT, and AUC were higher than the upper confidence limit of 125% (see sponsors table 8.1-1 below) Collectively, data indicate that ketoconazole exhibited no significant effect on exposure to temsirolimus; however, exposures to sirolimus metabolite were increased by approximately 2.2-fold for Cmax and 3.1-fold for AUC.

Table 8.1-1: Least Squares Geometric Mean Ratios of Temsirolimus and Sirolimus

Pharmacokinetic Metric	LSGM Ratio (% of Reference)	Lower 90% CI	Upper 90% CI
Temsirolimus Ln(C_{max})	93.53	87.87	99.55
Temsirolimus Ln(AUC_T)	109.41	103.41	115.76
Temsirolimus Ln(AUC)	110.19	105.22	115.4
Sirolimus Ln(C_{max})	223.69	197.52	253.32
Sirolimus Ln(AUC_T)	251.57	232.29	272.44
Sirolimus Ln(AUC)	309.6	277.49	345.43

Reference: single dose temsirolimus IV 5 mg alone
Test: single dose temsirolimus IV 5 mg + ketoconazole oral 200 mg QD
Abbreviations: LSMG = least squares geometric mean; CI = confidence interval; Ln = logarithmic normal; C_{max} = peak concentration; AUC_T = area under the concentration time-curve; AUC = total area under the concentration-time curve.

The most frequently occurring AEs (≥10% of all subjects) were headache (29.4%), neutropenia (23.5%), pain (11.8%), and insomnia (11.8%). There were no deaths and no SAEs. One (1) subject discontinued the study due to an IV infiltration that was reported as an AE.

4.1.12 149-US: Desipramine study

Title: An open-label, nonrandomized, 2-period, sequential, drug interaction study to evaluate the potential pharmacokinetic interaction between temsirolimus administered intravenously as a 30-minute infusion and desipramine to healthy subjects.

Investigators: [redacted]

Study Period: June 2005 to July 2005

Clinical Phase: 1

Objectives: The primary objective was to evaluate the effect of IV temsirolimus on the pharmacokinetic profile of desipramine in healthy subjects. The secondary objective was to assess the safety and tolerability of temsirolimus and desipramine coadministered acutely.

Design: Each subject received a single 50-mg oral dose of desipramine on study days 1 and 15. Each subject received a single 25-mg IV dose of temsirolimus via 30-minute infusion on study day 15. Pretreatment with IV diphenhydramine (25 mg) was administered approximately 30 minutes before the start of temsirolimus infusion. Desipramine administration occurred at the start of the temsirolimus infusion on day 15. All subjects fasted from 10 hours before dose administration until 4 hours after receiving the dose.

Formulation: Temsirolimus 25 mg/mL (formulation # 0932028J, batch # 2005B0018). Desipramine oral 50-mg tablets (Norpramin) is available commercially and was supplied by the study site.

Pharmacokinetics: Subjects were genotyped for CYP2D6 isozyme on admission to the study unit on day -1.

Venous blood samples were collected for determination of desipramine and its metabolite 2-hydroxy desipramine in plasma at the following times on days 1 and 15: predose, 0.5, 2, 4, 8, 10, 12, 16, 24, 32, and 48 hours after desipramine administration. In addition, a blood sample was collected from poor CYP2D6 metabolizers for desipramine PK measurements on days 12, 26, and 30 (approximately 264 hours and 360 hours after dose administration in period 1 and period 2).

Venous blood samples were collected for determination of temsirolimus and sirolimus in whole blood at the following times on study day 15: predose, 0.5 (immediately before end of infusion), 2, 4, 8, 24, 48, 264 (day 26), and 360 (day 30) hours after temsirolimus administration.

Urine samples were obtained for determination of desipramine and its metabolite concentrations on study days 1 and 15 before desipramine administration and at the following intervals: 0 to 12 hours, 12 to 24 hours, and 24 to 48 hours after dose administration.

