CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

20-981

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA Number:	22-981
Serial Number:	000
Received by CDER:	April 11, 2007
Product:	HYCAMPTIN®
Clinical Indication:	Relapsed small cell lung cancer
Sponsor:	GlaxoSmithKline Research Triangle Park, North Carolina 27709
Documents Reviewed:	N000, electronic submission
Review Division:	Division of Drug Oncology Products
Reviewer:	W. David McGuinn, Jr., M.S., Ph. D., D.A.B.T.
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Clinical Pharmacologist:	Sophia Abraham, Ph. D.
Review draft completed:	September 20, 2007
Date of review submission to DFS:	October 5, 2007

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Submitted Non-clinical Studies not reviewed

None

Studies reviewed by Dr. Alan Taylor, February 28, 1989, IND 32,693

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PP 002 CM: Chemotherapeutic Activity of SK&F 1 04864-A and SK&F 104864-E Against B16 and B16F10 Melanoma in Female B6D2 F1 Mice
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Studies reviewed by Dr. Doo Young Lee-Ham, July 25, 1995, IND 42,993

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Studies reviewed by Dr. W. David McGuinn, Jr., May 24, 1996, NDA 20-671.

PP 013 CM: Biochemical and Cellular Pharmacological Studies of SK&F 104864, a Specific Inhibitor of Eukaryotic Topoisomerase-I

Addendum to SK&F Report No. PP001CM (October 1995)

Addendum to SK&F Report No. PP004CM (October 1995)

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TP-1034:	SK&F 104864-A: 1 month intravenous toxicity study in dogs TP-1021: 5 day oral toxicity study in dog
BP-005C:	Determination of ^{14}C-SK&F 104864-A Binding to Protein in Human, Dog and Rat Plasma and the Blood to Plasma Ratio in Dog Blood

BF-1007:	^{14}C-SK&F 104864A: Studies of Plasma Protein Binding and Blood Cell Binding in vitro (Rat, Dog and Man)
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BF-1005:	The effect of SK&F 104864-A on hepatic levels of cytochrome P450 and related parameters in male and female Sprague Dawley rats after oral dosing at 0, 0.0023, 0.023 and 0.23 mg free base/kg/day
BP- 010:	An *in vitro* investigation of the inhibitory potential of topotecan CSKF 104864) on the human cytochrome P450 enzymes CYP IA2, CYP2A6, CYP2C8/9, CYP2D6, CYP2E, CYP3A and CYP4A
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BF-1004:	Excretion and plasma concentrations of drug related material following single intravenous administration of ^{14}C-SK&F 104864A to male and female rats and dogs at target levels of 1 mg pure free base/kg and 0.5 mg pure free base/kg, respectively Dog
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BP-1001: Validation of a sensitive assay for determination of SKF 104864 and total drug (SKF 104864 and SKF 105992) and drug stability verification in extracts of dog plasma

BF-1006: Analysis of topotecan and topotecan as the total of the lactone plus carboxylate form in human plasma using HPLC with fluorescence detection

BF-1001: Analysis of topotecan and topotecan as the total of the lactone plus carboxylate form in rat plasma using HPLC with fluorescence detection

BF-1002: Analysis of topotecan and topotecan as the total of the lactone plus carboxylate form in dog plasma using HPLC with fluorescence detection

EXECUTIVE SUMMARY

Recommendations

Recommendation on Approvability

The available Pharmacology and Toxicology information is adequate to support the approval of HYCAMPTIN™ for use in the proposed clinical indication.

Recommendation for Non-clinical studies

None

Recommendations on Labeling

Most of the sections of the product label that contain non-clinical information are the same as those for the IV formulation of Topotecan. This information applies appropriately to the oral formulation.

The following text should be added to the product label:

8.3 Nursing Mothers

Rats excrete high concentrations of Topotecan into milk. Lactating female rats given 4.72 mg/m^2 (about twice the clinical dose on a mg/m^2 basis), excreted Topotecan into milk at concentration up to 48-fold higher than those in plasma. It is not known whether Topotecan is excreted in human milk. [redacted] b(5)

[redacted]

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity testing of topotecan has not been done. Topotecan, however, is known to be genotoxic to mammalian cells and is a probable carcinogen. Topotecan was mutagenic to L5178Y mouse lymphoma cells and clastogenic to cultured human lymphocytes with and without metabolic activation. It was also clastogenic to mouse bone marrow. Topotecan did not cause mutations in bacterial cells.

Topotecan given to female rats prior to mating at a dose of [redacted] the clinical dose on a mg/m^2 basis) caused superovulation possibly related to inhibition of follicular atresia. This dose given to pregnant female rats also caused increased pre-implantation loss. Studies in dogs given 0.4 mg/m^2 (about 1/6th the clinical dose on a mg/m^2 basis) of topotecan daily for a month suggest that treatment may cause an increase in the incidence of multinucleated spermatogonial giant cells in the testes. Topotecan may impair fertility in women and men. b(5)

Summary of Non-clinical findings

Overview of Non-clinical findings

In basic solution, hydroxyl ions hydrolyze topotecan to the hydroxy carboxylate open ring form. At physiological pH, the in active open ring form predominates. Topotecan is a very potent inhibitor of DNA replication. The inhibitory binding constant of topotecan at the DNA site is approximately μM. Because of the hydrolysis reaction, the actual binding constant is probably much lower.

The sponsor conducted an array of preclinical studies in the mouse, rat, rabbit, and dog, thus they have adequately characterized the toxicities associated with topotecan. In mice given IV doses of topotecan daily for five days the LD_{10} is about 14 mg/m^2 and the LD_{90} is about 29 mg/m^2; the slope of the dose response curve is about what one would expect for such a cytotoxin. The LD_{50} in rats given five daily doses is about 14 mg/m^2, or about six times the proposed clinical dose on a mg/m^2 basis, suggesting an uncomfortably small therapeutic index. Dogs do not tolerate one-third the clinical dose given for 28 days. Dogs appear best to predict the toxicities of topotecan.

The dose limiting toxicities in all these species are myelosuppression and anemia. The onset of myelo-suppression is rapid; the degree of myelosuppression is duration and dose dependent. Dosing causes the microscopic damage usually associated with myelo-suppression; bone marrow hypocellularity, cellular depletion of the spleen, thymus, and lymph nodes. Treatment is also associated with mild anemia. These parameters usually recover to near control values within weeks after dosing stops.

Repeated dosing with topotecan caused increases in ALT and AST suggesting liver damage. It also causes transient changes in the urinalysis parameters, suggesting mild kidney damage. Damage to the GI was remarkably rare and usually seen only in long-term studies of oral dosing.

Topotecan is a teratogen and a fetotoxin at doses well below the proposed clinical dose in rats and rabbits. Dosing is associated with fetal resorption, post-implantation mortality, and fetal malformations of the eye, brain, skull and vertebrae. Dosing also possibly causes damage in the testes in dogs. Lactating rats secrete Topotecan into breast milk. Topotecan given to female rats prior to mating at a dose of 0.23 mg/m^2 caused superovulation possibly related to inhibition of follicular atresia. This dose given to pregnant female rats also caused increased pre-implantation loss. Studies in dogs given 0.4 mg/m^2 of topotecan daily for a month suggest that treatment may cause an increase in the incidence of multinucleated spermatogonial giant cells in the testes. Topotecan may impair fertility in women and men.

The sponsor did not do carcinogenicity tests with topotecan. Nevertheless, topotecan is genotoxic to mammalian cells *in vivo* and *in vitro*. It caused significant increases in mutations in mouse L5178Y lymphoma cells at 0.1 μg/ml (~2.4 μM) in the presence and absence off S9. It was also strongly positive in the mouse micronucleus test. It did not cause mutations in bacterial cells.

Intravenous doses of topotecan are distributed through the body in less than ten minutes. The elimination half-life, $t_{1/2}\beta$ in dogs is about 100 minutes. The rat eliminates topotecan faster than the dog; $t_{1/2}\beta$ is about 50 minutes. The studies in the rat suggest a second elimination phase, $t_{1/2}\gamma$ ~349 minutes. Studies in the mouse rat suggest significant diurnal enterohepatic recirculation. In all these species, AUC and C_{max} increases proportional to dose and repeat dose studies suggest significant accumulation. Sustained daily C_{max} values greater than about 1 μM were lethal to dogs in a three month study. The volume of distribution at steady state is greater

than total body water, 2.8 l/kg in the dog, suggesting depot sequestration. In all species, tested protein binding is between 25.3 and 39.7%.

Oral bioavailability of topotecan in the dog is about 35%. Rats with bile duct cannulae still secrete about 18% of an intravenous dose of topotecan in the feces. This suggests significant intestinal secretion. After 24 hours, rats excrete approximately 43% of a topotecan dose in the urine and about 41% in the feces as the parent compound. Less than 1% of the dose appears in the urine as the N-demethylated metabolite; about 3% of the dose appears in the feces as this metabolite. Excretion in the dog is similar. The N-demethylated compound is the only major metabolite formed by any species. Hepatic microsomes from rat, dog and human form the N-desmethyl metabolite from the parent compound. Cytochrome P450 CYP1A2 is possibly responsible for most of this reaction. Topotecan does not induce any major hepatic cytochrome P450 isozymes nor does it inhibit any major cytochrome P450 activities *in vitro.*

One toxicology study submitted is adequate to qualify the impurity, [redacted], in drug product batches of topotecan at a specification limit of [redacted] %. Two other studies that compared the toxicity of different batches of topotecan do not clearly demonstrate toxicological equivalence among those batches. b(4)

Pharmacological activity:

Topoisomerase I is a nuclear enzyme that relieves torsional strain in DNA by opening single strand breaks. Once topoisomerase I creates a single strand break, the DNA can rotate freely in front of the advancing replication fork created by helices during DNA synthesis. Topotecan binds reversibly to the Topoisomerase I - DNA to form a tertiary complex that appears to prevent religation of these single strand breaks. Double strand DNA damage occurs during DNA synthesis when cells are treated with Topotecan. Mammalian cells cannot efficiently repair these double strand breaks. This damage probably initiates apoptosis. From this mechanism, the sponsor and others have hypothesized that Topotecan clinical efficacy results because cancer cells divide more frequently than normal somatic cells, thus they will be differentially affected by topotecan toxicity. This pharmacological mechanism is also the major mechanism of Topotecan toxicity.

Non-clinical safety issues relevant to clinical use

Topotecan Oral is a potent cytotoxin. It causes dose level and dose frequency related myelosuppression, thrombocytopenia and anemia that is usually dose limiting. The onset of myelosuppression can be rapid (less than 8 days) and profound. Animals and humans can usually recover from this toxicity within two to three weeks after treatment ceases. Repeated dosing with topotecan can cause increases in ALT and AST suggesting liver damage. Histology did not confirm hepatic toxicity, probably because myelosuppression is dose limiting. There is also potential for damage to the kidneys and the gastrointestinal system. Topotecan is a potent mutagen and clastogen consistent with its mechanism of action. Studies to determine its potential as a carcinogen have not been done. Lastly, Topotecan is a fetotoxin and teratogen at doses lower than the proposed clinical dose.

PHARMACOLOGY/TOXICOLOGY REVIEW

Introduction and Drug History

NDA number	20-981
Review	number 1
Submission	000

Information location in EDR:

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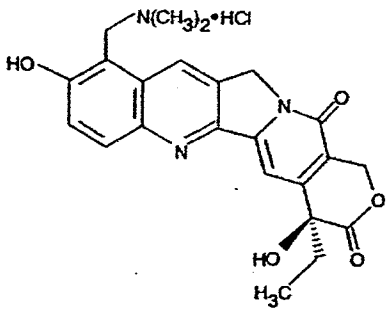
Information to sponsor	No
Sponsor	GlaxoSmithKline Research Triangle Park, North Carolina 27709
Reviewer name	W. David McGuinn, Jr., M.S., Ph. D., D.A.B.T.
Division name	Division of Drug Oncology Products
Review completion date	

Drug

Code Name:	SK&FS-104 864-A
Generic Name:	Topotecan
Trade Name:	HYCAMTIN®
Chemical Name:	(S)-10-[(dimethylamino)methyl]-4-ethyl-4,9-dihydroxy-1H-pyrano[3',4':6,7]indolizino[1,2-b]-quinoline-3,14-(4H,12H)-dione monohydrochloride
CAS Number:	123948-87-8

FW = ——— (free base) g/mole, b(4)

FW = 457.91 (hydrochloride salt) $C_{23}H_{23}N_3O_5 \bullet HCl$



Structure:

Relevant INDs & NDAs	IND 42,993; IND 32,693 NDA 20-671
Drug class:	Topoisomerase I inhibitor
Intended clinical population:	Relapsed small cell lung cancer

Clinical formulation: Hard gelatin capsules containing Topotecan HCl 0.25 and 1 mg

Table 1 Composition of Topotecan 0.25 mg and 1 mg Capsules

Component	Quantity (mg/capsule)		Function	Reference to Standard
Strength	0.25 mg	1 mg		
Topotecan Hydrochloride				GlaxoSmithKline
Hydrogenated Vegetable Oil				Supplier
Glyceryl Monostearate				USNF
Preprinted Hard Gelatin Capsule, Size				Supplier
				USNF
				USP

b(4)

Note:
1. The actual quantity of topotecan hydrochloride required to provide 0.25 mg or 1 mg as free base is calculated for each batch based on the assay (as free base) of the drug substance using the equation:
theoretical free base * 100 / assay = amount of topotecan hydrochloride to add to batch.
For example, 0.25 mg or 1 mg topotecan free base capsules manufactured with drug substance with an assay (as free base) of %, contain: mg topotecan hydrochloride.
2.

Route of administration Oral
Dose and schedule 2.3 mg/m^2/day orally once daily for 5 consecutive days
Repeated every 21 days

Disclaimer: I have reconstructed all tabular and graphical information directly from the sponsor's electronic and paper submissions unless otherwise specified. I calculated the percentage differences in physiological parameters in tables as:

(Value in exposed animal – value in control) ÷ (value in control)

I have used some information from my review of NDA 20-671 of IV topotecan, May 1996 and I have reviewed several studies submitted to IND 32,693 but not included in this NDA submission. I have also excerpted information directly from the sponsor's submissions.

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 20-981 are owned by GlaxoSmithKline or are data for which GlaxoSmithKline has obtained a written right of reference. Any information or data necessary for approval of NDA 20-981 that GlaxoSmithKline does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that GlaxoSmithKline does not own (or from FDA reviews or summaries of a previously approved

application) is for descriptive purposes only and is not relied upon for approval of NDA 20-981.

Pharmacology

Pharmacology Summary

Wall M.E. et al. (*J. Amer. Chem. Soc.* **88**: 3888-3890, 1966) isolated camptothecin from Camptotheca acuminata in 1966 based on activity against murine leukemia cells *in vitro* and in vivo. The US National Cancer Institute tested this compound in phase I and phase II trials, but abandoned it because of its toxicity; primarily hemorrhagic cystitis and unpredictable myelosuppression, emesis and diarrhea. During these early studies, investigators did not realize that the lactone was the active form of the drug, nor did they know that camptothecin bound tightly to human serum albumin. The formation of the active lactone in acidic urine probably caused the dose limiting hemorrhagic cystitis associated with camptothecin. Efficacy was probably diminished by the drug's formulation. The sponsor developed topotecan as derivative of camptothecin that is water soluble in the lactone form and binds less strongly to plasma proteins (Kingsbury W.D., et al., *J.Med. Chem.* **34**: 98-107, 1991). They anticipated that these changes would improve efficacy and decrease toxicity.

Topoisomerase I opens a single strand break in DNA at phosphodiester bonds. These single strand breaks allow the DNA to rotate and thus relieve torsional strain in supercoils caused by the moving replication fork. Topoisomerase I may also relieve torsional strain during transcription. Once the torsional strain is relieved and replication process is complete, topoisomerase I religates the phosphodiester bond.

Topotecan and related compounds inhibit DNA topoisomerase I by preventing the preventing this religation reaction. This potent cytotoxin forms a ternary complex with the topoisomerase I and DNA. Nevertheless, the binding of topotecan to topoisomerase I is reversible. This ternary complex can be isolated and characterized by electrophoresis. Camptothecins do not bind to DNA or topoisomerase I alone, but only to the DNA-topoisomerase I complex.

Topotecan stabilizes the single strand break opened in the DNA by topoisomerase 1. The interaction between this stabilized complex with the advancing replication fork of the DNA replication process has not been adequately described. Nevertheless, this interaction causes double strand breaks in the DNA. Eukaryotic cells cannot efficiently repair these double strand breaks and the cells die, possibly after initiating apoptosis. Inhibition of DNA replication with an inhibitor of DNA polymerase a such as aphidicolin blocks the ability of camptothecin and related compounds to produce double strand breaks and cytotoxicity without interfering with their effect on topoisomerase 1. Thus, topoisomerase I inhibitors are S-phase specific.

Topotecan is cytotoxic to human and murine tumor cells in culture. In the human tumor stem cell assay, 1 μg/ml of topotecan inhibited a significant proportion of primary tumor explants. Consistent with the S phase-specific mechanism of the drug, longer exposures caused greater inhibition. The sponsor and other investigators have tested topotecan against a variety of in vitro and in vivo tumor models. Topotecan is active against many of these tumors and as expected efficacy increases with dose and length of exposure.

In tests performed by the sponsor and others, topotecan showed no major pharmacological activity other than the anticipated topoisomerase I inhibition. It caused no

unexpected effects on locomotor activity, hexobabrital sleeping time, behavior, body temperature, electroshock response, pain response, or response to seizure producing drugs. Topotecan dosing caused only minor (~10%) and inconsistent changes in cardiac parameters (heart rate, blood pressure, left ventricular pressure) in dogs. Topotecan did not affect intestinal motility or contractility. Doses of 2.54 mg/kg in rats caused significant (22 to 34%) increases in urine volume 2 to 5 hours after dosing. Changes in urinary Na^+, K^+ and Cl^- accompanied this increase in volume. These minor changes may result from nephrotoxicity.

Pharmacology Review

No new studies

Safety Pharmacology

See my review of Topotecan IV, NDA 20-671.

Safety Pharmacology Review

1) Secretion of Drug-Related Material in the Milk of Lactating Rats Following a Single Intravenous Dose of ^{14}C-SK&F-104864-A to a Rat (*sic*) at a Nominal Dose Level of 4.72mg pfb/m^2 (0.8 mg pfb/kg)

Major findings

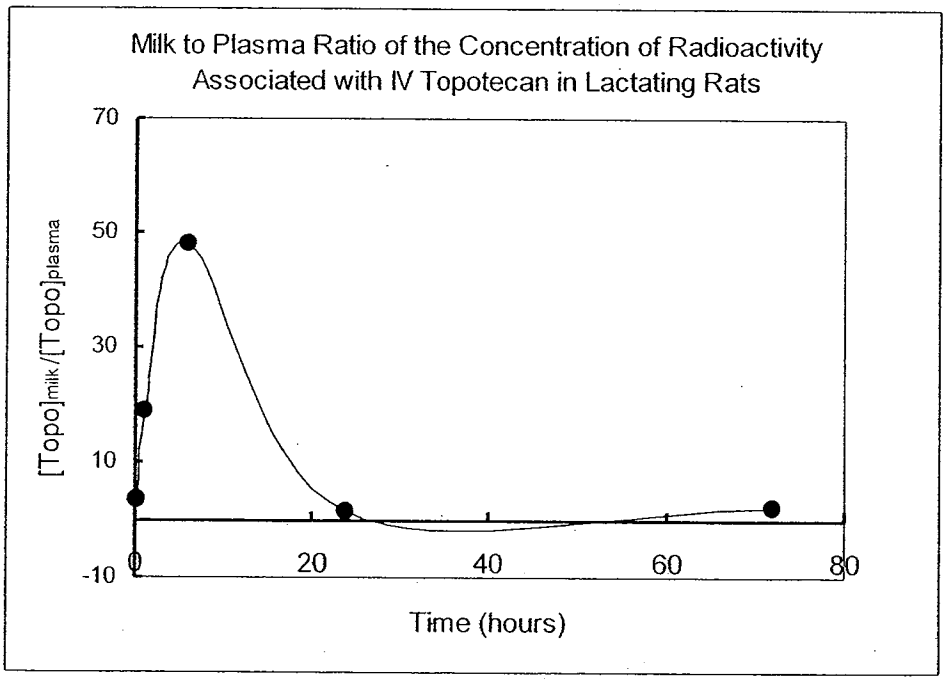
Lactating rats secrete the radioactivity associated with IV topotecan rapidly into milk. The ratios of the concentrations in milk to those in plasma are so high as to suggest an active process. The concentration in milk remains higher than that in plasma as longer than 72 hours after dosing.