Pharmacokinetic Analysis: The plasma concentration data for desipramine and 2-hydroxy-desipramine, and the whole blood concentration data for temsirolimus and sirolimus were analyzed for each subject using a noncompartmental method. Least squares geometric mean (LSGM) ratios between test and reference treatments, their 90% confidence intervals for Cmax, AUCT, and AUC, and 2 one-sided T-tests were determined using the WinNonlin Enterprise application version 4.1.

Safety: Routine safety and tolerability were evaluated from the results of reported signs and symptoms, scheduled physical examinations, vital sign measurements, ECGs, and clinical laboratory evaluations.

Assay Method: Temsirolimus and sirolimus concentrations were measured simultaneously using a validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) combination method with internal standard. This combination method was split to determine both a low range and a high range of concentrations.

The bioanalytical method for measuring desipramine and 2-hydroxy-desipramine in sodium

heparin-treated plasma and in urine employed a liquid chromatography/tandem mass spectrometry (LC/MS/MS) combination method. For samples drawn from subjects in the present study, concentrations of desipramine and 2-hydroxy-desipramine in plasma and urine were determined and reported.

Results: Of the 26 subjects enrolled, only 23 completed both periods. Genotyping for 2D6 isozyme identified subject 0021 as a poor metabolizer.

Median Tmax was consistent at 8 hr for desipramine and 4 hr for 2-hydroxy-desipramine, irrespective of treatment. The mean (SD) desipramine Vz/F was approximately 2974 (1821) and 3548 (1909) L for the 2 treatments. The apparent clearance of desipramine appeared to slightly increase from 123 to 144 L/hr (approximately 17%) when desipramine was administered together with temsirolimus; however, this increase did not manifest into a clinically relevant change in desipramine exposures (about 12.7%). There was only 4.3% increase in the mean apparent metabolic clearance of 2-hydroxy-desipramine metabolite in the presence of temsirolimus. The mean desipramine half-life was about 24.7 (21.5) hr following desipramine alone treatment and 29.5 (31.43) hr following the combination treatment.

The observed pharmacokinetic values of temsirolimus are similar to those determined in study 3066K1-151-US following administration of temsirolimus alone.

Findings from statistical comparison indicated that the 90% confidence intervals (CI) for LSGM ratios of Cmax, AUCT, and AUC of desipramine were within the range of 80 to 125%. Statistical comparison was repeated after excluding subject 0021, the poor metabolizer by 2D6 genotyping. The results reaffirmed that the 90% CI for LSGM ratios of Cmax, AUCT, and AUC of desipramine were still within the range of 80 to 125%. There was statistically significant difference of desipramine Cmax (p-value = 0.001) and Vz/F (p-value < 0.001) by treatment. These differences did not manifest into clinically relevant change as confirmed by the 20% CI testing assessment of Cmax and AUC since the difference in the LSGM ratio was less than 20%.

Table 8.1-1: Least Squares Geometric Mean Ratios of Desipramine and 2-Hydroxy-Desipramine Following Oral Desipramine

Pharmacokinetic Metric	LSGM Ratio (% of Reference)	Lower 90% CI	Upper 90% CI
Desipramine Ln(C_{max})	87	81	93
Desipramine Ln(AUC_T)	91	85	98
Desipramine Ln(AUC)	96	85	109
2-hydroxy-desipramine Ln(C_{max})	91	78	105
2-hydroxy-desipramine Ln(AUC_T)	92	85	100
2-hydroxy-desipramine Ln(AUC)	95	83	110
Re-analysis results after excluding subject 0021[a]			
Desipramine Ln(C_{max})	86	80	92
Desipramine Ln(AUC_T)	89	84	96
Desipramine Ln(AUC)	94	83	107
2-hydroxy-desipramine Ln(C_{max})	91	78	106
2-hydroxy-desipramine Ln(AUC_T)	91	84	99
2-hydroxy-desipramine Ln(AUC)	95	82	109

Reference: Single 50-mg oral dose of desipramine alone
Test: Single 50-mg oral dose of desipramine coadministered with a single 25-mg IV dose of temsirolimus
Abbreviations: LSMG = least squares geometric mean; CI = confidence interval; Ln = logarithmic normal; C_{max} = peak concentration; AUC_T = area under the concentration time-curve; AUC = total area under the concentration-time curve.
a: Subject 0021 was a poor metabolizer; the statistical analysis of desipramine was repeated excluding this subject.