Study number	6146-117 Submitted to IND 32,693, Feb. 20, 1998, IT Seq. 453
Conducting laboratory	[redacted]
Date of study initiation	September 1996
GLP compliance	Yes
QA report	Yes
Drug	^{14}C-SK&F-104864-A, Batch No. KG 27022-174A1 specific activity 0.098 mCi/mg, chemical purity 83.6% Radiochemical purity >99% as hydrochloride salt and SK&F-104864-A, Batch No. MM-19117-249 (used only for reference)
Methods	
Doses	4.72mg pfb/m^2 (0.8 mg pfb/kg)
Species	female Sprague Dawley rats ([redacted] CD(SD)BR), age 8 weeks, body weights ca 135-179 g b(4)
Number	15, 3 per sampling time
Route	IV, tail vein
Schedule	Single dose day 13-post partum

Formulation	3% (w/v) aqueous mannitol (pH 3.0)
Sampling time	10 min, 1, 6, 24, and 72 hours post dosing

The following table and graph show that lactating rats rapidly secrete the radioactivity associated with IV topotecan into milk at relatively high concentrations. The ratio of the concentration in milk to the concentration in plasma is so high at 1 and 6 hours as to suggest active secretion.

Time (hr)	[Topotecan] in Milk µg eq/g	SD	Milk to Plasma Ratio	SD
0.17	0.79	0.09	3.6	0.6
1	2	0.1	19	4
6	1.4	0.1	48	10
24	0.01	0.002	1.6	0.2
72	0.005	0.003	2	0.8



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2) Phototoxicity

The sponsor included the following text in the non-clinical overview:

"The potential for phototoxicity has not been addressed in previous submissions. Topotecan exhibited two peaks at the boundary of the UVA/UVB region of the absorption spectra (— and — nm; see NDA 20-671/S-013). Despite some retention of radioactivity in uveal tract/retina and skin in quantitative whole body autoradiography studies, no toxicity was identified in the eyes or skin of pigmented dogs during 6-month repeat dose oral toxicity studies. Additionally, there were no reports of skin or eye adverse events in recent continuous infusion cycle clinical studies. In light of this and the seriousness of the indication (relapsed SCLC), the risk of phototoxicity from oral treatment with topotecan is considered minimal."

b(4)

In the absence of definitive phototoxicity studies, I tentatively concur with this statement.

Pharmacokinetics and Toxicokinetics

Pharmacokinetics and Toxicokinetics Summary

Intravenous doses of topotecan are distributed through the body in less than ten minutes. The elimination half-life, $t_{1/2}\beta$ in dogs is about 100 minutes. The rat eliminates topotecan faster than the dog; $t_{1/2}\beta$ is about 50 minutes. The studies in the rat suggest a second elimination phase, $t_{1/2}\gamma$ ~349 minutes. Studies in the mouse suggest significant diurnal enterohepatic recirculation. In all these species, AUC and C_{max} increases proportional to dose at non-toxic doses. Repeat dose studies suggest significant accumulation at the highest doses, probably due to saturation of an essential metabolism and accumulation in fatty, i.e. acidotic, tissues. High repeat doses result in serious toxicity because of this accumulation. High oral doses may also saturate absorption. The volume of distribution at steady state is greater than total body water, 2.8 l/kg in the dog, suggesting depot sequestration. One hour after an intravenous dose in rats, the highest concentrations of radio labeled topotecan are in the liver, muscle and skin. Little of the dose remained anywhere in the animal after 120 hours. A small amount of topotecan may enter the brain. Because of the hydrolysis reaction, the time verses concentration curves for topotecan and that of total administered dose should diverge. They do, but not to the extent the equilibrium constant would suggest. Some factor in the plasma stabilizes the topotecan and it is possible that the lactone form is more highly bound than the open ring carboxylate. In all species tested, protein binding of total topotecan was between 25.3 and 39.7%. This is much less than the original topoisomerase I inhibitor, camptothecin.

Oral bioavailability of topotecan in the dog is about 35%. Rats with bile duct cannulae still secrete about 18% of an intravenous dose of topotecan in the feces. This suggests significant intestinal secretion. After 24 hours, rats excrete approximately 43% of a topotecan dose in the urine and about 41% in the feces as the parent compound. Less than 1% of the dose appears in the urine as the N-demethylated metabolite; about 3% of the dose appears in the feces as this metabolite. The N-demethylated compound is the only major metabolite formed by any species. Hepatic microsomes from rat, dog and human form the N-demethyl metabolite from the parent compound at about the same rate, 1 to 3 pmol/min/mg. Cytochrome P450 CYP1A2 is possibly responsible for most of this reaction. Less than 3% of the dose appears as unidentified polar metabolites. At 24 hours, dogs eliminate less of a topotecan dose in both the urine and the feces, but the proportion in each is about the same. Again, the N-demethyl compound is the only major

identified metabolite. Topotecan does not induce any major hepatic cytochrome P450 isozymes nor does it inhibit any major cytochrome P450 activities in vitro. The following table shows C_{max} in the dog after a single IV dose. If the inhibitory constant for topotecan is approximately μM these doses provide only just high enough concentrations of topotecan. Nevertheless, numbers are deceiving. The actual k_i at the enzyme active site is not known, intracellular pH may influence concentrations of topotecan at the active site.

Dose mg/m^2	C_{max} ng/ml	C_{max} μM
4	137	0.33
10	219	0.52
25	611	1.45

Pharmacokinetics and Toxicokinetics Review

1) Quantitative tissue distribution of drug-related material in the rat following a single oral administration of ^{14}C-SK&F 104864-A at a target dose level of 0.8 mg fb/kg (4.72 Mg Fb/m^2)

(fb refers to the free base of topotecan)

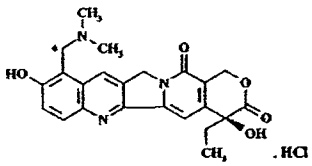
Major findings

Radioactivity distributed to all major tissues following oral administration of ^{14}C-SK&F-104864-A at 0.8 mg fb/kg to rats. Concentrations of radioactive material in most tissues declined rapidly and there was no evidence of long-term retention of radioactivity in any tissue. There were no differences in the distribution of radioactivity between males and females.

Study number	158817, Contractor's Report No. 14468 RSD-100J35-2, In the NDA Submission
Conducting laboratory	b(4)
Date of study initiation	September, 1996
GLP compliance	Yes
QA report	Yes
Drug	^{14}C-SK&F-104864-A, Batch No. KG 27022-174A1 specific activity 3.626 MBq/mg, chemical purity 90.8% Radiochemical purity >99% as hydrochloride salt and SK&F-104864-A, Batch No. MM-19117-249 (used only for reference)
Methods	
Doses	0.8 mg fb/mL (ca 4.72 Mg Fb/m^2) 10 mL/kg
Species	male and female Sprague Dawley rats (⁻ .CD(SD)BR) age 7-8 weeks, body weights ca 150-220 g
Number	18 per sex
Route	PO
Schedule	Single dose
Formulation	3% (w/v) aqueous mannitol (pH 3.0) non-radiolabeled compound was not added to radiolabeled compound

Methods

Three animals of each sex were killed at each of the following times after dosing, 0.5, 2, 6, 24, 48 and 96 h. Tissues and organs were excised from each animal. The concentration of total radioactivity was determined in each sample by liquid scintillation counting



*Site of radiolabel

Results

The following tables (sponsor's) show the average concentrations of radioactivity in µg equivalents per gram or mL for male and female rats with time. In most tissues (except GI and liver), the highest concentrations occur at the second time point, two-hours. Most of the radioactivity is gone by the six-hour time point. More radioactivity is found in brown fat relative to white fat consistent with topotecan's lipophilicity. Concentrations in brain tissue were difficult to distinguish from background (<30 dpm above average background) at all time points, suggesting exclusion from the CNS space. The concentration in blood and plasma is surprisingly low. There are no significant differences between males and females.

Table 1: Mean concentrations of total radioactivity in tissues at various times following single oral administration of [^{14}C]SK&F-104864-A to male rats at a target dose level of 0.8 mg fb/kg (4.72 mg fb/m^2)

Sample	Concentration (ug fb equiv/g or /mL)					
	0.5 h	2 h	6 h	24 h	48 h	96 h
Adrenals	0.016	0.039	0.009	0.014	0.006	0.005
Exorbital lachrymal glands	0.018	0.053	0.016	0.014	0.004	0.004
Aortic wall	°0.003	°0.014	°0.003	0.005	0.003	°0.002
Bone marrow	°0.022	0.064	0.014	0.016	°0.007	°0.004
Bone mineral	0.010	0.019	0.006	0.008	0.004	°0.003
Residual brain	°0.001	°0.004	°0.002	°0.003	°<0.001	°0.001
Cerebellum	°0.003	°0.005	0.002	0.003	°0.001	°0.001
Cerebrum	°0.003	0.003	0.002	0.003	0.001	0.001
Epididymis	0.013	0.019	0.007	0.007	0.003	0.003
Eyes	0.002	0.010	°0.002	0.002	°0.001	°0.001
Fat-brown	0.015	0.045	0.007	°0.004	0.004	0.003
Fat-white	0.007	0.015	0.003	0.004	0.003	0.003
Urinary bladder wall	0.050	0.079	0.036	0.021	°0.005	°0.002
Harderian gland	0.018	0.047	0.028	0.040	0.012	0.006
Heart	0.020	0.029	0.007	0.005	0.003	0.003
Kidneys	0.095	0.119	0.020	0.018	0.010	0.007
Liver	0.421	0.412	0.070	0.051	0.023	0.010
Lungs	0.031	0.047	0.012	0.009	0.006	0.004
Lymph nodes (mandibular)	°0.009	0.027	0.010	0.011	0.004	°0.003
Lymph nodes (mesenteric)	0.021	0.027	0.012	0.011	0.005	0.004
Medulla spinalis	°<0.001	°0.001	°0.001	°0.003	°0.001	°0.001
Intraorbital lachrymal glands	°0.005	°0.016	°0.006	0.008	°0.003	°0.001
Muscle	0.016	0.023	0.004	0.004	0.003	0.002
Pancreas	0.072	0.111	0.022	0.016	0.006	0.003
Pituitary	0.010	0.032	°0.009	°0.008	°0.003	°0.002
Preputial glands	°0.003	0.011	0.008	0.012	°0.005	°0.002
Prostate	0.019	0.030	0.013	0.014	0.006	0.003
Seminal vesicles	0.017	0.041	0.018	0.025	0.006	0.006
Skin	0.026	0.029	0.021	°0.014	0.007	0.004
Sub-lingual glands	0.010	0.023	0.017	0.011	°0.005	°0.002
Sub-mandibular glands	0.018	0.040	0.015	0.011	0.005	0.004
Spleen	0.038	0.063	0.013	0.013	0.006	0.005
Testes	0.006	0.016	0.012	0.007	0.003	0.002
Thymus	0.019	0.036	0.010	0.009	0.005	0.004
Thyroid	°0.004	°0.006	°0.013	°0.004	°0.003	°0.002
Stomach wall	5.715	0.482	0.050	0.251	0.076	0.004
Duodenal wall	2.211	0.582	0.097	0.144	0.036	0.005
Small intestinal wall	2.819	1.922	0.202	0.258	0.094	0.006
Caecum wall	0.326	3.239	6.339	1.490	0.038	0.017
Large intestinal wall	0.550	0.876	1.617	0.554	0.034	0.016
Blood	0.018	0.023	0.011	0.008	0.003	0.002
Plasma	0.016	0.022	0.007	0.012	0.003	0.002

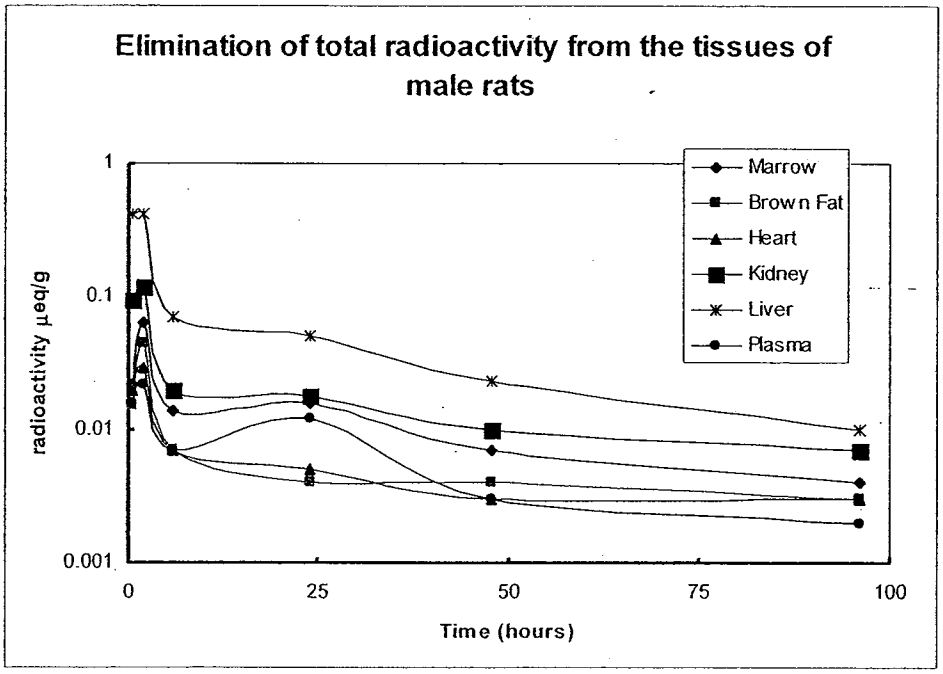
° = Mean includes results calculated from data less than 30 d.p.m. above background

Table 2: Mean concentrations of total radioactivity in tissues at various times following single oral administration of [^{14}C]SK&F-104864-A to female rats at a target dose level of 0.8 mg fb/kg (4.72 mg fb/m^2)

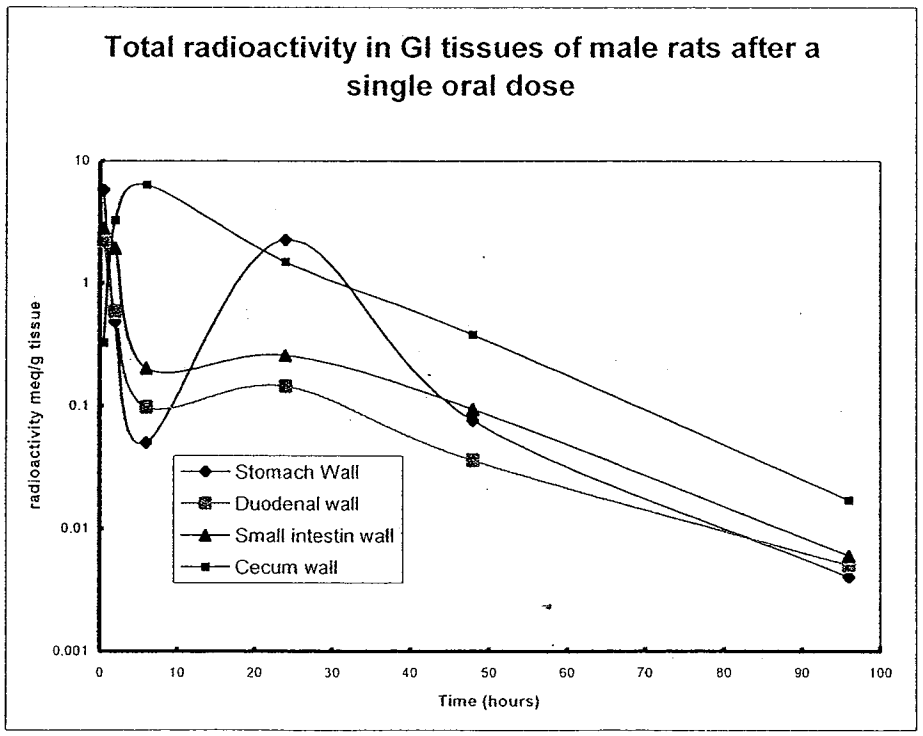
Sample	Concentration (ug fb equiv/g or /mL)					
	0.5 h	2 h	6 h	24 h	48 h	96 h
Adrenals	0.040	0.042	0.015	0.019	0.013	0.007
Exorbital lachrymal glands	0.071	0.176	0.055	0.035	0.008	0.004
Aortic Wall	0.008	0.011	0.006	0.006	0.004	°0.003
Bone marrow	0.057	0.058	0.019	0.018	°0.008	°0.003
Bone mineral	0.022	0.022	0.007	0.009	0.005	°0.005
Residual brain	°0.003	0.004	°0.002	°0.006	°0.001	°0.001
Cerebellum	°0.006	0.004	°0.003	0.004	°0.001	0.001
Cerebrum	0.003	0.003	0.003	0.004	0.002	0.001
Eyes	0.005	0.006	0.003	0.003	0.001	°0.001
Fat-brown	0.030	0.025	0.007	0.007	0.005	0.003
Fat-white	0.025	0.013	0.004	0.005	0.003	0.003
Urinary bladder wall	0.216	0.088	0.036	0.019	°0.012	°0.003
Harderian gland	0.031	0.054	0.033	0.049	0.017	0.006
Heart	0.035	0.032	0.008	0.007	0.004	0.002
Kidneys	0.242	0.110	0.022	0.024	0.010	0.005
Liver	0.374	0.240	0.062	0.048	0.017	0.009
Lungs	0.054	0.054	0.013	0.013	0.005	0.003
Lymph nodes (mandibular)	0.020	0.028	0.018	0.014	0.005	0.003
Lymph nodes (mesenteric)	0.044	0.035	0.010	0.032	0.007	0.004
Medulla spinalis	°0.001	°0.001	°0.001	°0.003	°0.001	°0.002
Intraorbital lachrymal glands	°0.008	°0.056	0.050	°0.011	°0.003	°0.002
Muscle	0.023	0.023	0.006	0.005	0.003	0.002
Ovaries	0.044	0.059	0.015	0.021	0.010	0.006
Pancreas	0.122	0.145	0.039	0.027	0.006	0.003
Pituitary	0.022	0.023	°0.006	°0.007	°0.002	°0.004
Preputial glands	°0.017	°0.018	°0.019	°0.013	°0.005	°0.003
Skin	0.025	0.027	0.009	0.007	0.013	0.003
Sub-lingual glands	0.024	0.038	0.052	0.015	°0.005	°0.002
Sub-mandibular glands	0.035	0.051	0.028	0.016	0.006	0.003
Spleen	0.071	0.065	0.016	0.016	0.007	0.004
Thymus	0.028	0.038	0.014	0.011	0.005	0.003
Thyroid	0.009	0.008	°0.005	°0.002	°0.003	°0.001
Uterus	0.036	0.023	0.010	0.016	0.005	0.003
Stomach wall	2.346	0.308	0.059	0.114	0.038	0.011
Duodenal wall	1.410	0.702	0.103	0.150	0.036	0.007
Small intestinal wall	5.121	3.491	0.220	0.279	0.050	0.010
Caecum wall	0.970	2.991	3.600	2.736	0.106	0.027
Large intestinal wall	3.681	1.201	1.200	0.930	0.052	0.022
Blood	0.032	0.027	0.011	0.016	0.003	0.002
Plasma	0.027	0.025	0.009	0.012	0.004	0.002

° = Mean includes results calculated from data less than 30 d.p.m. above background

The following graph shows the elimination of radioactivity from selected important tissues on a logarithmic scale. Again, the two-hour time point is usually the apparent Tmax and most of the drug is gone by 24 hours. The graph suggests the possibility of enterohepatic recirculation. This seems quite possible considering the stable location of the radiolabel and the lipophilicity of topotecan. Recalculating metabolites are probably the variants of the open ring lactone. While I have not fit the data to a pharmacokinetic model, the graph also suggests a two-phase elimination, a rapid elimination probably through both the kidneys and the GI and a slower elimination of material that partitioned into fat.



The following graph shows the distribution of radioactivity in all the GI tissues sampled. The graph shows that the exposure of these tissues was about 10 times greater than that of tissues distal to the portal circulation. The graph also provides strong evidence for enterohepatic recirculation.



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2) SK&F 104864-A: 6-Month Oral Toxicity Study in Rats.

Major findings

In this study in rats, topotecan lactone T_{max} varied between 0.25 and 4 hours. C_{max} and AUC increased proportionately with dose and at the low doses of the study remained relatively constant with time on study. Nevertheless, total topotecan (both forms) C_{max} and AUC decreased on day 176 as much as 70%. There is insufficient information to explain these changes.

Study Number	TP-1036, NDA 20-671, Seq. 000, Vol. 1.19, p 2. Repeated here from my review of NDA 20-671
Conducting laboratory	SmithKline Beecham Pharmaceuticals, King of Prussia, PA
Date of study initiation	
GLP compliance	Yes
QA report	Yes
Drug	SK&F 104864-A, Lot # MM-19163-58, 87.3% pure free base.
Method	
Animals	60 male and 60 female Sprague-Dawley rats
Dose	0, 0.0023, 0.023, or 0.23 mg/kg/day for six months (0, 0.0136, 0.136, or 1.36 mg/m^2/day) calculated as free base
N	12 rats/sex/dose, 3 rats/sex/dose for pharmacokinetic evaluation
Vehicle	3% mannitol$_{(aq)}$, pH 3, 10 ml/kg
Route	oral gavage

Pharmacokinetic — The rats used for pharmacokinetic studies on day 1 were a satellite group dosed with the main group. On day 1 of dosing, investigators drew blood (0.25 ml) before dosing and at 0.25, 0.5, 1, 1.5, 2, 4, and 6 hr from this group. On days 36, 121 and 176 they drew blood from rats in the main dosing study, before dosing, and at 0.5, 1, 2, 4 and 6 hours. Two rats/sex/dose group were used for each time point to generate composite pharmacokinetic profiles. Plasma topotecan was determined by the HPLC.