The most frequently occurring AEs (≥10% of all subjects) were stomatitis (50.0%), headache (23.1%), acne (23.1%), pain (15.4%), nausea (15.4%), pharyngitis (15.4%), rash (15.4%), accidental injury (11.5%), chills (11.5%), fever (11.5%), dry mouth (11.5%), and vomiting (11.5%).

4.1.13 151-US: Rifampin study

Title: A study to examine the potential effect of rifampin on the pharmacokinetics of intravenously and orally administered temsirolimus when administered concomitantly to healthy subjects.

Investigators: [redacted]

Study Period: July 2005 to August 2005

Clinical Phase: 1

Objectives: The primary objective was to evaluate the effects of multiple oral doses of rifampin on the PK profile of a single IV and a single PO dose of temsirolimus in healthy subjects. The secondary objective was to assess the safety and tolerability of the coadministration of rifampin and temsirolimus.

Design: This was an open-label, non-randomized, sequential, 2-period, parallel-group, study which enrolled healthy men and women. On Day 1 and 21 subjects received either:

- IV temsirolimus 25 mg infused over 30-mintues with diphenhydramine pretreatment 30 minutes prior.
- Oral temsirolimus 30 mg.

On days 15-27 subjects received 600 mg of rifampin each day.

Thirty-two subjects were enrolled and 16 subjects were assigned to receive IV temsirolimus and 16 subjects to PO temsirolimus.

Formulation: Temsirolimus 25 mg/mL (batch # 2005B0018). Temsirolimus oral 10mg (batch # 2004B0146). Rifampin 300 mg capsules are available commercially and was supplied by the study site.

Pharmacokinetics: Blood samples for the determination of temsirolimus and sirolimus in blood were collected on Day 1 and Day 21 at predose, 0.5 (immediately before end of infusion) 1, 2, 3, 8, 24, 48, 72, 96, 120, 144, and at 168 hours after temsirolimus administration.

Pharmacokinetic Analysis: The whole blood concentration data for temsirolimus were analyzed for each subject in both study periods using a noncompartmental method. Least squares geometric mean ratios between test and reference treatments, and their 90% confidence intervals for Cmax, AUCT, and AUC were determined using the WinNonlin Enterprise application version 4.1.

Safety: Routine safety and tolerability were evaluated from the results of reported signs and symptoms, scheduled physical examinations, vital sign measurements, ECGs, and clinical laboratory evaluations.

Assay Method: Temsirolimus and sirolimus concentrations were measured simultaneously using a validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) combination method with internal standard. This combination method was split to determine both a low range and a high range of concentrations.

Results: Of the 32 subjects enrolled, 3 subjects did not complete the study (1 from IV group, 2 from PO group). The pharmacokinetics of IV temsirolimus were comparable with concomitant rifampin treatment as compared to temsirolimus alone. The AUC and Cmax of sirolimus after IV temsirolimus administration was decreased approximately 65% and 56% respectively with concomitant rifampin administration. The mean (CV%) pharmacokinetic parameters of temsirolimus and sirolimus following a single 25 mg IV or 30 mg PO temsirolimus dose with or without rifampin are below:

	Cmax (ng/mL)	Tmax[a] (hr)	AUCt (hr ng/mL)	AUC (hr ng/mL)	Thalf (hr)
Temsirolimus					
IV					
without rifampin	512 (23)	0.5 (0.5, 1.0)	2044 (18)	2056 (18)	23.0 (10)
with rifampin	532 (9)	0.5 (0.0, 0.5)	1863 (21)	1879 (21)	22.6 (19)
PO					
without rifampin	5.81 (28)	1.0 (0.5, 2.00)	61.95 (38)	70.9 (34)	10.61 (31)
with rifampin	3.36 (19)	1.0 (0.5, 8.0)	40.5 (31)	49.8 (31)	14.5 (52)
Sirolimus					
IV					
without rifampin	50.6 (24)	2.0 (1.0, 24.0)	3652 (15)	4564 (16)	69.4 (19)
with rifampin	17.7 (18)	24 (1.0, 48.0)	1720 (19)	2004 (19)	55.1 (12)
PO					
without rifampin	17.4 (40)	2.0 (0.5, 3.0)	498 (32)	594 (31)	70.4 (20)
with rifampin	6.5 (35)	2.0 (1.0, 8.0)	193 (22)	235 (26)	76.4 (37)

a: Values for Tmax are median (min, max)

Findings from statistical comparison indicated that the 90% confidence intervals (CI) for LSGM ratios of Cmax, AUCT, and AUC of IV temsirolimus were within the range of 80 to 125%. For sirolimus, respective LSGM ratio values of Cmax, AUCT, and AUC were lower than the lower confidence limit of 80%, and yielded values of 36, 47, and 44%

Table 8.1.1-1: Least Squares Geometric Mean Ratios of Temsirolimus and Sirolimus following IV Administration of Temsirolimus

Pharmacokinetic Metric	LSGM Ratio (% of Reference)	Lower 90% CI	Upper 90% CI
Temsirolimus Ln(C_{max})	107	93	123
Temsirolimus Ln(AUC_T)	91	87	96
Temsirolimus Ln(AUC)	92	87	96
Sirolimus Ln(C_{max})	36	31	41
Sirolimus Ln(AUC_T)	47	43	52
Sirolimus Ln(AUC)	44	39	49

The Cmax of temsirolimus after an oral dose was 5.81 ng/mL and occurred approximately 1 hour after dosing. The AUC of temsirolimus is 70.9 ng hr/mL and for sirolimus is 594 ng hr/mL. The median Tmax for sirolimus was 2 hours. In the presence of rifampin the Cmax and AUC of temsirolimus decreased by 21% and 29% respectively. The sirolimus concentrations were also reduced in the presence of rifampin by 60% for Cmax and AUC which is similar to the IV results.

Results below from statistical comparison indicate that the 90% CI for LSGM ratios of Cmax, AUCT, and AUC of temsirolimus and sirolimus were lower than the lower confidence limit of 80% (80-125%) after oral administration of temsirolimus and rifampin.

Table 8.1.2-1: Least Squares Geometric Mean Ratios of Temsirolimus and Sirolimus following Oral Administration of Temsirolimus

Pharmacokinetic Metric	LSGM Ratio (% of Reference)	Lower 90% CI	Upper 90% CI
Temsirolimus Ln(C_{max})	59	54	65
Temsirolimus Ln(AUC_T)	67	55	82
Temsirolimus Ln(AUC)	71	61	84
Sirolimus Ln(C_{max})	40	36	45
Sirolimus Ln(AUC_T)	40	34	47
Sirolimus Ln(AUC)	40	34	47

Reference: single oral dose temsirolimus 30 mg alone
Test: single 30-mg oral dose of temsirolimus + rifampin oral 600 mg QD for 7 days after 6 days of rifampin pretreatment before the temsirolimus dose
Abbreviations: LSMG = least squares geometric mean; CI = confidence interval; Ln = logarithmic normal; C_{max} = peak concentration; AUC_T = area under the concentration time-curve evaluated to the last measurable timepoint; AUC = total area under the concentration-time curve.

The most frequently occurring AEs (≥10% of all subjects) were stomatitis/mucositis (53.1%), headache (40.6%), acne (21.9%), pain (15.6%), rash (12.5%), and pharyngitis (12.5%).