Topotecan concentration was below the limit of quantitation in all samples from controls and the 0.0136 mg/m^2/d dose groups. T_{max} varied between 0.25 and 4 hours. C_{max} and AUC increased proportionately with dose. The first table below shows that topotecan (lactone) C_{max} and AUC remained relatively constant with time on study. Nevertheless, the second table shows that total topotecan (both forms) C_{max} and AUC decreased on day 176. The decrease in AUC was as much as 70%. Parameters for male and female rats were similar. The male rats in the high dose group had increased serum glucose and decreased cholesterol and triglycerides as the study progressed. These changes might be related to the changes in C_{max} and AUC in the males, but the problem remains for the females. There is insufficient information to explain these changes.

Pharmacokinetics of the Lactone form of Topotecan in rats							
		Day 1		Day 36		Day 176	
Dose	Sex	C_{max}	AUC_{0-t}	C_{max}	AUC_{0-t}	C_{max}	AUC_{0-t}
mg/m²/d	n=3	ng/ml	ng*h/ml	ng/ml	ng*h/ml	ng/ml	ng*h/ml
0.136	male	0.23 ± 0.08	0.5 ± 0.1	NR	NR	0.23	0.31
1.36	male	1.7 ± 0.3	4.6 ± 0.9	1.17	4.02	1.54	5.95
0.136	female	0.26 ± 0.07	0.7 ± 0.2	0.25	0.64	0.23	0.51
1.36	female	2.0 ± 0.4	5.7 ± 1.3	1.77	5.98	2.69	7.38

Pharmacokinetics total of Topotecan (both forms) in rats							
		Day 1		Day 36		Day 176	
Dose	Sex	C_{max}	AUC_{0-t}	C_{max}	AUC_{0-t}	C_{max}	AUC_{0-t}
mg/m²/d	n=3	ng/ml	ng*h/ml	ng/ml	ng*h/ml	ng/ml	ng*h/ml
0.136	male	0.30 ± 0.08	1.4 ± 0.3	0.28	1.35	0.21	0.52
1.36	male	3.6 ± 0.8	10.3 ± 0.5	2.12	7.7	1.8	7.05
0.136	female	0.31 ± 0.02	1.3 ± 0.4	0.36	1.44	0.32	0.41
1.36	female	4.0 ± 0.8	12.5 ± 2.5	3.58	10.8	2.74	8.33

Dog

3) TP-1021: 5-day oral toxicity study in dog.

This study was submitted to IND submission 244, 6/16/95, Vol. 1.23, p. 2.

The following table shows the model independent pharmacokinetic parameters for topotecan following repeat oral administration of topotecan hydrochloride at 1.36 and 4 mg fb/m² to male and female dogs.

				C_{max} (µM)			
Dose mg/kg/day	Dose mg/m²/day	Dose (µmol/kg/day)		Male	sd	Female	sd
0.068	1.36	3.2	Day 1	0.0135	0.0024	0.0127	0.0017
0.2	4	9.5	Day 1	0.0396	0.0002	0.0313	0.0050
0.068	1.36	3.2	Day 5	0.0120	*	0.0118	0.0014
0.2	4	9.5	Day 5	0.0183	*	0.0261	*
				AUC_{0-inf} (µM*hr)			
Dose mg/kg/day	Dose mg/m²/day	Dose (µmol/kg/day)		male	sd	Female	sd
0.068	1.36	3.2	Day 1	0.0142	0.0057	0.0123	0.0045
0.2	4	9.5	Day 1	0.0448	0.0176	0.0496	0.0164
0.068	1.36	3.2	Day 5	0.0170	*	0.0156	0.0045
0.2	4	9.5	Day 5	0.0653	*	0.0555	*

Data are mean values ± SD of 3 animals except for * which are means of 2 animals.

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4) TP-1034: SK&F 104864-A: 1-month intravenous toxicity study in dogs.

This study was submitted to IND submission 244, 6/16/95, Vol. 1.21, p 2.

The following graph shows the model-independent pharmacokinetic parameters for total drug in dogs following repeated intravenous administration of topotecan hydrochloride for 28 days.

Parameter (units)	Day of study	Parameter value (mean ± S.D., n = 4-7)			
		Male		Female	
Dose (mg fb/m^2/day)		0.1	0.4	0.1	0.4
C_{max} (ng/mL)	1	2.67 ± 0.20	9.59 ± 4.44	2.87 ± 1.18	9.12 ± 1.03
	28	3.04 ± 0.19	11.7 ± 2.05	2.61 ± 0.22	11.8 ± 1.55
$AUC_{0\text{-}inf}$ (ng.h/mL)	1	6.18 ± 1.16	17.9 ± 4.4	6.34 ± 2.15	20.2 ± 4.6
	28	6.24 ± 0.54	27.1 ± 6.6	5.66 ± 0.53	29.7 ± 7.4

Total drug = topotecan plus SK&F 105992. Doses were administered as bolus injections.

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5) 3-Month Oral Toxicity study of SK&F-104864-A in Dogs

Major findings

Three months of daily oral topotecan at a dose of 60 mg/m^2/day was lethal to all dogs in this high dose group between days 30 and 71 of the study. Lethality was associated with daily maximal plasma concentrations of topotecan greater than about 1.3 μM. Daily dosing resulted in significant accumulation over the course of the study with accumulation factors between days 1 and 89 ranging from 7 to 30 fold. These results and the results of the 6-month study below strongly suggest that higher doses of topotecan saturate some essential metabolism of the drug, leading to severe toxicity. Also, AUC and C_{max} increased linearly with dose on day 1 but were less than dose proportional on day 89 suggesting saturation of absorption.

Study number	SKF-105685/TP-1010/1, Contractor's Report No. G91061 Submitted to IND 32,693, July 23, 2003, IT, Seq. 580 Report 9
Conducting laboratory	SmithKline Beecham Pharmaceuticals, King of Prussia, PA
Date of study initiation	November 1, 1991
GLP compliance	Yes
QA report	Yes
Drug	SK&F-104864-A, Lot CSL-14467-276, 99.5% pure
Methods	
Doses	0, 0.03, 0.3, 1 or 3 mg/kg/day (expressed as free base) 0, 0.6, 6, 20 or 60 mg/m^2/d

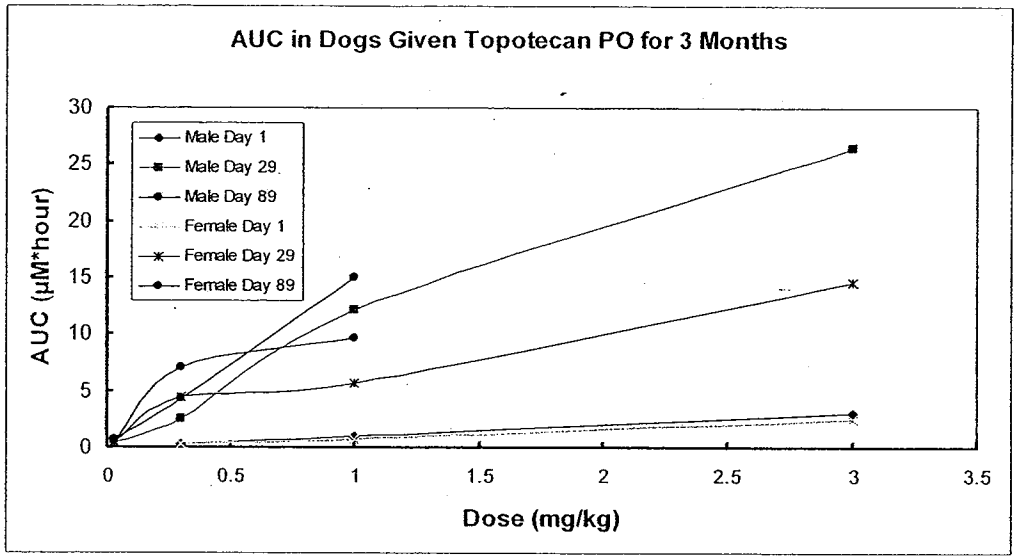
Species	male and female Beagle dogs age 7 to 8 months, weight 8.2 to 12.4 kg
Number	3 per sex per dose group
Route	PO
Schedule	daily for 90 days
Formulation	gelatin capsules
Necropsy	Days 90, 91, and 92 – no recovery groups
Histopathology	Adequate battery
Pharmacokinetics	Days 1, 29, and 89 serial samples. Samples were also taken pre-dose, days 15, 44, 61 and 75 to assess steady state concentrations.

The following table shows the results of this toxicokinetics study. The sponsor did not calculate the terminal half-life or volume of distribution or clearance. I calculated the clearance on day 1 to be about 3 L/kg/hr but only about 0.17 L/kg/hr on day 89. This difference results from the dramatic increase in AUC over the course of the study.

C_{max}	µM							
Males	Dose (mg/kg)	Dose mg/m^2/day	Male Day 1	sd	Male Day 29	sd	Male Day 89	sd
	0.03	0.6	ND		0.021	0.012	0.033	0.017
	0.3	6	0.020	0.002	0.131	0.033	0.209	0.104
	1	20	0.076	0.014	0.631	0.135	0.769	0.342
	3	60	0.256	0.050	1.322	0.569	Died	
Females	Dose (mg/kg)	Dose mg/m^2/day	Female Day 1	sd	Female Day 29	sd	Female Day 89	sd
	0.03	0.6	ND		0.026	0.005	0.033	0.009
	0.3	6	0.021	0.007	0.233	0.093	0.320	0.145
	1	20	0.066	0.014	0.282	0.017	0.460	0.064
	3	60	0.190	0.071	0.925	0.116	Died	
AUC	µM*hr							
Males	Dose (mg/kg)	Dose mg/m^2/day	Male Day 1	sd	Male Day 29	sd	Male Day 89	sd
	0.03	0.6	ND		0.43	0.23	0.68	0.44
	0.3	6	0.22	0.01	2.48	0.68	4.29	2.15
	1	20	0.99	0.29	12.18	3.13	15.04	6.63
	3	60	3.00	0.84	26.49	13.10	Died	
Females	Dose (mg/kg)	Dose mg/m^2/day	Female Day 1	sd	Female Day 29	sd	Female Day 89	sd
	0.03	0.6	ND		0.48	0.14	0.55	0.18
	0.3	6	0.22	0.09	4.42	2.05	6.95	3.15
	1	20	0.71	0.16	5.63	0.57	9.57	0.99
	3	60	2.48	0.61	14.56	5.17	Died	

ND = concentrations below detection limit

The following graph shows that AUC increase linearly with dose on day 1 and dosing resulted in only very low exposure. Exposure increased with significantly with continued dosing and evidently, steady state was not reached by day 29 particularly in females. C_{max} increased in a manner similar to AUC. This graph demonstrates significant accumulation over time with daily dosing.



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4) 6-Month Intravenous Toxicity study in Dogs

Major findings

At the very low does of this experiment, topotecan C_{max} and AUC increased linearly with dose and there was no accumulation of drug in plasma over the 180-day course of the experiment.

Study number	G95117, SKF-104864/100G3W/1 Submitted to IND 32,693, Dec. 20, 1996, IT, Seq. 371, p 130
Conducting laboratory	SmithKline Beecham Pharmaceuticals, King of Prussia, PA
Date of study initiation	October 4, 1995
GLP compliance	Yes
QA report	Yes
Drug	SK&F-104864-A, Lot JW-15880-118-2, 79.8% pure This drug lot is listed as batch U95023 in the CMC section The purity is not stated
Methods	
Doses	0, 0.001, 0.003, or 0.01 mg/kg/day (expressed as free base) 0, 0.020, 0.06 or 0.2 mg/m^2/day
Species	male and female Beagle dogs age 9 to 10 months, weight 10 to 12 kg males, 9 to 11 kg females
Number	4 per sex per dose group
Route	IV; dose volume 1 mL/kg
Schedule	daily for six months
Formulation	3% mannitol$_{(aq)}$, pH 3

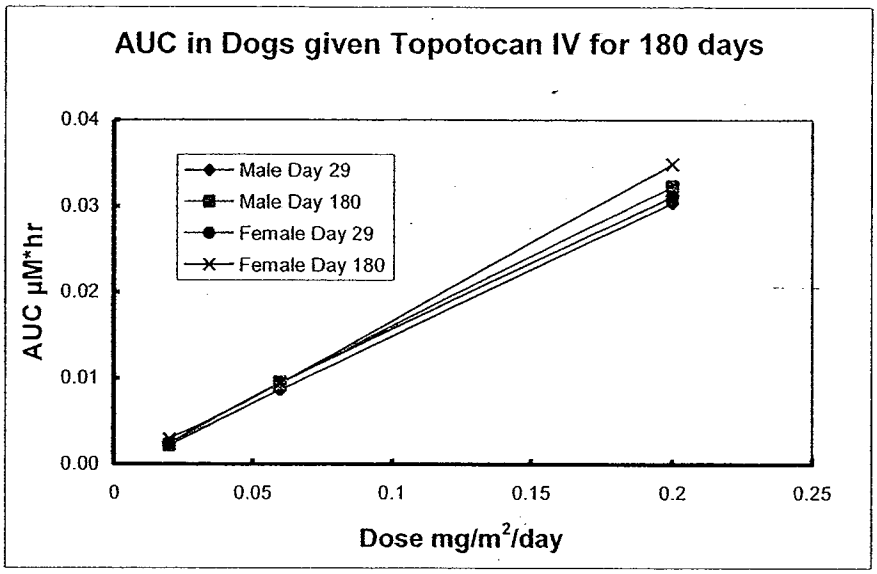
Necropsy	day 181 – no recovery groups
Histopathology	Adequate battery
Pharmacokinetics	Days 29, and 180, 0.25, 0.5, 1, 2, 4, and 6 hours post dosing See the section on Pharmacokinetics and Toxicokinetics above

Results

The following table shows the pharmacokinetic parameters obtained in this study. The sponsor did not calculate the terminal half-life or volume of distribution or clearance. The doses in this study are much lower than those of the 3-month study. There is little accumulation over the course of the study and little toxicity; the average of the accumulation factor for day 180 compared to day 29 is 1.1. The clearance is 0.86 L/kg/hr on day 29 and 0.8 L/kg/hr on day 180. This strongly suggests that significantly higher doses such as those in the 3-month study saturate some crucial metabolic process.

C_{max}	µM					
Males	Dose (mg/kg)	Dose mg/m^2/d	Male Day 29	sd	Male Day 180	sd
	0.001	0.02	0.001	0.000	0.002	0.000
	0.003	0.06	0.004	0.000	0.005	0.001
	0.01	0.2	0.014	0.002	0.016	0.003
Females	Dose (mg/kg)	Dose mg/m^2/d	Female Day 29	sd	Female Day 180	sd
	0.001	0.02	0.001	0.000	0.002	0.001
	0.003	0.06	0.004	0.000	0.005	0.001
	0.01	0.2	0.014	0.001	0.017	0.002
AUC	µM*hr					
Males	Dose (mg/kg)	Dose mg/m^2/d	Male Day 29	sd	Male Day 180	sd
	0.001	0.02	0.0022	0.0001	0.0022	0.0016
	0.003	0.06	0.0086	0.0005	0.0095	0.0036
	0.01	0.2	0.0304	0.0057	0.0323	0.0116
Females	Dose (mg/kg)	Dose mg/m^2/d	Female Day 29	sd	Female Day 180	sd
	0.001	0.02	0.0024	0.0008	0.0029	0.0004
	0.003	0.06	0.0094	0.0008	0.0094	0.0021
	0.01	0.2	0.0311	0.0026	0.0349	0.0031

The following graph shows that at these low doses AUC increased linearly with dose and the topotecan did not accumulate over the course of the experiment.



Pharmacodynamic drug interactions

1) The Effect of SK&F-104864-A on Hepatic Levels of Cytochrome P450 and Related Parameters in Male and Female Sprague Dawley Rats after Daily Intravenous Dosing at 0.115 and 0.230 mg pfb/kg/day (0.68 and 1.36 mg pfb/m2/day) for 10 Days.

Major findings

Treatment of rats with mildly toxic doses of IV topotecan daily for 10 days did not increase total microsomal protein nor did it induce the expression of cytochrome P450 1A1, 2B or 3A activity. This treatment did significantly induce the activity of cytochrome P450 2A1 in males, the increase in females did not reach statistical significance but I consider the effect toxicologically significant. Dosing also caused increases that did not reach significance in 2E1 4A activity in males. The sponsor did not consider these latter two effects toxicologically significant. I do, particularly the increase in 4A activity. The results suggest the potential for some enzymatic induction in humans.

Study number	6146-118
	Submitted to IND 32,693, Feb. 20, 1998, IT Seq. 453
Conducting laboratory	[redacted] b(4)
Date of study initiation	January 1997
GLP compliance	Yes
QA report	Yes
Drug	SK&F-104864-A, Lot MM-19163-58

b(4)

Methods

Doses	0, 0.115 and 0.230 mg fb/kg/day (0.68 and 1.36 mg fb/m^2/day)
Species	male and female Sprague Dawley rats (— CD(SD)BR) age 8 weeks, body weights ca 135-179 g
Number	3 per sex/dose group
Route	IV, tail vein
Schedule	Daily for 10 Days
Formulation	3% (w/v) aqueous mannitol (pH 3.0)

This treatment did not increase total microsomal protein in low dose males or females or in high dose females. The 16% increase seen in high dose males is not toxicologically significant. The treatment did not affect the mean hepatic microsomal cytochrome P450 concentration in any group neither did it affect the ethoxyresorufin O-deethylase activity or 6β-hydroxytestosterone production, 16β-hydroxytestrone production. Ten days of IV treatment did increase 7α-hydroxytestosterone production 2.5 fold in high dose males. The increase in females was greater than 20% but did not reach statistical significance. It increased lauric acid 11-hydroxylase activity in male by about 20% but again the increase did not reach statistical significance. It increased lauric acid 12-hydroxylase activity in low and high dose males by nearly 2 fold but the increase did not reach statistical significance.

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Toxicology

Multiple Dose Toxicology Summary

Study	Species	Schedule	Doses mg/kg	Doses mg/m^2	Mortality	Comment	Toxicity
TP 010 CM	Rat	Daily	0	0			
	6m+6f/group	5 Days	0.023	0.138	0/12	NOAEL	
		2 courses	0.068	0.408	0/12		
		IV	0.23	1.38	0/12	relatively low doses	Mild dose related decreases in red and white cell parameters, some signs of liver damage
R41080	Rat	Daily	0	0			
	10m+10f/group	5 Days	0.07	0.42			
		PO	0.13	0.78			
Part of a combination study, not all groups shown			0.23	1.38		relatively low doses	No significant toxicity
R41021	Rat	Daily	0	0			
	10m+10f/group	5 Days	0.23	1.38			
		PO	0.79	4.74			Weight loss, decreased platelets, reticulocytes, white cell count, neutrophils, lymphocytes, monocytes, and eosinophils. Increases in blood glucose, cholesterol and bilirubin and decreases in total protein, albumin. Microscopic damage was seen in the bone marrow, lymph nodes and GI.
Part of a combination study, not all groups shown			2.3	13.8	1/10 f		Weight loss, decreased platelets, reticulocytes, white cell count, neutrophils, lymphocytes, monocytes, and eosinophils. Increases in blood glucose, cholesterol and bilirubin and decreases in total protein, albumin. Microscopic damage was seen in the bone marrow, lymph nodes and GI.
TP-1017	Rat	Daily	0	0			
	15/sex/dose	28 Days	0.0023	0.0138	0/30		
		IV	0.023	0.138	0/30		
			0.023	0.138	0/30		Minor changes consistent with toxicities seen in the HD group Decreased body wt, decreased red and white cell parameters, decreased serum protein. Decreased thymus wt. Damage in thymus, spleen, marrow and nodes.
			0.23	1.38	0/30		
TP-1036	Rat	Daily	0	0			
	12m+12f/group	6 Months	0.0023	0.0138		Not a NOAEL	Increased glucose, deccreased cholesterol and triglycerides indicating metabolic derangement all males all dose groups
		Oral	0.023	0.138			
			0.23	1.38			Dose increased on day 121 due to lack of significant toxicity, decreased back after 7 doses
	Dose increase for HD group		0.907	5.442	1/12 m		Severe GI damage, Decreases in red and whit cell parameters
TP-1034	Dog	Daily	0	0			
	4m+4f mid dose groups 7m+7f control and HD	1 Month IV	0.001 0.005	0.02 0.1			
	3m+3f recovery		0.02	0.4	1/7 f d23		Decreased red and white cell parameters, decreased thymus wt with atrophy, hypocellular marrow, damage in GI and testes
TP-1010/1	Dog	Daily	0	0			
	3m+3f/group	3 months	0.03	0.6	0/6	Not a NOAEL	Decreases in red cell parameters. Clinical chemistry indications of liver damage
		Oral	0.3	6	0/6		Decreases in red cell parameters. Clinical chemistry indications of liver damage
			1	20	0/6		A dose dependent decrease in red cell parameters and damage to the liver and gastrointestinal system that increased with time and dose
			3	60	6/6		Deaths due to GI damage
G95117	Dog	Daily	0	0			
		6 Months	0.001	0.02	0/8		
	4m+4f/group	IV	0.003	0.06	0/8	NOAEL	
			0.01	0.2	0/8		Decreased red cell parameters 10% or less

Multiple Dose Toxicology

Rat

1) Five-Daily Dose Repeat Schedule Toxicity of SK&F 104864 in the Rat

Major findings

Male and female rats survived five daily doses of topotecan as high as 1.36 mg/m^2/day given over two courses. These doses are significantly less than the proposed clinical dose on a mg/m^2 basis. Dosing was associated with significant myelosuppression and thrombocytopenia and mild anemia. There were also indications of mild hepatic toxicity. These toxicities were reversible.