4.1.14 145-US: Single ascending dose study

Title: An open-label, nonrandomized, ascending single-dose PK/PD study of mTOR inhibition after a 30-mintue IV infusion of temsirolimus in healthy subjects.

Investigators: [redacted]

Study Period: September 2004 to November 2004

Clinical Phase: 1

Objectives: The primary objective was to quantify the exposure/response relationship of temsirolimus after IV administration using p-S6RP activation status in the blood. The secondary objective was to determine the pharmacokinetics (PK) of temsirolimus and metabolite in whole blood and plasma after a single IV dose. Another secondary objective was to determine the inter-subject variability of the biological response. In addition, the safety and tolerability of ascending single IV doses of temsirolimus in healthy subjects were assessed.

Design: Single IV doses of temsirolimus ranging from 1 to 25 mg via 30-minute infusion were evaluated in healthy subjects. On study day 1, each subject received a single dose of temsirolimus. Each subject received IV pretreatment of diphenhydramine (50 mg) at approximately 30 minutes before temsirolimus administration. All subjects fasted from 10 hours before dose administration until 4 hours after receiving the dose. Temsirolimus was

administered at only 1 dose level at a time, and administration at the next higher dose level was not begun until the safety and tolerability of the preceding dose had been evaluated through 5 days for each dose group and deemed acceptable.

Formulation: Temsirolimus 25 mg/mL (formulation # 0931851J; batch # 2004B0050).

Pharmacokinetics: Blood samples for the determination of temsirolimus and sirolimus in blood were collected on study Day 1 before dose administration (predose) and 0.25, 0.5 (immediately before end of infusion), 1, 2, 4, 8, 12, 24, 48, 72, 120, 168, 264, and 336 hours after temsirolimus administration.

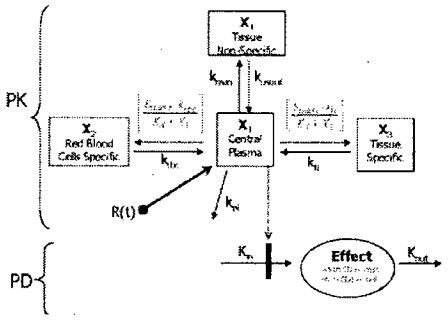
Hematocrit levels for the calculation of distribution of blood concentrations to erythrocytes were obtained at the following times: predose and at 0.25, 0.5 (immediately before end of infusion), 1, 2, 4, 8, 12, 24, 48, 72, 120, 168, 264, and 336 hours after test article administration.

Blood samples were collected to measure concentrations of p-S6RP on study day 1 before dose administration (predose) and 0.25, 0.5 (immediately before end of infusion), 2, 8, 24, 72, 168, 264, and 336 hours after test article administration.

Pharmacokinetic Analysis: The whole blood concentration data for temsirolimus were analyzed for each subject in both study periods using a noncompartmental method.

To determine a structural PK model, the nonlinear aspect of temsirolimus disposition related to FKBP-12 specific binding in blood cells and tissues required characterization. Composite concentrations of temsirolimus plus sirolimus (with units of temsirolimus equivalents) versus time in whole blood and plasma were analyzed by writing differential equations for a model with saturable distribution and solving using ADAPT II. The model also included a PD component in which drug concentrations in the central compartment were used to modulate a nonlinear inhibitory function of drug effect in circulating lymphocytes. Sirolimus is known to be equipotent to temsirolimus at inhibiting downstream signaling mediated through mTOR, so metabolite exposure was included in the model. To minimize mathematical complexity, the exposures of parent drug and metabolite were therefore combined for both the PK and PD aspects of the model.

Figure 2.3.2.2-1: PKPD Model of Temsirolimus and Sirolimus (Study 3066K1-145-US)



Safety: Routine safety and tolerability were evaluated from the results of reported signs and symptoms, scheduled physical examinations, vital sign measurements, ECGs, and clinical

laboratory evaluations.

Assay Method: Temsirolimus and sirolimus concentrations were measured simultaneously using a validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) combination method with internal standard. This combination method was split to determine both a low range and a high range of concentrations.

Results: Data from 30 subjects was available with 6 subjects per dosing group at 1-, 3-, 10-, 15-, or 25-mg. Below are the pharmacokinetic parameter derived from the non-compartmental analysis:

Dose (mg)	N	Cmax (ng/mL)	tmax (h)	t1/2 (h)	AUCt (ng.h/mL)	AUC (ng.h/mL)
Temsirolimus						
1	6	121 (10)	0.5 (0.5, 0.5)	20.1 (14)	360.4 (8.6)	373 (8.9)
3	6	278 (2.5)	0.5 (0.5, 0.5)	18.1 (15)	839 (8.2)	857 (8.4)
10	6	425 (13)	0.5 (0.5, 0.5)	15.8 (12)	1562 (17)	1612 (19)
15	6	476 (5.7)	0.5 (0.25, 0.5)	19.7 (11)	2048 (7.6)	2064 (7.6)
25	6	595 (7.0)	0.5 (0.25, 0.5)	16.6 (7.1)	2329 (17)	2339 (17)
Sirolimus						
1	6	3.78 (31)	4.0 (4.0, 4.0)	102 (5.1)	327 (17)	371 (14)
3	6	11.1 (24)	4.0 (4.0, 4.0)	100 (12)	976 (27)	1079 (27)
10	6	35.7 (6.6)	2.0 (2.0, 4.0)	67.9 (18)	2540 (11)	2564 (11)
15	6	47.8 (11)	2.0 (1.0, 4.0)	69.5 (12.5)	3895 (17)	4050 (17)
25	6	73.2 (21)	1.0 (1.0, 2.0)	65.6 (14)	6169 (21)	6386 (22)

Results from the modeled analysis indicated that the nonlinearity of exposure with dose was well explained by the provision of saturable and specific binding relationships in the blood cells and the peripheral tissue. The dissociation constant Kd was 14.2 µg, or 5.14 ng/mL in plasma, and indicates the concentration at which 50% of all specific binding sites are occupied by drug.

Maximal receptor occupancy was achieved during the infusion period, and dropped off rapidly following cessation of infusion. Maximal receptor occupancy was not achieved with the 1 mg dose (~56% by end of infusion), but was observed with the higher doses. Differences in the absolute profiles among the dose groups were probably due to differences in peripheral distribution with dose. Mean (10th, 90th percentile) maximal specific binding in blood cells was 1.4 mg (0.47, 2.5 mg) and tissue was 5 mg (0.94, 9.9 mg). These findings are important since they suggest that mean IV doses greater than 6.4 mg (1.41, 12.4 mg) (derived from the sum of 1.4 mg in blood plus 5 mg in tissue) would be needed to saturate specific central and peripheral tissue binding sites. It is thought that doses below this range could limit drug activity since specific binding is active and distribution is attenuated, while doses above this range would maximize peripheral tissue penetration and optimize therapy.

Individual profiles of p-S6RP versus time indicated that CD19+ cells were more profoundly

inhibited by temsirolimus than were CD3+ cells at lower (1 mg and 3 mg) doses. Results from modeling indicate a near consistently higher magnitude of values for IC50 (drug concentration for half-maximal response) in CD19+ cells at all doses as compared to CD3+ cells. This finding directly influenced the assessment of sensitivity to treatment, and was thought to arise from differences between CD3+ and CD19+ cell responsiveness to phorbal ester (PMA) induction during analytical processing of the blood sample.

Data indicate that following the 25-mg dose, S6RP is 20% inhibited from phosphorylation for 203 hours and is 50% inhibited for 82 h. Higher degrees of inhibition (greater than approximately 75%) were not attainable based on the observed data or by the global model.

4.2 QT REVIEW

For the Phase 3 studies ECGs were performed in triplicate at:

- the screening visit;
- during first treatment cycle
    - immediately prior to the end of pretreatment medication
    - immediately prior to the end of temsirolimus infusion (Cmax);
- 3 months after the first dose of temsirolimus
    - immediately prior to the end of temsirolimus infusion
- at the final evaluation.