Study Number	TP 010 CM, NDA 20-671, Vol. 1.16, p 279 Repeated here from my review of NDA 20-671
Conducting laboratory	SmithKline Beecham Pharmaceuticals, King of Prussia, PA
GLP compliance	Yes
QA report	Yes
Drug	SK&F 104864-A, Lot # JW-14479-101-3, 87.3% pure free base This drug batch is not listed in the CMC submission
Methods	
Animals	male and female Sprague-Dawley rats
Dose	0, 0.023, 0.068, or 0.230 mg/kg/day X5 days (0, 0.136, 0.401, 1.36 mg/m^2/day) calculated as free base
N	six rats/sex/dose, given on days 1 through 5 and again on days 22 through 26
Vehicle	0.9% saline, pH 2.60 to 2.65 with 0.1 N HCl, 1 ml/kg
route	i.v. tail vein
Observations	
physical exam	d-21, 3, 9, 17, 23, 29, 38, 43
clinical signs	daily
body weight	d-6, 1, 8, 15, 22, 29, 36, 43
food cons	d-3, 5, 12, 19, 26, 33, 40
ophthalmology	d-18, 30, 44
hematology	d-14 or -13, 8, 15, 29, 43
clinical chemistry	d-14 or -13, 8, 15, 29, 43
urinalysis	d-12, 9, 16, 30, 44
termination	3 rats/sex day 30, 3 rats/sex day 44
necropsy	gross signs
histopathology	

Results

Mortality	all rats survived to scheduled necropsy
Clinical signs	mild hair thinning between days 9 and 43
Body weight	no dose related changes
Food Cons	no dose related changes
Ophthalmology	no dose related changes

Hematology	On day 8, dose related decrease in Hbg in females, Hbg and RBC remained low in these females on d15 and continued to decrease after the second course. These values returned to normal by day 44. This decrease was less than 7% of pretreatment values. WBC decreased as much as 50% from pre-treatment values in the high dose group and as much as 20% in the mid dose group. All white cell types were affected with a nadir observation on d29. Cell counts were returning to normal by day 43 but had not completely recovered.
Clinical chemistry	These results were ambiguous. Alkaline phosphatase decreased in some rats and the decrease was most pronounced in the high dose group, but a clear dose response was not established. ALT and AST increased in some rats to as much as 3 fold greater than the upper normal limit, but again a clear dose response was not established.
Urinalysis	no dose related changes
Gross Pathology	Day 30, dose related incidence of thymic atrophy Day 44, no drug induced lesions
Histopathology	Day 30, round aggregates of small lymphocytes in thymic medulla of 5 dosed rats. The incidence did not increase with dose but was not seen in controls. Day 44, hyperkeratosis and follicular atrophy in three rats probably related to topotecan.
Bone Marrow	Topotecan caused treatment related increase in marrow fat, hypoplasia of the granulocytic and megakaryocytic progenitors, and erythroid hyperplasia in high dose rats. These changes were reversible.

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2) SK&F 104864-A: 6-Month Oral Toxicity Study in Rats

Major findings

Doses as high as 1.36 mg/m^2 given daily for six months caused only slight increases in serum glucose (~15% in male rats) and decreases in serum cholesterol (13 to 26%). Triglycerides were decreased 10 to 42% in males in all dose groups (0.0136, 0.136, or 1.36 mg/m^2/day) throughout the study. These changes indicate some derangement of metabolism that is probably drug related. The doses in this study were too low to provide toxicologically meaningful information.

Study Number	TP-1036, NDA 20-671, Seq. 000, Vol. 1.19, p 2 Repeated here from my review of NDA 20-671
Conducting laboratory	SmithKline Beecham Pharmaceuticals, King of Prussia, PA
GLP compliance	Yes
QA report	Yes
Drug	SK&F 104864-A, Lot # MM-19163-58, 87.3% pure free base This drug lot is listed as Batch Number U94036 Manufactured February 1994, purity not stated
Method	

Animals	60 male and 60 female Sprague-Dawley rats
Dose	0, 0.0023, 0.023, or 0.23 mg/kg/day for six months (0, 0.0138, 0.138, or 1.38 mg/m^2/day) calculated as free base
N	12 rats/sex/dose, 3 rats/sex/dose for pharmacokinetic evaluation
Vehicle	3% mannitol$_{(aq)}$, pH 3, 10 ml/kg
Route	oral gavage
Toxicokinetics	See above in the Pharmacokinetic and Toxicokinetic section

Observations

physical exam	pre-study
clinical signs	daily
body weight	d-6, 1, 8, 15, 22, 29, 36, 43
food cons	daily
ophthalmology	d-16 and d175
hematology	d15, 28, 89, 133 (only control and high dose) and 180 (all)
clinical chemistry	d15, 28, 89, 133 (only control and high dose) and 180 (all)
urinalysis	d16, 29, 91 and just before termination
necropsy	gross signs
histopathology	see table

On day 121, none of the rats showed any signs of toxicity in any of the dose groups. The investigators decided to increase the high dose four fold to 5.44 mg/m^2/d. After seven days of dosing at this level, male rats showed significant toxicity that evidently threatened the study. The investigators decreased the dose back to the original 1.36 mg/m^2/d for the remainder of the study.

Mortality	One high dose male was moribund on day 134 after seven days of dosing at 5.44 mg/m^2/d. On necropsy, this rat had erosions in the stomach and duodenum, necrosis of the crypt epithelium in the jejunum and ileum and regenerative glandular hyperplasia in the colon and cecum. Microscopically, there was bilateral testicular degeneration and necrosis and lymphoid depletion of the thymus.
Clinical signs	None except for the except those associated with the 5.44 mg/m^2/d regimen (see above).
Body weight	High dose male rats lost a significant but small amount of weight (2%) during the 5.44 mg/m^2/d regimen. This weight loss was reversible when the dose was lowered to the original 1.36 mg/m^2/d.
Food Cons	Anorexia associated with the weight loss during the 5.44 mg/m^2/d regimen. The male that died ate little to nothing after this regimen. This anorexia was probably associated with the severe damage to the GI tract.
Ophthalmology	no dose related changes
Hematology	Mean Hbg, RBC, Hct decreased ~6% in females after the 5.44 mg/m^2/d regimen (measured d133). After this dosing regimen, the WBC in these females increased more than 50% and the absolute neutrophil count increased 3 fold. These paradoxical increases could be secondary to the GI toxicity or rebound from transient myelosuppression.
Clinical	On day 180, glucose was increased ~15% in all male rats. The serum cholesterol was decreased 13 to 26%. Triglycerides were decreased 10 to 42% in males in all dose groups throughout the study. These changes indicate some derangement of metabolism that is probably drug related.

Urinalysis	No dose related changes
Organ Weight	No toxicologically significant changes
Gross Pathology	No drug related gross changes
Histopathology	No drug related microscopic changes

Dog

3) SK&F 104864-A: One-Month Intravenous Toxicity Study in Dogs.

Major findings

A dose of 0.4 mg/m^2 given daily for one month was lethal to 1 of 7 female dogs. All other dogs survived to scheduled necropsy. The high dose caused decreases in red and white cell parameters, decreased thymus weight with atrophy, hypocellular marrow, and damage in GI and testes. Lower doses caused little or no toxicity.

Study number	TP-1034, submitted to IND 32,693 submission 244, 6/16/95, Vol. 1.21, p 2. Repeated here from my review of NDA 20-671
Conducting laboratory	SmithKline Beecham Pharmaceuticals, King of Prussia, PA
GLP compliance	Yes
QA report	Yes
Drug	SK&F 104864-A, Lot # MM-19163-58, 87.3% pure free base This drug lot is listed as Batch Number U94036 Manufactured February 1994, purity not stated
Methods	
Animals	Forty four purebred beagle dogs
Dose	0, 0.02, 0.1, 0.4 mg/m^2/day one months (groups 1, 2, 3 and 4) 0, 0.4 mg/m^2/d (groups 5 and 6, recovery)
N	four dogs/sex groups 1 to 4 respectively 0, 0.4 mg/m^2 three dogs/sex/group dosed and held for 8-week recovery groups 5 and 6 respectively
Toxicokinetics	d1 & d28, blood samples pre-dose, 0.25, 0.5, 1, 2, 4, & 6 hr See review in Pharmacokinetics and Toxicokinetics Section
Vehicle	3% mannitol$_{(aq)}$, pH 3
Route	i.v., 1 ml/kg cephalic vein
Observations	
physical exam	pre-study
clinical signs	daily
body weight	weekly
food cons	weekly
ECG	pre-study and day 23
ophthalmology	pre-study and day 26
hematology	pre-study, d9, d27, d42, d56, d70, d84
clinical chemistry	pre-study, d9, d27, d42, d56, d70, d84
urinalysis	pre-study and d27

necropsy	day 29 or 30 for Groups 1 through 4, on day 89 for groups 5 & 6
histopathology	Adequate battery

Samples of liver were reserved to determine Cytochrome P450 Analysis.

Mortality	one female high dose dog was killed on day 23, this dog suffered several days of anorexia and weight loss
Clinical signs	no consistent dose related symptoms of toxicity
Body weight	no statistically significant changes
Food Consumption	variable decreases to 65% in high dose dogs
Ophthalmology	no effects
ECG	no effects
Hematology	Hct, RBC & Hbg in high dose males decreased 9 and 15% respectively WBC in high dose females decreased ~50% Recovery group, Hct, RBC & Hbg recovered by d28 (males) and d42 (females) post treatment. WBC recovered by day 42 (d14 post treatment).
Homeostasis	no effects
Clinical Chem	inconsistent decreases in serum cholesterol
Urinalysis	no toxicology significant effects
Organ Weight	dose related decrease in mean absolute and relative thymus weight in females, this decrease was not completely reversed after eight weeks.
Gross Pathology	decreased thymus size in 5 of 8 high dose dogs, this decrease remained in 4 of 6 recovery dogs after eight weeks.
Histopathology	Thymus: atrophy consistent with decreased thymus weight, remained in 1 of 6 recovery dogs. Lymph nodes: All high dose dogs had minimal to mild follicular atrophy, reversible. Bone Marrow: Mild to severe atrophy, hypocellularity in one high dose male and in the female killed on day 23. Colon: Minimal atrophy of the mucosa in 7 of 8 high dose dogs. Testes: incidence of multinucleated spermatogonial giant cells suggests possible dose related toxicity, but the data is equivocal. Symptom remained in 1of 3 recovery high dose dogs

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4) 3-Month Oral Toxicity study of SK&F-104864-A in Dogs

Major findings

Three months of daily oral topotecan at a dose of 60 mg/m^2/day was lethal to all dogs in this high dose group between days 30 and 71 of the study. The investigators did not attempt to decrease the dose in this group. These dogs probably died from toxicity to the gastrointestinal tract seen microscopically at all levels and damage to the liver.

All the dogs dosed with 0.6, 6 or 20 mg/m^2 survived to the end of the study but demonstrated a dose dependent decrease in red cell parameters and damage to the liver and gastrointestinal system that increased with time and dose.

Study number	SKF-105685/TP-1010/1, Contractor's Report No. G91061 Submitted to IND 32,693, July 23, 2003, IT, Seq. 580 Report 9
Conducting laboratory	SmithKline Beecham Pharmaceuticals, King of Prussia, PA
Date of study initiation	November 1, 1991
GLP compliance	Yes
QA report	Yes
Drug	SK&F-104864-A, Lot CSL-14467-276, 99.5% pure This drug lot is not listed in the CMC section
Methods	
Doses	0, 0.03, 0.3, 1 or 3 mg/kg/d (expressed as free base) 0, 0.6, 6, 20 or 60 mg/m^2/d
Species	male and female Beagle dogs age 7 to 8 months, weight 8.2 to 12.4 kg
Number	3 per sex per dose group
Route	PO
Schedule	daily for 90 days
Formulation	gelatin capsules
Necropsy	Days 90, 91, and 92 – no recovery groups
Histopathology	Adequate battery
Pharmacokinetics	Days 1, 29, and 89 serial samples. Samples were also taken pre-dose, days 15, 44, 61 and 75 to assess steady state concentrations. See the section on Pharmacokinetics and Toxicokinetics above

Mortality – All dogs in the high dose group (60 mg/m^2/day) died or were killed humanely between days 30 and 71 of the study. The investigators did not attempt to decrease the dose in this group. There were no deaths in any of the other groups.

The following table shows the major results of this study. The study report says that the investigators analyzed the results by ANOVA. The statistics report indicates that none of the parameters were statistically different from control. Although, I did not do any further statistical analysis on any of the parameters, some appear to show a dose response that would be toxicologically relevant. The results show a clear dose dependent decrease in red cell parameters in the male dogs that died before scheduled necropsy.

Parameter	Observation time	0.6 mg/m^2	6 mg/m^2	20 mg/m^2	60 mg/m^2
Mortality		0/6	0/6	0/6	6/6
Clinical Signs	Daily				
Emesis					Frequent and severe, foamy, red, usually within one hour after dosing
Abnormal Stool		Slight increased incidence	Slight increased incidence	Incidence increased with dose and with time on study	Frequent soft mucoid stool, sometimes hemocult positive
Excessive salivation					Pre and post dosing, high incidence
Body Weight	Weekly	NTS	NTS	NTS	NTS
Food consumption	Day -6, 7, 42, 84	NTS	NTS	NTS	Anorexia prior to death in some dogs
ECG	Pre-dose, days 26 and 86	NTS	NTS	NTS	NTS
Ophthalmoscopy	Pre-dose, days 51 and 86	NTS	NTS	NTS	NTS
Hematology	Day -16, 15, 57, 85				
RBC male	Day 15				-16.1%
Hgb male	Day 15				-8.5%
Hct male	Day 15	-0.5%	-6.8%	-4.2%	-13.0%
RBC male	Day 57	4.3%	-6.1%	-8.9%	
Hgb male	Day 57	1.2%	-6.4%	-10.5%	
Hct male	Day 57	0.4%	-6.1%	-9.8%	
RBC female	Day 57	-6.0%	-9.4%	-10.8%	
Hgb female	Day 57	-5.2%	-10.3%	-7.5%	
Hct female	Day 57	-5.6%	-8.2%	-6.4%	
RBC male	Day 85	7.1%	-5.1%	-4.5%	
Hgb male	Day 85	1.6%	-6.4%	-7.6%	
Hct male	Day 85	2.9%	-4.6%	-4.8%	
Platelets male	Day 85	6.1%	4.2%	57.6%	
RBC female	Day 85	-10.3%	-5.9%	-11.7%	
Hgb female	Day 85	-10.1%	-7.3%	-9.1%	
Hct female	Day 85	-10.5%	-4.8%	-6.7%	
Platelets female	Day 85	-7.5%	29.4%	40.1%	
Clotting Parameters	Day -16, 15, 57, 85	NTS	NTS	NTS	NTS
Clinical Chemistry	Day -16, 15, 57, 85				
Ca^{++} male	Day 15	-1.2%	-2.8%	-2.2%	-6.9%
Urea Nitrogen male	Day 15	0.0%	7.6%	-7.6%	-15.9%
Creatinine male	Day 15	-3.0%	-7.0%	-3.0%	-13.0%
Glucose male	Day 15	-8.7%	5.4%	-2.2%	-10.1%
Cholesterol male	Day 15	1.1%	-13.3%	-29.4%	-48.9%
Triglycerides male	Day 15	3.7%	-28.6%	-38.0%	-55.1%
Total Protein male	Day 15	4.0%	-5.8%	-5.3%	-13.5%
Albumin male	Day 15	1.9%	-1.3%	-4.4%	-20.2%
ALP male	Day 15	5.5%	60.1%	-3.1%	17.2%
ALT male	Day 15	-3.8%	7.9%	9.7%	81.5%
AST male	Day 15	1.1%	0.0%	-8.5%	13.3%
Urea Nitrogen female	Day 15	6.5%	13.1%	0.0%	-10.5%
Cholesterol female	Day 15	3.5%	-10.0%	-32.2%	-50.3%
Triglycerides female	Day 15	3.9%	-26.4%	-44.1%	-51.2%
Total Protein female	Day 15	-0.7%	-1.2%	-5.9%	-11.6%
Albumin female	Day 15	-4.2%	-7.1%	-5.0%	-11.9%
Cholesterol male	Day 85	-11.4%	-39.2%	-62.1%	
Triglycerides male	Day 85	-12.0%	-27.2%	-50.1%	
Albumin male	Day 85	-3.0%	-11.2%	-21.2%	
Cholesterol female	Day 85	-2.1%	-53.3%	-57.5%	
Triglycerides female	Day 85	9.5%	-60.8%	-61.5%	
Albumin female	Day 85	-4.0%	-10.2%	-12.4%	
ALT male	Day 85	-0.8%	72.8%	222.9%	
AST male	Day 85	4.7%	7.7%	50.4%	
ALT female	Day 85	2.4%	69.0%	185.2%	
AST female	Day 85	-5.7%	29.0%	52.5%	
Urinalysis	Day -16, 15, 57, 85	NTS	NTS	NTS	NTS
Organ Weights					
Adrenals male		9.2%	12.3%	33.1%	
Adrenals female		-1.8%	3.2%	27.7%	
Microscopic Pathology					
Spleen foam cells			1 m, 1 f	3 m, 3 f	3 m, 3 f
Thymic Atrophy					2 f
Mesenteric lymph node foam cells			1 m	3 m, 2 f	2 m
Popliteal lymph node foam cells			1 f	3 m, 2 f	
Liver hepatocellular vacuolization					3 m, 3 f
Liver Kupffer cell vacuolization					2 m, 2 f
Duodenum acute congestion					1 m, 3 f
Jejunum acute congestion					3 m, 3 f
Ileum acute congestion					2 m, 3 f
Colon glandular dilation					3 f
Colon acute congestion				1 m, 1 f	2 m, 3 f
Cecum acute congestion				1 m	3 f
Rectum glandular dilation					2 m, 2 f
Rectum acute congestion				1 m, 1 f	2 m, 3 f

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5) 6 Month Intravenous Toxicity study in Dogs

Major findings

Daily IV doses of topotecan for 180 days at the high dose of 0.2 mg/m^2/day caused a minor decrease (<10%) in red cell parameters in male dogs consistent with decreased red cell production. There were no other toxicologically significant changes in high dose animals or animals treated with 0.06 or 0.02 mg/m^2/day. The doses in this study were too low to provide toxicologically meaningful information.

Study number	G95117, SKF-104864/100G3W/1 Submitted to IND 32,693, Dec. 20, 1996, IT, Seq. 371, p 130
Conducting laboratory	SmithKline Beecham Pharmaceuticals, King of Prussia, PA
Date of study initiation	October 4, 1995
GLP compliance	Yes
QA report	Yes
Drug	SK&F-104864-A, Lot JW-15880-118-2, 79.8% pure This drug lot is listed as batch U95023 in the CMC section The purity is not stated
Methods	
Doses	0, 0.001, 0.003, or 0.01 mg/kg/day (expressed as free base) 0, 0.020, 0.06 or 0.2 mg/m^2/day
Species	male and female Beagle dogs age 9 to 10 months, weight 10 to 12 kg males, 9 to 11 kg females
Number	4 per sex per dose group
Route	IV, dose volume 1 mL/kg
Schedule	daily for six months
Formulation	3% mannitol$_{(aq)}$, pH 3
Necropsy	day 181 – no recovery groups
Histopathology	Adequate battery
Pharmacokinetics	Days 29, and 180, 0.25, 0.5, 1, 2, 4, and 6 hours post dosing See the section on Pharmacokinetics and Toxicokinetics above

Results

Mortality –	None
Clinical Observations –	None
Body Weight –	No effect
Food Consumption –	No effect
ECG –	No effect
Ophthalmology –	No effect
Hematology –	Mean Hbg in HD males 7 to 8% lower than controls day 177 RBC and Hct in HD males 6 to 10% lower than controls day 177 No other hematological findings
Clotting parameters –	No toxicologically significant changes

Clinical Chemistry –	No toxicologically significant changes
Urinalysis –	No effect
Organ Weight –	No effect
Gross Pathology –	Red discoloration at the injection site, no other changes
Histopathology –	Fibrosis and inflammation at the injection site, no other toxicologically significant changes

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6) GF120918A and SK&F 104864-A: 5-Day Oral Combination Toxicity Study in the Sprague Dawley® Rat

Major findings

The highest dose of topotecan (as the free base) given alone orally daily for five days (the proposed clinical schedule) to rats was only 1.36 mg/m^2, well below the clinical dose of 2.3 mg/m^2 recommended in this NDA. Neither this dose nor lower doses caused significant toxicity. The study provides little information useful to this NDA.