In the Phase 1 clinical studies subjects were required to complete a standard 12-lead ECG at multiple time points after receiving a single dose of temsirolimus on study day 1 as follows:

- 133-US - predose (-2 hours); after study drug administration (0.5, 1, 2, 8, 24, 120, 192, and 264 hours postdose); and at the final study evaluation (336 hours postdose).
- 145-US - predose (-2 hours); after study drug administration (0.25, 0.5, 1, 2, 8, 24, 48, and 168 hours postdose); and at the final study evaluation (336 hours postdose).

QT results from the Phase 3 data were calculated using Fridericia and Bazett's correction for heart rate. Results for subjects in the Phase 1 studies were calculated using Bazett's correction for heart rate.

Study 304 - advanced renal cell carcinoma

The following treatment arms were studied:

- INF
- Temsirolimus 25 mg
- Temsirolimus 15mg/INF

The results for QTcF categorical analysis are below in Table 9. There were no patients at any time point with QTcF >500 msec. Following the first dose no patients in any treatment arm had a QTcF increase from baseline >30-60 msec or >60 msec.

After 3 months of treatment there were no patients in any treatment arm with QT or QTcF >450 msec (men) or >470 msec (women). One patient in the temsirolimus 25 mg arm had a QTcF increase from baseline > 60 msec after three months of treatment. The patient's mean QTc(F) was 337 msec predose and 410 msec at 3 months post first dose, which was within normal range (a 73 msec increase in QTc(F) from baseline).

At the withdrawal visit there were no patients in any treatment arm with QT or QTcF >450 msec

(men) or >470 msec (women). One patient in the temsirolimus/INF arm had an QTcF increase from baseline >60msec. This patient's mean QTc(F) was 349 msec predose and 423 msec at the withdrawal visit, which was within normal range. The patient had received the last dose of temsirolimus approximately 1 week before the withdrawal visit.

TABLE 22. Incidence of potentially clinically important values in QTcF interval in Study 304

	INF	TEMSR 25 mg	TEMSR 15mg/IFN
First Dose (n)	**8**	**15**	**11**
QTcF Interval >450 msec (♂) or >470 msec (♀)		1	
QTcF Interval <450 msec (♂) or <470 msec (♀)	8	14	11
Increase from baseline 0-30 msec	5	6	6
3 Months post dose (n)		**5**	**3**
QTcF Interval <450 msec (♂) or <470 msec (♀)		4	1
Increase from baseline 0-30 msec		3	1
Increase from baseline >60 msec		1	
Withdrawal Visit (n)	**4**	**10**	**5**
QTcF Interval <450 msec (♂) or <470 msec (♀)	4	10	5
Increase from baseline 0-30 msec	1	2	1
Increase from baseline >30-60 msec	1	2	1
Increase from baseline >60 msec			1

No overall tendencies were evident in QT changes over time. No statistically significant change from baseline in QTc(F) interval was seen within any treatment arm ($p>0.05$). Pairwise treatment comparisons did not show any statistically significant difference between either the temsirolimus 25 mg or temsirolimus 15 mg/IFN arm versus the IFN arm ($p>0.05$).

Regarding cardiovascular treatment-emergent adverse events, there were no reports of torsade de pointes. Four (4) patients had arrhythmia (3 in the IFN arm and 1 in the temsirolimus 15 mg/IFN arm). All but 1 case of arrhythmia was rated grade 1 in severity, and none resulted in treatment discontinuation. The remaining case of arrhythmia, which occurred in the temsirolimus 15 mg/IFN arm, was grade 2 atrial fibrillation as reported by the investigator and thus not related to an effect on cardiac repolarization.

Study 305 - Mantle Cell Lymphoma Study

Patients were randomized to receive temsirolimus IV 175 mg (3 successive weekly doses) followed by either temsirolimus 75 mg or 25 mg weekly, or patients could receive investigators choice of single-agent treatment. QT data was obtained from the following patients:

- 27 patients in the Temsirolimus 175/25 mg treatment arm
- 26 patient in the Temsirolimus 175/75 mg treatment arm
- 16 patients in the investigators choice arm.

The results for QTcF categorical analysis are below in Table 10. There were no patients with a QTc(F) interval greater than 500 msec.

Following the first dose there were three patients in the temsirolimus 175/75 mg arm who had longer than normal QTcF values (absolute value >450 msec [men] or >470 msec [women]). Of the 67 patients with QT data, eight patients in the temsirolimus 175/25 mg arm, three patients in the 175/75 mg arm and patients in the investigator choice arm had

At 3-months of treatment two patients in the temsirolimus 175/75 mg arm had a QTc(F) interval

>450 msec [men]or >470 msec [women]. One of these patients had a QTcF change from baseline >30-60 msec. This patient was a male who had a predose QTcF of 432 msec and a QTcF of 463 at 3-months.

At the withdrawal visit one patient in the temsirolimus 175/25 mg arm and one patient in the 175/25 mg arm had QTc(F) interval >450 msec [men]or >470 msec [women]. Each of these patients had a QTcF increase from baseline >30-60 msec. One patient was a male randomized to the temsirolimus 175/75 mg arm who had an QTc(F) of 487 msec at the withdrawal visit. The patient's mean predose QTc(F) was 435 msec (52 msec change). The patient had discontinued treatment approximately 5 weeks earlier, had a pacemaker, and was receiving concomitant Sotacar.

The other patient was a female randomized to the temsirolimus 175/25 mg arm, had a QTc(F) of 478 msec at the withdrawal visit, which occurred approximately 1 week after the last dose of study drug. The patient's mean predose QTc(F) was 438 msec (40 msec change). Notably, the patient's ECGs were obtained once at each time point rather than in triplicate. The patient had cardiac arrhythmia (right bundle branch block) at study withdrawal, which the investigator judged to be probably not related to treatment. The patient did not experience syncope, seizure, or dizziness while on treatment or at the withdrawal visit.

TABLE 23. Incidence of potentially clinically important values in QTcF interval in Study 305

	TEMSR 175/25 mg	TEMSR 175/75 mg	Investigator Choice
First Dose (n)	**26**	**26**	**15**
QTcF Interval >450 msec (♂) or >470 msec (♀)		3	1
Increase from baseline 0-30 msec		2	1
QTcF Interval <450 msec (♂) or <470 msec (♀)	26	23	16
Increase from baseline 0-30 msec	8	3	7
3 months post dose (n)	**7**	**8**	**0**
QTcF Interval >450 msec (♂) or >470 msec (♀)		2	
Increase from baseline 0-30 msec		1	
Increase from baseline >30-60 msec		1	
QTcF Interval <450 msec (♂) or <470 msec (♀)	7	6	0
Increase from baseline 0-30 msec	3	3	
Withdrawal Visit (n)	**10**	**7**	**7**
QTcF Interval >450 msec (♂) or >470 msec (♀)	1	1	
Increase from baseline >30-60 msec	1	1	
QTcF Interval <450 msec (♂) or <470 msec (♀)	9	6	7
Increase from baseline 0-30 msec	1	4	2

Analysis of within-treatment differences did not reveal any overall tendencies in QTc(F) interval. Pairwise treatment comparisons showed no statistically significant differences in QTc(F) between temsirolimus arms at any post-baseline time point ($p>0.05$).

Phase 1 studies

Only Bazett's correction was used to analyze the data from the Phase 1 studies. ECGs were obtained for 6 healthy subjects in study 133 and 30 healthy subjects in study 145 who Of these, 6 subjects in each study received temsirolimus 25 mg. No subject who received temsirolimus in these studies had a clinically noteworthy value for QTc(B) interval at any time point in the study. There were no reports of torsade de pointes or other arrhythmia in these studies.

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