A dose of 250 mg/kg of GF120918A alone caused no significant toxicity. Co-administration of GF120918A with topotecan at doses of 0.07, 0.13, or 0.23 mg/kg/day (0.4, 0.8, or 1.36 mg/m^2/day, respectively) significantly increased the toxicity of topotecan. GF120918A co-administration caused a dramatic increase in topotecan exposure implying an inhibition of topotecan metabolism. The spectrum to toxicities of topotecan in combination with GF120918A (decreases in red and white cell parameters secondary to damage to the bone marrow and lymphatic organs and damage to the liver) was comparable to that seen after administration of higher oral doses of topotecan. Following 19 days of recovery, these treatment-related toxicities appeared reversible. This experiment did not determine a NOAEL for topotecan dosing.

Study number	GlaxoSmithKline Study No. R41080 [redacted] Number 7274-196 Submitted electronically to this NDA
Conducting laboratory	[redacted] Inc.
Date of study initiation	June 24, 2002
GLP compliance	Yes
QA report	Yes
Drug	GF120918A, Batch Number MDRI1004 SK&F 104864-A Batch Number QS-44967-117 This batch number is not listed in the CMC section
Methods	
Doses	The following dose table is from the study report

b(4)

Group	No. of Animals Male	Female	Dose[a] GF120918X (mg/kg/day)	Dose[a] SK&F 104864 (mg/kg/day)	(mg/m²/day)[b]
Toxicity Animals					
1 Vehicle	10	10	0[c]	0[c]	0[c]
2 GF120918A	10	10	250[d]	0[d]	0[d]
3 SK&F 104864-A	10	10	0[c]	0.07[e]	0.4[e]
4 SK&F 104864-A	10	10	0[e]	0.13[e]	0.8[e]
5 SK&F 104864-A	10	10	0[e]	0.23[e]	1.36[e]
6 GF120918A and SK&F 104864-A	10	10	250[f]	0.07[f]	0.4[f]
7 GF120918A and SK&F 104864-A	10	10	250[f]	0.13[f]	0.8[f]
8 GF120918A and SK&F 104864-A	10	10	250[f]	0.23[f]	1.36[f]
Toxicokinetic Animals					
9 Vehicle	3	3	0[c]	0[c]	0[c]
10 GF120918A	9	9	250[d]	0[d]	0[d]
11 SK&F 104864-A	9	9	0[e]	0.07[e]	0.4[e]
12 SK&F 104864-A	9	9	0[e]	0.13[e]	0.8[e]
13 SK&F 104864-A	9	9	0[e]	0.23[e]	1.36[e]
14 GF120918A and SK&F 104864-A	9	9	250[f]	0.07[f]	0.4[f]
15 GF120918A and SK&F 104864-A	9	9	250[f]	0.13[f]	0.8[f]
16 GF120918A and SK&F 104864-A	9	9	250[f]	0.23[f]	1.36[f]

a Dose levels are expressed for the free base GF120918X (a correction factor of 1.10) and for the free base SK&F 104864 (a correction factor of 1.2515).
b Approximate dose levels on a mg/m²/day basis were calculated by multiplying the dose in mg/kg by a factor of 6.
c Groups 1 and 9 received the control article for GF120918A first, closely followed by the control article for SK&F 104864-A only.
d Groups 2 and 10 received GF120918A first, closely followed by the control article for SK&F 104864-A.
e Groups 3 through 5 and 11 through 13 received the control article for GF120918A first, closely followed by the SK&F 104864-A.
f Groups 6 through 8 and 14 through 16 received GF120918A first, closely followed by SK&F 104864-A.

Species	Rat/—.: Sprague Dawley® SD Male: 318 to 380 g, Female: 221 to 275 g (about 12 weeks)
Number	10 per sex per dose main group 5 per sex per dose recovery group 9 per sex per dose toxicokinetic group (3 in control groups)
Route	PO gavage
Schedule	Daily for five days
Formulation	GF120918A: 0.5% (w/w) hydroxypropyl methylcellulose/1% (w/w) ———— in sterile water for injection SK&F 104864-A: 3% (w/w) aqueous mannitol in sterile water for injection adjusted to a pH of 3.0 ± 0.2 using 0.1N HCl 10 mL/kg
Necropsy	day 8 main group, day 22 main group
Histopathology	Adequate battery
Pharmacokinetics	

b(4)

Results

I have excerpted the following series of tables from the sponsor's study report. The tables demonstrate that at the doses of this study topotecan causes little toxicity in male or female rats when given daily for five days PO. The toxicity of topotecan increases significantly when given with GF120918A. The table showing toxicokinetics of topotecan demonstrates that this increase in toxicity is due to an significant increase in exposure to topotecan when co-administered with GF120918A, probably due to a decrease in topotecan metabolism.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.07	0.13	0.23	0.07	0.13	0.23	0	0	0.07	0.13	0.23	0.07	0.13	0.23
Noteworthy Findings: (Noteworthy findings are those considered biologically significant. Data that are statistically significant but not biologically significant may be excluded from this table.)																
Number of Unscheduled Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1[1]	1[2]
Clinical Observations	Beginning on Day 4, hunched appearance, liquid, nonformed or few feces, and yellow or brown haircoat in the perineal or ventral abdominal area were noted predominantly in females given GF120918A and 0.13 or 0.23 mg/kg/day SK&F 104864; other observations in these dose groups included red haircoat and red discharge from eyes; these observations were no longer evident after Day 12.															
Body Weight (g)	Group Mean															
Day 1	352	352	345	345	350	348	353	353	247	244	243	246	246	245	243	251
Day 5[3]	354	353	353	350	351	343*	344*	336*	243	243	245	243	241	236*	232*	216*
Terminal Necropsy, Day 8[3]	336	338	337	333	335	332	322*	309*	231	231	233	230	229	223	215*	186*
Recovery Necropsy, Day 24[3]	372	371	373	379	381	381	367	373	248	244	245	244	246	243	241	233
Organ Weights (Terminal)																
Spleen Absolute (g)	0.8068	0.7851	0.7592	0.7967	0.8984	0.8589	0.6964	0.6141*	0.6387	0.6498	0.6347	0.6229	0.5863	0.5938	0.5270*	0.4068*
Relative (%)	0.2322	0.2318	0.2311	0.2473	0.2674	0.2544	0.2187	0.2009	0.2755	0.2839	0.2788	0.2709	0.2590	0.2771	0.2432	0.2141*
Thymus Absolute (g)	0.5077	0.4742	0.4816	0.3721*	0.4536	0.3279*	0.1640*	0.1026*	0.3081	0.3202	0.2951	0.3230	0.2721	0.1342*	0.0872*	0.0495*
Relative (%)	0.1460	0.1402	0.1468	0.1149*	0.1351	0.0974*	0.0516*	0.0336*	0.1330	0.1405	0.1291	0.1408	0.1197	0.0627*	0.0399*	0.0259*

[1] Animal died following blood collection; death was attributed to technical error.

[2] Animal found dead on Day 8.

[3] Covariate adjusted means for Days 5, 8 and 24. Fasted body weights presented for Days 8 and 24.

* Group mean value for parameter significantly different from the vehicle control group ($p < 0.05$)

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.07	0.13	0.23	0.07	0.13	0.23	0	0	0.07	0.13	0.23	0.07	0.13	0.23
Hematology																
Red blood cells ($E^6/\mu L$)																
Day 8, Terminal	8.46	8.30	8.46	8.44	8.48	7.99*	8.13	7.91*	7.98	7.84	7.90	7.90	7.95	7.10*	7.12*	8.18
Day 24, Recovery	8.36	8.46	8.59	8.57	8.39	8.07	8.48	7.97	7.82	8.01	7.97	7.81	7.79	7.48	7.50	7.28
Hemoglobin (g/dL)																
Day 8, Terminal	15.7	15.4	15.9	15.8	15.6	15.2	14.9	14.9*	14.8	14.9	15.0	15.1	14.9	13.5*	13.4*	15.1
Day 24, Recovery	15.5	15.5	15.7	15.6	15.4	15.3	15.4	15.5	14.8	15.3	15.3	15.0	14.9	14.8	14.8	14.8
Hematocrit (%)																
Day 8, Terminal	46.4	45.6	46.5	46.4	46.2	44.5	43.7*	43.6*	44.0	44.3	44.4	44.6	44.4	39.9*	39.9*	45.7
Day 24, Recovery	46.0	45.9	46.6	46.5	45.3	45.5	46.0	45.2	43.7	45.0	45.3	44.4	44.1	43.7	43.7	43.0
Platelets ($E^3/\mu L$)																
Day 8, Terminal	989	939	953	945	963	1085	1106	863	976	946	970	968	1003	1189*	869	390*
Day 24, Recovery	905	987	1005	969	957	945	1029	1151	961	950	972	884	920	875	915	904
Reticulocytes ($E^3/\mu L$)																
Day 8, Terminal	190	152	146	150	158	134	95*	0*	157	127	187	173	131	68*	0*	0*
Day 24, Recovery	182	176	173	194	193	177	207	205	160	176	153	177	154	159	167	174

* Group mean value for parameter significantly different from the vehicle control group ($p < 0.05$)

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.07	0.13	0.23	0.07	0.13	0.23	0	0	0.07	0.13	0.23	0.07	0.13	0.23
	Group Mean								Group Mean							
Hematology (continued)																
White blood cells ($E^3/\mu L$)																
Day 8, Terminal	8.8	9.0	8.8	8.7	8.6	7.6	5.5*	5.2*	6.0	6.6	6.0	6.5	6.2	5.2	4.7	3.9
Day 24, Recovery	8.3	8.4	8.6	6.6	7.4	8.0	7.6	6.4	4.5	5.3	5.5	6.7*	5.3	6.2	4.2	4.0
Neutrophils ($E^3/\mu L$)																
Day 8, Terminal	1.1	1.2	1.3	1.3	1.1	1.1	0.6*	0.1*	0.6	0.7	0.6	0.7	0.7	0.3*	0.1*	0*
Day 24, Recovery	1.0	1.4	1.1	0.9	1.0	0.9	1.5	1.0	0.6	0.6	0.6	0.7	0.6	0.7	0.4	0.5
Lymphocytes ($E^3/\mu L$)																
Day 8, Terminal	7.5	7.6	7.2	7.3	7.3	6.3	4.8*	5.0*	5.3	5.8	5.2	5.6	5.3	4.8	4.5	3.9
Day 24, Recovery	7.1	6.8	7.3	5.5	6.1	6.9	5.8	5.1	3.8	4.6	4.8	5.9*	4.6	5.3	3.6	3.2
Eosinophils ($E^3/\mu L$)																
Day 8, Terminal	0.1	0.1	0.2	0.1	0.1	0.1	0*	0*	0.1	0.1	0.1	0.1	0.1	0*	0*	0*
Day 24, Recovery	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2*
Red Blood Cell Distribution Width (%)																
Day 8, Terminal	15.1	14.5*	14.8	15.1	14.7	14.7	14.9	14.1*	14.5	14.2	14.1	14.3	14.1	14.0	13.3*	13.7*
Day 24, Recovery	16.4	15.7	16.2	15.7	16.0	17.0	17.2	17.8*	14.8	15.1	15.3	14.7	15.3	15.3	17.0*	18.1*

* Group mean value for parameter significantly different from the vehicle control group ($p < 0.05$)

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.07	0.13	0.23	0.07	0.13	0.23	0	0	0.07	0.13	0.23	0.07	0.13	0.23
	Group Mean								Group Mean							
Clinical Chemistry																
Total protein (g/dL)																
Day 8, Terminal	7.2	7.2	7.1	7.2	6.9	7.2	6.8	6.6*	7.0	7.1	7.2	7.1	7.1	6.6	6.7	5.8*
Day 24, Recovery	6.9	7.0	7.0	7.0	6.9	7.0	7.1	6.8	6.9	7.2	7.2	7.0	6.9	6.9	7.1	6.9
Albumin (g/dL)																
Day 8, Terminal	4.7	4.7	4.7	4.7	4.6	4.7	4.5	4.6	4.8	4.9	4.9	4.9	4.9	4.5*	4.6	3.8*
Day 24, Recovery	4.7	4.7	4.6	4.8	4.6	4.6	4.7	4.6	4.9	5.1	5.1	5.0	4.8	4.9	5.0	4.9
Globulin (g/dL)																
Day 8, Terminal	2.5	2.5	2.4	2.4	2.3	2.4	2.3	2.0*	2.2	2.2	2.3	2.2	2.2	2.1	2.1	2.0
Day 24, Recovery	2.2	2.3	2.4	2.2	2.3	2.4	2.4	2.2	2.1	2.1	2.2	2.0	2.1	2.0	2.1	2.0
Albumin/globulin ratio																
Day 8, Terminal	1.9	1.9	2.1	1.9	2.0	2.0	2.0	2.3*	2.3	2.3	2.2	2.2	2.3	2.1	2.2	2.1
Day 24, Recovery	2.1	2.0	2.0	2.2	2.0	2.0	1.9	2.1	2.3	2.4	2.4	2.5	2.3	2.4	2.4	2.4

* Group mean value for parameter significantly different from the vehicle control group ($p < 0.05$)

Dose (mg/Kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.07	0.13	0.23	0.07	0.13	0.23	0	0	0.07	0.13	0.23	0.07	0.13	0.23
	Group Mean								Group Mean							
Clinical Chemistry (cont'd)																
Cholesterol (mg/dL)																
Day 8, Terminal	87	90	87	81	79	94	81	75	69	78	78	76	76	69	71	108*
Day 24, Recovery	86	82	84	85	80	87	90	87	67	68	83	71	76	75	70	70
Total Bilirubin (mg/dL)																
Day 8, Terminal	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2*	0.2	0.2	0.2*	0.2	0.2	0.4*	0.8*	0.6*
Day 24, Recovery	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2
Pathology Findings	Incidence of Findings															
Number of Rats Examined	5	5	5	5	5	5	5	5	5	5	5	5	5	5	6[1]	6[2]
Macroscopic, Terminal																
Skin																
Stains, perineum/perianal	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	4
Stains, haircoat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3
Lymph node, mandibular																
large	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
mottled	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
diffusely red	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Thymus																
small	0	0	0	0	0	0	0	3	0	0	0	0	1	2	4	6

* Group mean value for parameter significantly different from the vehicle control group ($p < 0.05$)
[1] Includes one female that died following blood collection on Day 8.
[2] Includes one female found dead on Day 8.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.07	0.13	0.23	0.07	0.13	0.23	0	0	0.07	0.13	0.23	0.07	0.13	0.23
Pathology Findings	Incidence of Findings															
Macroscopic Recovery																
Number of Rats Examined	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4	4
Lymph node, mandibular																
diffusely red	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Microscopic, Terminal																
Number of Rats Examined	5	5	0	0	5	5	5	5	5	5	0	0	5	5	6[1]	6[2]
Marrow, femur																
congestion, sinusoidal	0	0	-	-	0	0	1	5	0	0	-	-	0	0	5	3
hypocellular, marrow	0	0	-	-	0	0	5	5	0	0	-	-	0	0	5	6
Marrow, sternum																
congestion, sinusoidal	0	0	-	-	0	0	0	5	0	0	-	-	0	0	5	4
hypocellular, marrow	0	0	-	-	0	0	0	5	0	0	-	-	0	0	5	5
Number of Rats Examined	5	5	0	0	5	5	4	5	5	5	0	0	5	5	6[1]	6[2]
Thymus																
depletion, lymphocytic often with lymphonecrosis	0	0	-	-	0	0	3	5	0	0	-	-	0	5	6	6
hemorrhage	1	2	-	-	2	1	0	0	1	0	-	-	3	1	2	2
hyperplasia, reticuloendothelial	0	0	-	-	0	2	3	0	0	0	-	-	0	5	3	0

\- Not applicable/not examined
[1] Includes one female that died following blood collection on Day 8.
[2] Includes one female found dead on Day 8.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.07	0.13	0.23	0.07	0.13	0.23	0	0	0.07	0.13	0.23	0.07	0.13	0.23
Pathology Findings	Incidence of Findings															
Microscopic, Terminal (cont'd)																
Number of Rats Examined	5	5	0	0	5	5	5	5	5	5	0	0	5	5	6[1]	6[2]
Spleen																
depletion, lymphocytic	0	0	-	-	0	0	0	0	0	0	-	-	0	0	0	1
Lymph node, mandibular																
depletion, lymphocytic	1	0	-	-	0	0	0	4	0	0	-	-	0	0	4	5
edema	0	0	-	-	0	1	0	0	0	0	-	-	0	0	0	0
hemorrhage	0	1	-	-	0	3	0	1	0	0	-	-	1	1	1	0
necrosis	0	0	-	-	0	0	0	0	0	0	-	-	0	0	0	1
Lymph node, mesenteric																
depletion, lymphocytic	0	0	-	-	0	4	5	5	0	0	-	-	0	3	5	4
infiltrates, macrophage, vacuolated	2	3	-	-	0	0	0	4	0	1	-	-	2	0	1	6
Small Intestine (Duodenum, Jejunum, Ileum)																
degeneration/necrosis, mucosal with hyperplasia and atrophy	0	0	-	-	0	0	0	0	0	0	-	-	0	0	0	5[3]

- Not applicable/not examined
1 Includes one female that died following blood collection on Day 8.
2 Includes one female found dead on Day 8.
3 Incidence is 6 for Ileum.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.07	0.13	0.23	0.07	0.13	0.23	0	0	0.07	0.13	0.23	0.07	0.13	0.23
Pathology Findings	Incidence of Findings															
Microscopic, Terminal (cont'd)																
Number of Rats Examined	5	5	0	0	5	5	5	5	5	5	0	0	5	5	6[1]	6[2]
Large Intestine (Cecum, Colon, Rectum)																
degeneration/necrosis, mucosal with hyperplasia	0	0	-	-	0	0	0	0	0	0	-	-	0	0	0	6[3]

- Not applicable/not examined
1 Includes one female that died following blood collection on Day 8.
2 Includes one female found dead on Day 8.
3 Incidence is 5 for Cecum

The following table compiled by the investigators shows the results of the toxicokinetic investigation in this study. The table shows that the exposure to topotecan increases dramatically in the presence of GF120918A, implying an inhibition of topotecan metabolism.

Dose (mg/kg/day)	Male								Female							
GF120918A[a]	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A[b]	0	0	0.07	0.13	0.23	0.07	0.13	0.23	0	0	0.07	0.13	0.23	0.07	0.13	0.23
Number of Animals																
Main	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Recovery	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5[f]	5[f]
Toxicokinetic	3	9	9	9	9	9	9	9	3	9	9	9	9	9	9	9
GF120918X																
AUC_{0-t} (ng.h/mL)																
Day 1	NA	1480	NA	NA	NA	1627	1733	2106	NA	7851	NA	NA	NA	7675	7268	8013
Day 5	NA	1833	NA	NA	NA	1776	2136	2132	NA	9290	NA	NA	NA	12124	12007	10099
C_{max} (ng/mL)																
Day 1	NA	85.3	NA	NA	NA	86.1	116	120	NA	385	NA	NA	NA	409	407	508
Day 5	NA	108	NA	NA	NA	81.7	111	100	NA	487	NA	NA	NA	572	620	488
SK&F 104864																
AUC_{0-t} (ng.h/mL)																
Day 1	NA	NA	6.87	6.89	10.4	42.8	70.3	125	NA	NA	6.39	13.8	15.8	50.5	94.2	169
Day 5	NA	NA	3.44	6.87	11.5	37.8	64.7	124	NA	NA	5.64	9.47	15.6	65.6	114	200
C_{max} (ng/mL)																
Day 1	NA	NA	1.04	1.43	2.08	5.04	8.97	11.1	NA	NA	2.63	3.76	4.64	8.19	15.4	25.0
Day 5	NA	NA	0.72	1.60	2.33	5.09	7.02	11.9	NA	NA	1.19	2.44	4.68	8.97	12.4	20.2

[a] Dose levels are for GF120918A (dose levels expressed as the free base, GF120918X) or GF120918A vehicle.
[b] Dose levels are for SK&F 104864-A (dose levels expressed as the free base, SK&F 104864) or SK&F 104864-A vehicle.
NA Not applicable
[f] Only 4 females survived to end of recovery in these groups.

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7) GF120918A and SK&F 104864-A: 5-Day Oral Combination Toxicity Study in the Sprague Dawley® Rat

Major findings

A dose of 13.8 mg/m^2/day for five days of topotecan PO was lethal to one female rat. This dose and the mid dose of 4.74 mg/m^2 caused weight loss, decreased platelets, reticulocytes, white cell count, neutrophils, lymphocytes, monocytes, and eosinophils. These doses also caused increases in blood glucose, cholesterol and bilirubin and decreases in total protein, albumin. Microscopic damage was seen in the bone marrow, lymph nodes and GI.

Giving GF120918A seriously exacerbated the toxicity of topotecan necessitating unscheduled necropsy of all mid and high dose animals on day 5.

Study number	GlaxoSmithKline Study No. R41021 [redacted] Number 7274-141 Submitted electronically to this NDA
Conducting laboratory	[redacted] b(4)
Date of study initiation	February 7, 2002
GLP compliance	Yes
QA report	Yes
Drug	GF120918A, Batch Number F030941 SK&F 104864-A Batch Number 104864-14-03M

This drug batch is not listed in the CMC section, but a Batch 104864-A4-02M is listed in association with Batch U01028 purity 97.8%

Methods

Doses

Dose Group	Dose GF120918A mg/kg	Topotecan mg/kg	Topotecan mg/m²
Toxicology			
Vehicle 1	0	0	0
GF120918A 2	250	0	0
Topotecan 3	0	0.23	1.38
Topotecan 4	0	0.79	4.74
Topotecan 5	0	2.3	13.8
GF120918A + Topotecan 6	250	0.23	1.38
GF120918A + Topotecan 7	250	0.79	4.74
GF120918A + Topotecan 8	250	2.3	13.8
Toxicokinetics			
Vehicle 9	0	0	0
GF120918A 10	250	0	0
Topotecan 11	0	0.23	1.38
Topotecan 12	0	0.79	4.74
Topotecan 13	0	2.3	13.8
GF120918A + Topotecan 14	250	0.23	1.38
GF120918A + Topotecan 15	250	0.79	4.74
GF120918A + Topotecan 16	250	2.3	13.8

Species	Rat/ ——Sprague Dawley® SD Male: 335 to 379, Female: 192 to 225 (about 12 weeks)
Number	10 per sex per dose main group 5 per sex per dose recovery group 9 per sex per dose toxicokinetic group (3 in control groups)
Route	PO gavage
Schedule	Daily for five days
Formulation	GF120918A: 0.5% (w/w) hydroxypropyl methylcellulose/1% (w/w) ——— in sterile water for injection SK&F 104864-A: 3% (w/w) aqueous mannitol in sterile water for injection adjusted to a pH of 3.0 ± 0.2 using 0.1N HCl 10 mL/kg
Necropsy	day 8 main group; day 22 main group
Histopathology	Adequate battery
Pharmacokinetics	See Pharmacokinetic and Toxicokinetics section

b(4)

Results

The following table shows that all the rats given GF120918A plus 0.79 or 2.3 mg/kg of topotecan (groups 7 and 8) and one female given GF120918A plus 0.23 mg/kg topotecan (group 6) were moribund by day five. The investigators killed these animals humanely. They also appropriately opted to kill the animals in the high and mid-dose topotecan only groups (groups 4 and 5) to serve as controls for the animals given both drugs. One female given 2.3 mg/kg topotecan only (group 4) also died; all other animals given topotecan alone or GF120918A alone survived to the end of the study. The clinical signs seen in animals prior to death were similar to those seen with high dose topotecan; that is, GF120918A alone caused little toxicity but modified the toxic dose response curve of topotecan. The table also shows a dose dependent decrease in body weight in animals given the drug combination and animals given topotecan alone.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.23	0.79	2.3	0.23	0.79	2.3	0	0	0.23	0.79	2.3	0.23	0.79	2.3
Noteworthy Findings: (Noteworthy findings are those considered biologically significant. Data that are statistically significant but not biologically significant may be excluded from this table.)																
Number of Unscheduled Deaths	0	0	0	0[c]	0[c]	0	10	10	0	0	0	0[d]	1[d]	1	10	10
Clinical Observations	Beginning as early as Day 3, hunched appearance; liquid, nonformed, or few feces; and yellow or brown haircoat in the perineal or ventral abdominal area were noted in animals given GF120918A and 0.79 or 2.3 mg/kg/day SK&F 104864; other observations in these dose groups included red haircoat, hypoactive behavior, squinting, cold to touch, thinness, clear discharge from the eyes, and discolored/dark red eyes. In general, the incidence of these observations increased with additional days of dosing. Beginning on Day 5 or 8, females given GF120918A and 0.23 mg/kg/day SK&F 104864 had discolored haircoat, liquid or nonformed feces, and hunched appearance; these observations were no longer evident after Day 13. One of these females (found dead on Day 8) also appeared thin and was squinting. No test article-related clinical observations were noted in animals given 0.23 or 0.79 mg/kg/day SK&F 104864 or GF120918A alone. Observations in animals administered 2.3 mg/kg/day SK&F 104864 alone were similar to those noted in animals given GF120918A and 0.79 or 2.3 mg/kg/day SK&F 104864.															
Body Weight (g)	Group Mean								Group Mean							
Day 1	357	359	359	354	353	359	357	359	209	206	205	206	207	205	207	207
Day 5	362	366	363	343*	328*	339*	301*	293*	216	210	208	201*	175*	188*	171*	157*
Day 7	371	372	368	-	-	330*	-	-	219	225	217	-	-	175*	-	-
Day 21	381	391	393	-	-	400	-	-	233	233	230	-	-	229	-	-

[c] Animals given GF120918A and 0.79 or 2.3 mg/kg/day SK&F-104864 were euthanized in a moribund condition on Day 5; all animals given 0.79 mg/kg/day SK&F-104864 alone and all surviving animals given 2.3 mg/kg/day SK&F-104864 alone were also euthanized on Day 5 to allow comparison with the respective combination groups. These animals were not fasted prior to clinical pathology blood sample collection/necropsy.

* Group mean value for parameter significantly different from the vehicle control group ($p < 0.05$)

\- No value

The following table shows that the red cell parameters in high and mid dose combination drug animals (groups 7 and 8) increase slightly by day five probably because of hemo-concentration secondary to dehydration. Platelets decrease in these animals. Red cell parameters and platelets decrease in low dose topotecan only animals but the decrease did not reach significance. These parameters are also decreased in low dose combination animals.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.23	0.79	2.3	0.23	0.79	2.3	0	0	0.23	0.79	2.3	0.23	0.79	2.3
Hematology																
Red blood cells ($E^6/\mu L$)																
Day 5, Unscheduled	-	-	-	8.43	9.57	-	11.93	12.46	-	-	-	7.22	9.39	-	10.59	12.07
Day 8, Terminal	8.74	8.68	8.44	-	-	8.26*	-	-	7.82	7.64	7.87	-	-	8.35	-	-
Day 22, Recovery	8.58	8.36	8.20	-	-	7.94*	-	-	7.75	7.41	7.78	-	-	7.10*	-	-
Hemoglobin (g/dL)																
Day 5, Unscheduled	-	-	-	15.4	16.7	-	21.0	21.8	-	-	-	14.3	17.9	-	20.1	21.8
Day 8, Terminal	15.7	15.7	15.6	-	-	15.0*	-	-	15.4	15.1	15.1	-	-	15.6	-	-
Day 22, Recovery	15.4	15.2	15.0	-	-	15.2	-	-	15.1	14.9	15.1	-	-	14.8	-	-
Hematocrit (%)																
Day 5, Unscheduled	-	-	-	44.3	48.3	-	60.2	62.0	-	-	-	40.5	51.1	-	57.0	61.1
Day 8, Terminal	45.8	45.5	45.2	-	-	43.6*	-	-	44.6	43.9	43.9	-	-	45.5	-	-
Day 22, Recovery	45.4	44.4	44.5	-	-	44.5	-	-	43.8	42.8	43.9	-	-	42.4	-	-
Platelets ($E^3/\mu L$)																
Day 5, Unscheduled	-	-	-	1168	1104	-	908	643	-	-	-	1219	1085	-	789	631
Day 8, Terminal	1068	1031	972	-	-	799*	-	-	1119	1125	1215	-	-	487*	-	-
Day 22, Recovery	1087	1097	1033	-	-	1045	-	-	1119	1046	1095	-	-	1017	-	-
Reticulocytes ($E^3/\mu L$)																
Day 5, Unscheduled	-	-	-	18	0	-	0	0	-	-	-	9	0	-	0	0
Day 8, Terminal	206	160	214	-	-	3*	-	-	178	187	166	-	-	0*	-	-
Day 22, Recovery	171	155	208	-	-	217	-	-	162	154	155	-	-	169	-	-

* Group mean value for parameter significantly different from the vehicle control group ($p < 0.05$)

\- No value

The study investigators constructed the following table to demonstrate the effect of treatment on white cell parameters. Treatment with topotecan alone significantly lowered white blood cell counts while combination treatment severely exacerbated this toxicity. This toxicity probably contributed significantly to the deterioration of the condition of the animals killed prior to scheduled necropsy.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.23	0.79	2.3	0.23	0.79	2.3	0	0	0.23	0.79	2.3	0.23	0.79	2.3
	Group Mean								Group Mean							
Hematology (continued)																
White blood cells ($E^3/\mu L$)																
Day 5, Unscheduled	-	-	-	5.9	2.5	-	1.4	1.0	-	-	-	5.3	1.7	-	1.1	0.6
Day 8, Terminal	6.5	6.9	7.4	-	-	4.7*	-	-	6.3	5.8	5.8	-	-	2.6*	-	-
Day 22, Recovery	5.2	5.4	6.4	-	-	6.2	-	-	5.3	4.2	4.2	-	-	4.0	-	-
Neutrophils ($E^3/\mu L$)																
Day 5, Unscheduled	-	-	-	0.6	0.1	-	0.1	0.0	-	-	-	0.3	0.0	-	0.0	0.0
Day 8, Terminal	0.9	1.1	1.1	-	-	0.2*	-	-	0.6	0.9	0.6	-	-	0.1*	-	-
Day 22, Recovery	0.9	0.8	0.8	-	-	1.1	-	-	0.7	0.5	0.4*	-	-	0.7	-	-
Lymphocytes ($E^3/\mu L$)																
Day 5, Unscheduled	-	-	-	5.2	2.3	-	1.3	0.8	-	-	-	4.9	1.6	-	1.0	0.5
Day 8, Terminal	5.2	5.4	5.9	-	-	4.4	-	-	5.4	4.6	4.9	-	-	2.5*	-	-
Day 22, Recovery	3.9	4.1	5.1	-	-	4.5	-	-	4.3	3.5	3.5	-	-	2.9	-	-
Monocytes ($E^3/\mu L$)																
Day 5, Unscheduled	-	-	-	0.1	0.0	-	0.1	0.1	-	-	-	0.1	0.0	-	0.1	0.1
Day 8, Terminal	0.3	0.3	0.4	-	-	0.1*	-	-	0.2	0.2	0.2	-	-	0.0*	-	-
Day 22, Recovery	0.3	0.4	0.4	-	-	0.5*	-	-	0.2	0.2	0.2	-	-	0.3	-	-
Eosinophils ($E^3/\mu L$)																
Day 5, Unscheduled	-	-	-	0.0	0.0	-	0.0	0.0	-	-	-	0.0	0.0	-	0.0	0.0
Day 8, Terminal	0.1	0.1	0.1	-	-	0.0*	-	-	0.1	0.1	0.1	-	-	0.0*	-	-
Day 22, Recovery	0.1	0.1	0.1	-	-	0.2	-	-	0.1	0.1	0.1	-	-	0.2	-	-

* Group mean value for parameter significantly different from the vehicle control group ($p < 0.05$)
\- No value

The investigators constructed the following table to demonstrate the changes in clinical chemistry parameters associated with dosing. The table shows that high doses of topotecan given daily for five days caused significant hyperglycemia. The investigators did not mark this change as significant because they did not have a vehicle control group for comparison, nevertheless the magnitude of the changes is sufficient to consider the changes toxicologically significant. This toxicity is probably a direct result of toxic insult to the pancreas. Again, co-administration of GF120918A exacerbated this toxicity. BUN and creatinine also increased with dosing suggesting significant kidney damage. Total protein and albumin decreased suggesting damage to the liver.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.23	0.79	2.3	0.23	0.79	2.3	0	0	0.23	0.79	2.3	0.23	0.79	2.3
	Group Mean								Group Mean							
Clinical Chemistry																
Glucose (mg/dL)																
Day 5, Unscheduled	-	-	-	136	148	-	263	290	-	-	-	163	184	-	173	257
Day 8, Terminal	98	97	97	-	-	106	-	-	97	105	105	-	-	125*	-	-
Day 22, Recovery	95	96	97	-	-	95	-	-	100	109	97	-	-	102	-	-
Urea nitrogen (mg/dL)																
Day 5, Unscheduled	-	-	-	16	21	-	44	68	-	-	-	17	30	-	34	86
Day 8, Terminal	20	20	19	-	-	22	-	-	20	21	20	-	-	31*	-	-
Day 22, Recovery	20	20	19	-	-	20	-	-	20	21	20	-	-	18	-	-
Creatinine (mg/dL)																
Day 5, Unscheduled	-	-	-	0.8	0.7	-	0.9	1.2	-	-	-	0.8	0.7	-	0.8	1.2
Day 8, Terminal	0.8	0.8	0.8	-	-	0.8	-	-	0.8	0.9	0.8	-	-	0.7*	-	-
Day 22, Recovery	0.9	0.8	0.8	-	-	0.8	-	-	0.9	0.9	0.9	-	-	0.8	-	-
Total protein (g/dL)																
Day 5, Unscheduled	-	-	-	7.2	5.9	-	6.2	6.7	-	-	-	6.6	5.8	-	5.9	6.3
Day 8, Terminal	7.3	7.3	7.1	-	-	6.2*	-	-	7.2	7.1	7.0	-	-	5.7*	-	-
Day 22, Recovery	7.3	7.1	7.4	-	-	7.0	-	-	7.3	7.4	7.3	-	-	7.2	-	-
Albumin (g/dL)																
Day 5, Unscheduled	-	-	-	4.4	3.7	-	3.9	3.8	-	-	-	4.4	3.9	-	3.8	3.8
Day 8, Terminal	4.5	4.5	4.5	-	-	4.1*	-	-	4.7	4.7	4.7	-	-	4.0*	-	-
Day 22, Recovery	4.7	4.6	4.7	-	-	4.6	-	-	4.9	5.1	5.1	-	-	5.0	-	-

* Group mean value for parameter significantly different from the vehicle control group ($p < 0.05$)
\- No value

The following table is a continuation of the previous table, and again the changes in the parameters are consistent with significant drug induced hepatic damage.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.23	0.79	2.3	0.23	0.79	2.3	0	0	0.23	0.79	2.3	0.23	0.79	2.3
Clinical Chemistry (cont.)	Group Mean								Group Mean							
Globulin (g/dL)																
Day 5, Unscheduled	-	-	-	2.8	2.2	-	2.3	2.9	-	-	-	2.2	1.9	-	2.1	2.5
Day 8, Terminal	2.8	2.9	2.6	-	-	2.1*	-	-	2.5	2.4	2.3	-	-	1.7*	-	-
Day 22, Recovery	2.7	2.6	2.7	-	-	2.4	-	-	2.4	2.3	2.2	-	-	2.3	-	-
A/G ratio																
Day 5, Unscheduled	-	-	-	1.6	1.7	-	1.7	1.4	-	-	-	2.0	2.1	-	1.8	1.6
Day 8, Terminal	1.6	1.6	1.7	-	-	2.1*	-	-	1.9	2.0	2.0	-	-	2.4*	-	-
Day 22, Recovery	1.8	1.8	1.8	-	-	1.9	-	-	2.1	2.2	2.3	-	-	2.2	-	-
Total bilirubin (mg/dL)																
Day 5, Unscheduled	-	-	-	0.2	0.2	-	0.3	0.2	-	-	-	0.2	0.2	-	0.3	0.4*
Day 8, Terminal	0.1	0.1	0.1	-	-	0.3*	-	-	0.1	0.1	0.1	-	-	0.4*	-	-
Day 22, Recovery	0.1	0.1	0.1	-	-	0.1	-	-	0.1	0.1	0.2*	-	-	0.1	-	-
Serum bile acids (µmol/L)																
Day 5, Unscheduled	-	-	-	76	71	-	84	115	-	-	-	35	61	-	95	125
Day 8, Terminal	9	9	9	-	-	14	-	-	9	20	11	-	-	13	-	-
Day 22, Recovery	12	12	9	-	-	9	-	-	11	14	10	-	-	10	-	-
Cholesterol (mg/dL)																
Day 5, Unscheduled	-	-	-	86	100	-	151	164	-	-	-	95	117	-	125	113
Day 8, Terminal	74	80	74	-	-	78	-	-	78	86	78	-	-	69	-	-
Day 22, Recovery	84	78	84	-	-	81	-	-	87	67	86	-	-	73	-	-

* N = 1
* Group mean value for parameter significantly different from the vehicle control group ($p < 0.05$)
\- No value

The following table demonstrates dose related damage to the gastrointestinal tract, spleen and thymus. Again, combination dosing with GF120918A increased the incidence of toxicity in these target organs.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.23	0.79	2.3	0.23	0.79	2.3	0	0	0.23	0.79	2.3	0.23	0.79	2.3
Pathology Findings	Incidence of Findings															
Day of Necropsy	8	8	8	5	5	8	5	5	8	8	8	5	5	8	5	5
Number of Rats Examined	5	5	5	10	10	5	10	10	5	5	5	10	10[f]	5[g]	10	10
Macroscopic, Days 5 and 8																
Stains																
perineum/perianal	0	0	0	0	3	0	10	7	0	0	0	0	5	1	8	1
skin/haircoat	0	0	0	0	0	0	2	3	0	0	0	0	2	1	5	9
Discharge																
ocular	0	0	0	0	0	0	2	1	0	1	0	0	2	0	1	7
nasal	0	0	0	0	0	0	2	3	0	0	0	0	0	0	1	1
oral	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	2
Gastrointestinal tract																
light fluid content	0	0	0	0	3	0	7	7	0	0	0	0	1	0	5	8
light dry content	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1
Spleen																
small	0	0	0	0	0	0	4	7	0	0	0	0	1	1	9	4
Thymus																
small	0	0	0	0	6	3	6	9	0	0	0	1	5	2	10	6
Ileum																
diffusely red	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

[f] One female was found dead on Day 5.
[g] One female was found dead on Day 8.

Again, the following table is a continuation of the previous table. It also shows dose dependent damage in the gastrointestinal system and almost universal damage to the bone marrow in the high and mid dose groups. This is consistent with the severe decreases in white cell parameters demonstrated above.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.23	0.79	2.3	0.23	0.79	2.3	0	0	0.23	0.79	2.3	0.23	0.79	2.3
Pathology Findings	Incidence of Findings															
Day of Necropsy	8	8	8	5	5	8	5	5	8	8	8	5	5	8	5	5
Number of Rats Examined	5	5	5	10	10	5	10	10	5	5	5	10	10[f]	5[g]	10	10
Macroscopic, Days 5 and 8 (continued)																
Cecum																
diffusely red	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
thickened wall	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Thin animal	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	4
Mesenteric lymph node																
small	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Microscopic, Days 5 and 8																
Marrow, femur																
congestion, sinusoidal	0	1	0	7	10	5	10	10	0	2	1	10	10	5	10	10
hypocellular, marrow	0	0	0	6	10	5	10	10	0	0	0	10	10	5	10	10
Marrow, sternum																
congestion, sinusoidal	0	0	0	7	10	5	10	10	0	0	0	10	10	5	10	10
hypocellular, marrow	0	0	0	2	10	3	10	10	0	0	0	8	10	5	10	10
Spleen																
depletion, lymphocytic	0	0	0	0	0	0	10	10	0	0	0	0	5	2	10	10

[f] One female was found dead on Day 5.
[g] One female was found dead on Day 8.

The following three tables demonstrate significant dose related microscopic damage to lymphoid system, gastrointestinal tract and skin.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.23	0.79	2.3	0.23	0.79	2.3	0	0	0.23	0.79	2.3	0.23	0.79	2.3
Pathology Findings	Incidence of Findings															
Day of Necropsy	8	8	8	5	5	8	5	5	8	8	8	5	5	8	5	5
Number of Rats Examined	5	5	5	10	10	5	10	10	5	5	5	10	10[f]	5[g]	10	10
Microscopic, Days 5 and 8																
Thymus																
depletion, lymphocytic	0	0	0	10	9	4	9	10	0	0	0	10	10	4	9	10
Lymph node, mandibular																
depletion, lymphocytic	0	0	4	10	9	3	10	10	0	0	2	8	10	5	10	10
Lymph node, mesenteric																
depletion, lymphocytic	0	0	0	0	1	0	3	8	0	0	0	0	2	2	6	10
Stomach, nonglandular																
hypertrophy/ hyperplasia, mucosa	0	0	0	0	0	0	2	4	0	0	0	0	0	0	0	4
edema	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	1
inflammation, chronic	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Duodenum																
degeneration/necrosis with hyperplasia and atrophy, mucosal	0	0	0	2	10	1	10	10	0	0	0	0	10	2	10	10

[f] One female was found dead on Day 5.
[g] One female was found dead on Day 8.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.23	0.79	2.3	0.23	0.79	2.3	0	0	0.23	0.79	2.3	0.23	0.79	2.3
Pathology Findings	**Incidence of Findings**															
Day of Necropsy	8	8	8	5	5	8	5	5	8	8	8	5	5	8	5	5
Number of Rats Examined	5	5	5	10	10	5	10	10	5	5	5	10	10[f]	5[g]	10	10
Microscopic, Days 5 and 8 (continued)																
Jejunum																
degeneration/necrosis with hyperplasia and atrophy, mucosal	0	0	0	4	10	1	10	10	0	0	0	3	10	1	10	10
Ileum																
degeneration/necrosis with hyperplasia and atrophy, mucosal	0	0	1	6	10	1	10	10	0	0	0	6	10	2	10	10
Cecum																
degeneration/necrosis with hyperplasia and atrophy, mucosal	0	0	0	3	10	0	10	10	0	0	0	3	10	2	10	10

[f] One female was found dead on Day 5.
[g] One female was found dead on Day 8.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.23	0.79	2.3	0.23	0.79	2.3	0	0	0.23	0.79	2.3	0.23	0.79	2.3
Pathology Findings	**Incidence of Findings**															
Day of Necropsy	8	8	8	5	5	8	5	5	8	8	8	5	5	8	5	5
Number of Rats Examined	5	5	5	10	10	5	10	10	5	5	5	10	10[f]	5[g]	10	10
Microscopic, Days 5 and 8 (continued)																
Colon																
degeneration/necrosis with hyperplasia and atrophy, mucosal	0	0	0	1	9	0	9	10	0	0	0	0	10	1	9	10
Rectum																
degeneration/necrosis with hyperplasia and atrophy, mucosal	0	0	0	1	8	0	9	10	0	0	0	0	10	0	8	10
Skin																
atrophy, adnexal	0	0	0	0	5	0	0	7	0	0	0	0	6	1	1	8
necrosis, single cell, adnexa	0	0	0	0	0	0	5	8	0	0	0	0	0	0	3	4

[f] One female was found dead on Day 5.
[g] One female was found dead on Day 8.

The following table shows that not all damage to the lymphatic system was completely healed at the end of the recovery period.

Dose (mg/kg/day)	Male								Female							
GF120918A	0	250	0	0	0	250	250	250	0	250	0	0	0	250	250	250
SK&F 104864-A	0	0	0.23	0.79	2.3	0.23	0.79	2.3	0	0	0.23	0.79	2.3	0.23	0.79	2.3
Pathology Findings	Incidence of Findings															
Number of Rats Examined	5	5	5	0	0	5	0	0	5	5	5	0	0	5	0	0
Microscopic, Recovery																
Lymph node, mandibular depletion, lymphocytic	0	0	1	-	-	2	-	-	0	0	0	-	-	0	-	-

- No value

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8) Evaluation of the Toxicity of Degradants in Oral Topotecan Formulations in the Dog for 1 Month (Two 5-Day Cycles of Drug Treatment) - Study 1

Major findings

The doses in this study were considerably less than the proposed clinical dose on a mg/m^2 basis rendering the study of questionable value. It appears that Formulation 1 may cause more myelotoxicity than Formulation 2, but Formulation 2 may be somewhat more hepatotoxic. The exposure to Formulation 1 is greater that that for Formulation 2 to a degree greater than one would expect because it is the more pure of the two formulations. Exposure to Formulation 2 is greater than dose proportional. Formulation 1 contained —— impurities (—— % to —— %) whereas Formulation 2 contained —— to ——%). b(4)

Because there are some differences in the toxicity profile and the exposure, I cannot say that this experiment demonstrates that these two "Formulations" are toxicologically similar. Thus, I cannot say that this experiment qualifies the impurities in Formulation 2.

Study number	GlaxoSmithKline Study No. SKF-104864/RSD-100S2S/1 Protocol G97027 Submitted electronically to this NDA
Conducting laboratory	SmithKline Beecham
Date of study initiation	September, 1997
GLP compliance	Yes
QA report	Yes
Drug	SK&F 104864-A "Formulation 1" Lot U97066 Standard SK&F-104864-A "Formulation 2" Lot GAC-41374-025

Methods

Animals	Dogs
N	three dogs per sex per dose group
Doses	Group 1 – control, empty gelatin capsules Group 2 – 0.02 mg/kg/day (0.4 mg/m^2/day) formulation 2 Group 3 – 0.06 mg/kg/day (1.2 mg/m^2/day) formulation 2 Group 4 – 0.06 mg/kg/day (1.2 mg/m^2/day) formulation 1
Schedule	Daily for five days followed by 16 days recovery followed by five additional daily doses
Formulation	gelatin capsules filled with powdered topotecan
Clinical signs	Daily

Body Weight	Weekly
ECG	Prior to dosing and day 27
Ophthalmology	Prior to dosing and day 23
Clinical Pathology	Prior to dosing, day 7, 14, 21, and 28
Toxicokinetics	Days 5 and 26, 0.5, 1, 1.5, 2, 4, 8, 12 and 24 hours
Necropsy	Days 28 or 29

Formulation 1 is a relatively "clean" lot of topotecan evidently similar to lots used in previous non-clinical studies. Formulation 2 is a much more contaminated lot of topotecan. The following table shows the concentration of various contaminants in Formulation 1 and 2 used in this study.

Degradant	Formulation 1 Lot U97066 (Standard)	Formulation 2 Lot GAC-41374-025

b(4)

* Key degradants/levels evaluated in this study.

Results

Mortality – All animals survived to scheduled necropsy

Clinical signs – High dose animals given both formulations showed signs of anorexia and abnormal stool following each dosing period. "Red-tinged stool" and emesis was observed sporadically in group 3 and 4 dogs.

Body Weight – Mean body weight was decreased 4 to 9% compared to predose values in males given formulation 1 or 2 at the high dose (Groups 3 and 4) and in females in all treatment groups (Groups 2, 3, and 4)

Food cons. – Mean food consumption was decreased (up to 63%) during one or both treatment periods compared to the mean of pre-dose values for males given formulation 1 or 2 at 1.2 mg/m^2/day and for females in all treatment groups (Groups 2, 3, and 4)

ECG – No treatment related effects

Ophthalmology No treatment related effects

Clotting – No treatment related effects

Hematology –

Male Reticulocytes	Day 7	12%	-43%	-85%
Male Reticulocytes	Day 21	-5%	28%	57%
Male Reticulocytes	Day 28	-23%	-32%	-54%
Female Reticulocytes	Day 7	65%	-81%	-84%
Female Reticulocytes	Day 21	105%	40%	5%
Female Reticulocytes	Day 28	72%	-64%	-76%
Male WBC	Day 7	-36%	-47%	-53%
Male WBC	Day 21	-41%	-13%	12%
Male WBC	Day 28	-34%	-44%	-45%
Female WBC	Day 7	-31%	-34%	-55%
Female WBC	Day 21	6%	7%	-6%
Female WBC	Day 28	-26%	-24%	-54%
Male Lymphocytes	Day 28	-36%	-41%	-52%
Female Lymphocytes	Day 28	-11%	-13%	-36%
Male Neutrophils	Day 28	-37%	-47%	-40%
Female Neutrophils	Day 28	-30%	-28%	-59%
Male Monocytes	Day 28	-33%	-67%	-73%
Female Monocytes	Day 28	-58%	-39%	-85%

Clin. Chem. Serum alanine aminotransferase (ALT) values were increased 2- to 14-fold compared to pre-drug values of 29-39 U/L on Days 7 and/or 14 for 1/3 male dogs and 2/3 female dogs given formulation 2 at 1.2 mg/m^2/day and 1/3 female dogs given formulation 2 at 0.4 mg/m^2/day; values were increased 2- to 3-fold for 2 of these dogs on Day 28. The serum aspartate aminotransferase (AST) value was increased 3-fold on Day 7 for the female dog with the highest ALT value.

Male ALT	Day 7	17%	38%	28%
Male ALT	Day 21	19%	73%	31%
Male ALT	Day 28	14%	75%	4%
Female ALT	Day 7	-9%	503%	-15%
Female ALT	Day 21	12%	30%	-21%
Female ALT	Day 28	41%	6%	-21%

Urinalysis – No treatment related effects

Organ Wt	Group 2	Group 3	Group 4
Male Liver	9%	-18%	-19%
Female Liver	-11%	-3%	-18%
Testes	-1%	-5%	-9%
Male Thymus	11%	-48%	-15%
Female Thymus	-31%	25%	-55%
Ovaries Paired	-41%	-24%	-33%

Organ Wts. –
Gross Path – No drug related changes
Histopath – Microscopic damage in GI of one female given Formulation 1

The following table (sponsor's) demonstrates that the exposure to Topotecan and the maximum concentration after dosing were consistently lower in dogs receiving formulation 2 except for AUC in females on day 5 which was comparable to the exposure in dogs receiving formulation 1. The decrease in exposure is greater than one would expect from the fact that the actual

concentration of topotecan in Formulation 2 is less than that in Formulation 1 (maximum amount of impurities = ⟶% for Formulation 1 vs ~% for Formulation 2). The increase in exposure with formulation 2 was greater than dose proportional as was C_{max} on both day 5 and 26. This suggests the possibility of saturation of metabolism.

	Male (n=3)				Female (n=3)			
	Cmax [ng/mL]		AUC(0-t) [ng·h/mL]		Cmax [ng/mL]		AUC(0-t) [ng·h/mL]	
Dose (mg/m^2/day)	Day 5	Day 26	Day 5	Day 26	Day 5	Day 26	Day 5	Day 26
0.4*	2.21	2.09	6.54	6.32	4.18	3.89	13.5	11.8
1.2*	12.7	10.6	43.7	37.0	10.9	10.5	49.7	40.0
1.2**	14.2	12.2	58.3	59.6	13.9	13.3	48.9	58.9

* formulation 2
** formulation 1 (standard)

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9) Evaluation of the Toxicity of Degradants in Oral Topotecan Formulations in the Dog for 1 Month (Two 5-Day Cycles of Drug Treatment) - Study 2

Major findings

The doses in this study are relatively low; thus they caused little toxicity and the sample size is low. This renders comparison of the results equivocal. Nevertheless, there is some suggestion of differences in the spectrum and extent of toxicities. Thus, these studies do not establish the toxicological equivalence of the various formulations.

Study number	GlaxoSmithKline Study No. SKF-104864/RSD-100S2T/1 Protocol G97212 Submitted electronically to this NDA
Conducting laboratory	SmithKline Beecham
Date of study initiation	February, 1998
GLP compliance	Yes
QA report	Yes
Drug	SK&F 104864-A "Formulation 1" Lot U97065 Standard SK&F-104864-A "Formulation 2" Lot GAC-41374-187 SK&F-104864-A "Formulation 3" Lot GAC-41374-135

Methods

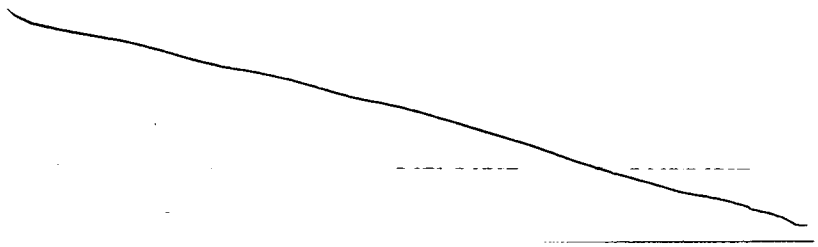
Animals	Dogs
N	three dogs per sex per dose group
Doses	Group 1 – control, empty gelatin capsules Group 2 – 0.06 mg/kg/day (1.2 mg/m^2/day) formulation 2 Group 3 – 0.06 mg/kg/day (1.2 mg/m^2/day) formulation 3 Group 4 – 0.06 mg/kg/day (1.2 mg/m^2/day) formulation 1
Schedule	Daily for five days followed by 16 days recovery followed by five additional daily doses

Formulation	gelatin capsules filled with powdered topotecan
Clinical signs	Daily
Body Weight	Weekly
ECG	Prior to dosing and day 27
Ophthalmology	Prior to dosing and day 23
Clinical Pathology	Prior to dosing, day 7, 14, 21, and 28
Toxicokinetics	None
Necropsy	Days 28 or 29

This experiment is very similar to the previous one in that it attempts to establish the toxicological equivalence of two "Formulations" of topotecan containing a spectrum of contaminants that different from that of a pure reference "Formulation" evidently similar to that used in most of the non-clinical studies. Nevertheless, note that the Lot numbers of all three "Formulations" are different from those in the previous experiment. The concentrations of the various contaminants are also different. The two lots referred to as Formulation 1 in both experiments are similar in composition.

Degradant	Formulation 1 Lot U97065 (Standard)	Formulation 2 Lot GAC-41374-187	Formulation 3 Lot GAC-41374-135, Group A

b(4)



* Key degradants/levels evaluated in this study.

Results

Mortality – All dogs survived to scheduled necropsy

Clinical Signs – abnormal stool consistency, red-tinged/discolored stool or absence of stool were observed in all drug-treated groups

Body Wt. – Decreased in all dosed animals 3 to 8% on day 27

Food cons. – Decreased up to 90 % in dosed animals following both dosing periods

ECG – No toxicologically significant changes

Ophthalmology No toxicologically significant changes

Hematology – decreases (~10%) in mean hemoglobin, total red cell count and hematocrit were observed for female dogs given formulation 1 (standard). For male or female dogs in all drug-treated groups, mean reticulocyte counts were decreased 64-83% (Days 8 and/or 28), mean platelet counts were increased 1.4- to 1.6-fold on Day 21 and mean total white cell counts were decreased 17-47% (Days 8 and 28).

Clin Chem. – Serum Alanine aminotransferase (ALT) values were increased 3- to 76-fold for 1 male and 1 female dog given formulation 2. The serum aspartate aminotransferase (AST) value was increased 3- to 13-fold for the male dog. These increases were not associated with histological lesions in the liver. These

	increases were not observed in dogs given formulation 3, which contained comparable or higher levels of key degradants.
Clotting –	No toxicologically significant changes
Urinalysis –	No toxicologically significant changes
Organ Wts. –	No toxicologically significant changes
Gross Path. –	No toxicologically significant changes
Histopath. –	Epithelial necrosis or regenerative epithelial activity or both in the colon was observed in dogs given formulation 1 (standard) or formulation 3.

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10) Impurity Evaluation in a 14 Day Intravenous Toxicity Study in Female Rats

Major findings

The sponsor identified an organic impurity, ———, in drug product batches of topotecan at a specification limit of —%. This specification limit exceeds the concentration of ——— contained in batches of drug used in previous intravenous toxicology experiments.

This experiment determined that a batch of topotecan containing — to —% — ——— caused a spectrum and intensity of toxicities in female rats toxicologically indistinguishable from that of the previous batches of topotecan used in non-clinical experiments at the statistical resolution of the experiment. b(4)

Study number	GlaxoSmithKline Study No. SKF-104864/RSD-1018KS/1 Submitted electronically to this NDA
Conducting laboratory	SmithKline Beecham, King of Prussia, PA
Date of study initiation	February, 2000
GLP compliance	Yes
QA report	Yes
Drug	Impure batch SK&F 104864-A Number (Batch/Lot No. JW43970-014A1), ——— range — to —% Control SK&F 104864-A, batch MM-19117-249, — ———, range — to —%

The sponsor submitted the following table describing these batches to the CMC portion of the NDA.

Table 15 Batch Analysis Data for Batches of Topotecan Drug Substance used in Support of GLP Nonclinical Studies

Batch Number	JW43970-014A1	MM-19117-249
Batch Size (g)	[redacted]	
Date of Manufacture	11 Feb 2000	20 Jul 1993
Site of Manufacture	GSK R&D (UM)	GSK R&D (UM)
Use	Preclinical	Preclinical
Description	Conforms	Conforms
Identification		
- IR	Conforms	Conforms
- HPLC	Conforms	Conforms
Topotecan content (% w/w, oasfb[1])	92.9	91.1
Related impurities (% area)[2]		
[redacted]		
- Total	[redacted]	
Residual solvents(% w/w)		
[redacted]	Not tested Not tested Not tested Not tested	Not tested Not tested
[redacted]		
[redacted]	Not tested	
[redacted]	Not tested	
[redacted]	Not tested	
[redacted]	Not tested	

b(4)

b(4)

Note:
1. oasfb = on anhyrous and solvent-free basis
2. Testing for individual compounds not performed at release, but performed as part of reanalysis.

Structure of topotecan impurities

Supplied by Dr. Brian Rogers (chemist), submitted to the CMC package of the NDA

2 Page(s) Withheld

✓ Trade Secret / Confidential (b4)

_____ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox- 1

Genotoxicity

Genotoxicity Summary

The sponsor did not do carcinogenicity tests with topotecan in laboratory animals. Nevertheless, topotecan is genotoxic to mammalian cells *in vivo* and *in vitro*. In mouse L5178Y lymphoma cells, 0.1 µg/ml of topotecan caused a four fold increase in mutations over controls. This dose was cytotoxic and increases in mutations occurred in the presence of S9. In human lymphocytes *in vitro*, 0.5 µg/ml of topotecan caused an eleven fold increase in the percentage of cells with chromosome breaks. In some cases, DNA damage caused by topotecan was greater than that caused by the positive controls. In mice, *in vivo*, 0.16 mg/kg of topotecan caused a 34 fold increase in the percentage of cells with micronuclei. Topotecan did not cause mutations in bacterial cells.

Genotoxicity Review

I have copied these review from my review of from NDA20-671

In vitro Studies

1) TP-0013: SK&F 104864-A: Bacterial Mutation Assay. Vol. 1.25, p 132

Drug	SK&F 104864, Batch JW-15827-73-2, tested purity 92.6 % w/w.
Concentrations	15 to 5000 µg/plate, range finding study 312.5, 625, 1250, 2500, to 5000 µg/plate, main study
Test Organisms	S. typhimurium TA1535, TA1537, TA1538, TA98 and TA100 All strains were defective in DNA repair (*uvrB*) and have a defective lipopolysacccharide barrier on the cell wall (*rfa*) E. coli, tryptophan-dependent auxotrophic mutant
Solvent	DMSO
Negative Control	DMSO
Positive Controls	9-aminoacridine, N-ethyl-N-nitro-N-nitrosoguanidine, 2-nitrofluorene, with S-9 2-Aminoanthracene
S9	phenobarbital and β-naphthaflavone in Sprague Dawley rats.
GLP	included and signed, [redacted] b(4)

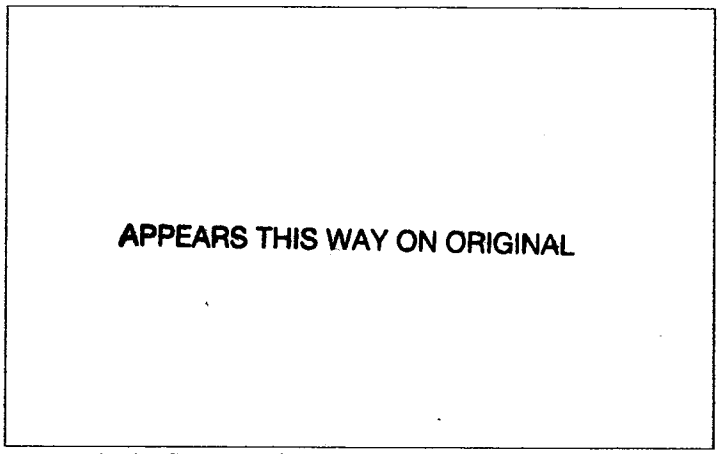
Topotecan was not toxic to any of the strains to doses of 5000 µg/plate. Positive controls all gave positive results. Concentrations of topotecan to 5000 µg/plate did not cause increases in the number of revertants in any of the strains. S-9 activation did not increase the number of revertants. The investigators did not establish whether topotecan passed the cell membrane of these strains.

2) TF-1002: SK&F 104864-A: Report of mutation tests with L5178Y mouse lymphoma cells at the TK locus, Vol. 1.25, p 163

Cells	Mouse L5178Y 3.7.2.C, heterozygous at the thymidine kinase gene locus (TK +/-)
Drug	SK&F 104864, batch MM-19007-84, 91.5% pure
Concentrations	0.08 to 1250 µg/ml toxicity 0.08 to 80 µg/ml with S9 0.0375 to 0.1 µg/ml without S9
Vehicle	DMSO
Positive control	ethyl methansulphonate (EMS) without S9 benzo(a)pyrene (Bap) with S9 assay 1 3-methylcholanthrene (3MC) with S9 assay 2
Negative control	DMSO
S9	Arochor 1254 in Sprague Dawley rats.
GLP	included and signed, SKB, The Frythe, UK These test were done twice

Cells were exposed to topotecan for four hours. They were then allowed to recover and express mutations for two days. Mutants (TK-/-) were selected in agar containing trifluorothymidine and survival was measured in non-selective agar. Colonies were counted on selective agar for TK-/- mutation and on non-selective agar for survival.

Cells grew at an acceptable rate only at concentrations below 0.16-µg/mL in the absence of S9 and below 80-µg/mL in the presence of S9. Mutation frequencies for positive and negative controls were within expected laboratory norms. Inhibition of growth was dose dependant.



In the first mutation assay, topotecan caused a significant dose related increase in mutation frequency above controls with and without S9. With S9, this increase was 4.9 fold above controls at 80-µg/ml and 1.9 fold at the lowest dose, 0.08 µg/ml. The following tables show the results for both assays with and without S9. The results in the second assay were similar. Clearly topotecan causes mutations in mammalian cells at clinically relevant concentrations.

FIRST MUTATION ASSAY - TREATMENT MEANS[a]

METABOLIC ACTIVATION: YES

TREATMENT µg/ml	% RELATIVE TOTAL GROWTH (b)	MUTATION FREQUENCY PER 10^5 cfu (b)	FOLD INCREASE IN MF OVER CONTROLS
DMSO 10 µl/ml	100.0	5.3	-
SK&F 104864-A 80.0	5.1	25.9*	4.9
SK&F 104864-A 0.4	11.5	10.5*	2.0 (1.98)
SK&F 104864-A 0.2	13.6	9.1*	1.7
SK&F 104864-A 0.08	19.3	10.3*	1.9
B(a)P 1.25	50.4	12.8*	2.4

METABOLIC ACTIVATION: NO

TREATMENT µg/ml	% RELATIVE TOTAL GROWTH (b)	MUTATION FREQUENCY PER 10^5 cfu (b)	FOLD INCREASE IN MF OVER CONTROLS
DMSO 10 µl/ml	100.0	14.2	-
SK&F 104864-A 0.1	5.7	56.2*	4.0
SK&F 104864-A 0.085	8.4	39.4*	2.8
SK&F 104864-A 0.075	9.7	38.5*	2.7
SK&F 104864-A 0.050	20.8	26.2*	1.8
EMS 600	33.3	74.2*	5.2

* Significantly different from controls p <0.005.
[a] Derived from Appendix Tables C4, C5, C8 and C9.
b See Methodology Section for calculation formulae.

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3) TP-1016: SK&F 104864-A: Chromosomal aberrations assay with human lymphocytes *in vitro*. Vol. 1.25, p 199

Major findings

Cells cultured human lymphocytes in RPMI 1640 (Dutch Modification) medium supplemented with fetal bovine serum, glutamine and antibiotics.

Drug	SK&F 104864, batch JW-15827-73-2
Concentrations	0.5 to 64 µg/ml toxicity in the presence of S9
	0.02 to 2.0 µg/ml toxicity without S9
	0.06 to 1.0 µg/ml assay without S9
	0.03 to 0.5 µg/ml assay with S9
Vehicle	DMSO
Positive control	cyclophosphamide with S9
	mitomycin C without S9
Negative control	DMSO
S9	Arochor 1254 in Sprague Dawley rats.
analysis	mitotic index and chromosome aberrations assessed microscopically
GLP	included and signed, b(4)
	These test were done twice

Topotecan was very toxic to human lymphocytes *in vitro*. It prevented cells from entering metaphase at all concentrations greater than 0.5 µg/ml with and without S9. In cultures

without S9, concentrations of 0.13 and 0.25-µg/ml reduced the mitotic index. Concentrations of 0.25 µg/ml caused visible damage to cells.

In these assays, the mitomycin C positive control was not clastogenic. This was because the experimenter inadvertantly applied 0.05 instead of 0.5-µg/ml. Nevertheless, topotecan was strongly clastogenic in this test, so a positive control is unnecessary. Cyclophosphamide was strongly clastogenic.

In test 1, cells were treated for 4 hours with and without S9 and harvested at 24 hours and 48 hours. Under these conditions, topotecan caused significant structural chromosomal aberrations at 0.5-µg/ml. Cells allowed to recover for 48 hours were more damaged than those allowed to recover for 24 hours. This was probably because the cells could express the damage due to topotecan after they had resumed division. The following tables summarize these results.

TABLE 5

SK&F 104864-A: Summary of Results of First Assay With S9 Mix

Post Treatment Harvest	Agent	Conc. (µg.ml⁻¹)	Accessory Data (%)		% Cells with Structural Damage to Chromosomes				%Cells with Changes in Chromosome Number
			Cells with Gaps	Hypodiploid Cells	Breaks	Rearrange-ments	Others	Total	Hyperdiploid
24 h	Negative Control Dimethylsulphoxide	0.5%	1.00	11.50	1.00	0.00	-	1.00	0.00
	SK&F 104864-A	0.13	1.50	19.00	10.50	0.00	-	10.50	0.00
	Positive Control Cyclophosphamide*	21	8.00	16.67	28.00	2.00	-	28.67	0.00
48 h	Negative Control Dimethylsulphoxide	0.5%	1.50	18.50	1.00	0.00	-	1.00	0.00
	SK&F 104864-A*	0.5	7.00	26.00	37.00	11.00	-	42.00	0.00
	Positive Control Cyclophosphamide	21	2.50	18.00	18.50	4.00	-	21.50	0.00

* Reduced number of cells (150 with CPA and 100 with SK&F 104864-A) scored because of high frequency of aberrations

TABLE 6

SK&F 104864-A: Summary of Results of First Assay Without S9 Mix

Post Treatment Harvest	Agent	Conc. (µg.ml⁻¹)	Accessory Data (%)		% Cells with Structural Damage to Chromosomes				% Cells with Changes in Chromosome Number
			Cells with Gaps	Hypodiploid Cells	Breaks	Rearrange-ments	Others	Total	Hyperdiploid
24 h	Negative Control Dimethylsulphoxide	0.5%	0.50	23.50	1.50	0.50	-	2.00	0.00
	SK&F 104864-A	0.13	1.00	9.00	6.00	1.00	-	7.00	0.00
	Positive Control Mitomycin C	0.05	1.00	25.00	2.50	0.00	-	2.50	0.00
48 h	Negative Control Dimethylsulphoxide	0.5%	1.50	23.00	2.00	0.00	-	2.00	0.00
	SK&F 104864-A*	0.5	1.92	38.46	22.12	7.69	-	27.88	0.00
	Positive Control Mitomycin C	0.05	2.00	20.50	1.50	0.00	-	1.50	0.00

* Reduced number of cells (104) scored because of high frequency of aberrations and toxicity

In test 2, cells were treated for 4 hours with and without S9, but were harvested only at 48 hours. Under these conditions, topotecan caused significant damage to chromosomes at 0.25 µg/ml. Microsomal activation increased this incidence of this damage in both test 1 and test 2.

TABLE 7

SK&F 104864-A: Summary of Results of Second Assay

With S9 Mix, 48 h Post Treatment Harvest

Agent	Conc. ($\mu g.ml^{-1}$)	Accessory Data (%)		% Cells with Structural Damage to Chromosomes				% Cells with Changes in Chromosome Number
		Cells with Gaps	Hypodiploid Cells	Breaks	Rearrange-ments	Others	Total	Hyperdiploid
Negative Control Dimethylsulphoxide	0.5%	0.75	9.75	2.00	0.00	-	2.00	0.00
SK&F 104864-A	0.03	1.00	13.00	2.00	0.50	-	2.00	0.00
	0.06	1.50	6.50	2.50	0.00	-	2.50	0.00
	0.13	0.50	6.50	4.00	0.00	-	4.00	0.00
	0.25	0.50	12.50	5.00	2.50	-	7.50	0.00
Positive Control Cyclophosphamide*	14	2.21	12.50	13.97	2.94	-	16.18	0.00

* = Reduced number of cells (136) scored because of toxicity

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In vivo Studies

4) TF-1001: SK&F 104864-A: Report of a micronucleus test in the mouse by the intravenous route (single-dose study). Vol. 1.25, p 253.

TF-1003: SK&F 104864-A: Report of a single dose micronucleus test in the mouse by the iv route to determine the no-effect dose, Vol 1.25, p 298.

These two studies of the induction of micronuclei and chromosomal aberrations in mouse erythrocytes are very similar. The sponsor conducted both tests under GLP guidelines and in both used SKF-104864A batch MM-19007-184, 91.5% purity. In both the sponsor dosed groups of 4 or 5 mice with a single i.v. dose or topotecan. After 24 or 48 hours the mice were killed and marrow from femora was removed, fixed on slides with methanol and stained with Giemsa. The slides were scored for the proportion of polychromatic cells, mature cells and the number of micronuclei in each group.

In the first experiment, topotecan killed some mice at doses of 50 mg/kg or above. Thus the dose range studied is appropriately high. The following table shows that topotecan caused a significant increase in the percent of erythrocytes with micronuclei at all non-lethal doses. The percent of cells with micronuclei is higher at lower doses. This inverted dose response is probably due to toxicity. High doses of topotecan probably prevented the cells from entering S-phase. Thus, micronuclei could not form in increasing numbers. Recovery to 48 hours did not increase the percent of cells with micronuclei and the results for both sexes were similar.

TABLE B2: SUMMARY OF MAIN TEST RESULTS (APPENDIX, TABLES C2.1-C2.8) #

Treatment	Dosage (mg/kg)	Sex	No. of Animals	Mean Terminal Bodyweight (g)	POLYCHROMATIC CELL DATA			MATURE CELL DATA			Polychromatic Cells (% of Total Cells Scored)*
					Cells Scored	With Micronuclei	% With Micronuclei	Cells Scored	With Micronuclei	% With Micronuclei	
Negative control (pH-adjusted saline)	0	M	10	31.46	5000	5	0.1	8336	2	0.02	37.49
SK&F 104864A	0.25	M	5	30.24	2500	67	2.68	6660	1	0.02	27.29
	0.5	M	5	30.64	2500	68	2.72	5873	4	0.07	29.86
	1.0	M	5	30.60	2500	18	0.72	6475	7	0.11	27.86
	2.0	M	5	31.50	2500	32	1.28	7310	6	0.08	25.48
	4.0	M	5	31.26	2500	26	1.04	6326	11	0.16	27.10
	8.0	M	5	30.54	2500	33	1.32	8136	5	0.06	23.51
	16.0	M	5	30.52	2500	22	0.88	11989	8	0.07	17.25

* Accessory data only

Figures may differ from those given in (Appendix Tables C2.1-C2.8) due to rounding errors and minor differences in calculating values

Because the response decreased with dose in the first experiment, the sponsor could not determine a dose of topotecan that did not cause micronucleus aberration in mouse erythrocytes. Thus, they conducted the second study at much lower doses. At 0.04 mg/kg and below, the response is indistinguishable from control. A dose of 0.08 mg/kg causes a large increase in the percent of cells with micronuclei. Twice this dose causes the maximum response. The response then decreases with higher doses, consistent with the finding in the first experiment. The degree of response is similar to that of the first experiment, i.e. the results is reproducible.

TABLE B1: SUMMARY OF THRESHOLD TEST RESULTS (APPENDIX, TABLES C2.1-C2.9) #

Treatment	Dosage (mg/kg)	Sex	No. of Animals	Mean Terminal Bodyweight (g)	POLYCHROMATIC CELL DATA			MATURE CELL DATA*			Polychromatic Cells (% of Total Cells Scored)*
					Cells Scored	With Micronuclei	% With Micronuclei	Cells Scored	With Micronuclei	% With Micronuclei	
Negative control (sterile saline)	0	M	4	30.98	8000	8	0.10	15297	6	0.04	34.34
SKF 104864A	0.0025	M	4	31.20	2000	3	0.15	4425	4	0.09	31.13
	0.005	M	4	30.78	2000	3	0.15	3930	2	0.05	33.73
	0.01	M	4	30.78	8000	7	0.09	14534	11	0.08	35.50
	0.02	M	4	31.73	8000	14	0.18	12409	7	0.06	39.20
	0.04	M	4	31.70	8000	8	0.10	17486	8	0.05	31.39
	0.08	M	4	32.05	5000	22	0.44	7599	5	0.07	39.69
	0.16	M	4	30.78	2000	69	3.45	2844	3	0.11	41.29
	0.32	M	4	30.40	2000	35	1.75	3658	1	0.03	35.35

* Accessory data only

Figures may differ from those given in (Appendix Tables C2.1-C2.8) due to rounding errors and minor differences in calculating values

As expected, topotecan causes a significant increase in chromosome aberrations in rapidly dividing cells. This increase is as much as 34 fold at 0.16 mg/kg (0.48 mg/m^2) in mice. Half this dose caused a four-fold increase, thus the dose response curve for this toxicity is very steep.

Carcinogenicity

The sponsor has not done carcinogenicity studies with topotecan.

Reproductive Toxicology

See review of NDA 20212

Overall Summary and Analysis

Topotecan (HYCAMTIN®) is a semi-synthetic derivative of camptothecin. Camptothecin is a natural product extracted from the bark of the tree Camptotheca *acuminata*. The chemical name for this compound is (S)-10-[(dimethylamino)methyl]-4-ethyl-4,9-dihydroxy-1 H - pyrano[3',4':6,7] indolizino[1,2-b]quinoline-3,14-(4H,12H)-dione monohydrochloride. Topoisomerase I is a nuclear enzyme that relieves torsional strain in DNA by opening single strand breaks. Once topoisomerase I creates a single strand break, the DNA can rotate in front of the advancing replication fork. Topotecan binds reversibly to the Topoisomerase I - DNA to form a tertiary complex that prevents religation of these single strand breaks. Current research suggests that double strand DNA damage occurs during DNA synthesis when progressing

replication enzymes interact with the ternary complex formed by topotecan, topoisomerase I and DNA. Mammalian cells cannot efficiently repair these double strand breaks. This damage probably initiates apoptosis.

In basic solution, hydroxyl ions hydrolyze topotecan to the hydroxy carboxylate open ring form. At physiological pH, the open ring form predominates. Topotecan, the closed ring lactone form, is stabilized by acid, that is when pH 4 or less. The open ring form does not bind to Topoisomerase I and does not cause DNA damage. The equilibration between the open ring form and topotecan is relatively slow. When topotecan is prepared in [redacted] (the clinical formulation of the IV drug product) and then added to buffer at physiological pH, the active lactone equilibrates with the inactive hydroxy carboxylate in about an hour. Thus, an hour after injection into a patient the concentrations of topotecan should be much smaller than the total concentration of both lactone and hydroxy carboxylate. AUC values from single dose kinetics in the mouse, rat and dog suggest that the concentration of topotecan in plasma remains within a factor of three of the concentration of total drugs, not different by several orders of magnitude as predicted by the equilibrium reaction. Plasma proteins possibly stabilize topotecan in the lactone form. The available studies did not explain this discrepancy between *in vivo* and *in vitro* behavior. Nevertheless, using the total drug concentrations in cell culture assays, I estimated the inhibitory binding constant of topotecan at the DNA site to be approximately μM. Because of the hydrolysis reaction, the actual binding constant is probably much lower. Topotecan is a very potent inhibitor of DNA replication.

b(4)

The sponsor conducted an array of preclinical studies in the mouse, rat, rabbit and dog, thus they have characterized the toxicities associated with topotecan. In early studies, the sponsor calculated the single dose LD_{10} to be 74 mg/m^2. They also estimated the LD_{50} in rats given five daily IV doses to be 13.8 mg/m^2, or about six times the proposed clinical dose. Rats survive 28 daily doses approximately equivalent to the clinical dose on a mg/m^2 basis. Dogs do not tolerate one-third the clinical dose given for 28 days. Dogs appear best to predict the toxicities of topotecan.

The dose limiting toxicities in all these species are myelosuppression and anemia. The onset of myelo-suppression is rapid; the degree of myelosuppression is duration and dose dependent. High repeat doses can cause profound decreases in WBC and platelet count within eight days of the start of treatment. All white cell types are affected. These changes are usually reversible when treatment ceases. Sometimes the platelet count and the neutrophil count will rebound to higher than normal, but this increase usually returns to normal within two weeks. RBC, Hgb, and Hct also decreased with dose and duration of treatment. These toxicities appear to result from decreased erythropoesis rather than hemolysis. These parameters usually recover to near control values within weeks after dosing stops.

Repeated dosing with topotecan caused increases in ALT and AST suggesting liver damage. Histology did not confirm hepatic toxicity, probably because myelosuppression is dose limiting. Thus, the doses were not high enough to demonstrate clear dose related damage. Similarly, repeated dosing sometimes caused transient changes in the urinalysis parameters, suggesting mild kidney damage; but again the doses were not high enough to demonstrate clear dose dependent toxicity.

Topotecan caused the microscopic damage usually associated with myelo-suppression, bone marrow hypocellularity, cellular depletion of the spleen, thymus, and lymph nodes. Damage to the GI was remarkably rare and usually seen only in long-term studies of oral dosing. Some dogs given 0.4 mg/m^2/d for one month developed multinucleated spermatogonial giant cells in the testes. In rats, a dose of 0.23 mg/kg/day given for 14 days before mating through gestation day six caused a 75% increase in the number of corpora lutea. The mechanism causing this super-ovulation was not determined. Weight loss is dose dependent and associated with

anorexia. Alopecia is dose dependent, but does not occur in all animals. Microscopically the hair follicles were atrophic.

The sponsor did not do carcinogenicity tests with topotecan in laboratory animals. Nevertheless, topotecan is genotoxic to mammalian cells *in vivo* and *in vitro*. In mouse L5178Y lymphoma cells, 0.1 µg/ml of topotecan caused a four-fold increase in mutations over controls. This dose was cytotoxic and increases in mutations occurred in the presence of S9. In human lymphocytes *in vitro*, 0.5 µg/ml of topotecan caused an eleven-fold increase in the percentage of cells with chromosome breaks. In some cases, DNA damage caused by topotecan was greater than that caused by the positive controls. In mice, *in vivo*, 0.16 mg/kg of topotecan caused a 34-fold increase in the percentage of cells with micronuclei. Topotecan did not cause mutations in bacterial cells.

Topotecan caused embryonic and fetal death in rabbits and rats. In rabbits, a dose of 0.32 mg/kg/day on days six through 20 of gestation causes fetal resorption. This dose caused significant maternal toxicity. In rats, a dose of 0.23 mg/kg/day given for 14 days before mating through gestation day six caused 99.6% fetal resorption, 24% pre-implant loss, and mild maternal toxicity. A dose of 0.10 mg/kg/day (0.4 times the clinical dose on a mg/m^2 basis) given to rats on days six through 17 of gestation caused a 2.5 fold increase in post-implantation mortality. This dose also caused a 3.8 fold increase in total fetal malformations. The most frequent malformations were of the eye (microphthalmia, anophthalmia, rosette formation of the retina, coloboma of the retina, ectopic orbit), brain (dilated lateral and third ventricles), scull and vertebrae. Topotecan given to female rats prior to mating at a dose of 0.23 mg/m^2 caused superovulation possibly related to inhibition of follicular atresia. This dose given to pregnant female rats also caused increased pre-implantation loss. Studies in dogs given 0.4 mg/m^2 of topotecan daily for a month suggest that treatment may cause an increase in the incidence of multinucleated spermatogonial giant cells in the testes. Topotecan may impair fertility in women and men.

Rats excrete high concentrations of Topotecan into milk. Lactating female rats given 4.72 mg/m^2 (about twice the clinical dose on a mg/m^2 basis), excreted Topotecan into milk at concentration up to 48-fold higher than those in plasma.

Intravenous doses of topotecan are distributed through the body in less than ten minutes. The elimination half-life, $t_{1/2}\beta$ in dogs is about 100 minutes. The rat eliminates topotecan faster than the dog; $t_{1/2}\beta$ is about 50 minutes. The studies in the rat suggest a second elimination phase, $t_{1/2}\gamma$ ~349 minutes. Studies in the mouse suggest significant diurnal enterohepatic recirculation. In all these species, AUC and C_{max} increases proportional to dose and repeat dose studies suggest significant accumulation with repeat dosing.

The volume of distribution at steady state is greater than total body water, 2.8 l/kg in the dog, suggesting depot sequestration. Topotecan should accumulate in fat or in hypoxic, i.e., acidotic, tissues. One hour after an intravenous dose in rats, the highest concentrations of radiolabeled topotecan are in the liver, muscle and skin. Little of the dose remained anywhere in the animal after 120 hours. A small amount of topotecan may enter the brain. Because of the hydrolysis reaction, the time verses concentration curves for topotecan and that of total administered dose should diverge. They do, but not to the extent that the equilibrium constant would suggest, as mentioned above. Some factor in the plasma stabilizes the topotecan. In all species, tested protein binding is between 25.3 and 39.7%. This is much less than the original topoisomerase I inhibitor, camptothecin.

Oral bioavailability of topotecan in the dog is about 35%. Rats with bile duct cannulae still secrete about 18% of an intravenous dose of topotecan in the feces. This suggests significant intestinal secretion. After 24 hours rats excrete approximately 43% of a topotecan dose in the urine and about 41% in the feces as the parent compound. Less than 1% of the dose appears in the urine as the N-demethylated metabolite; about 3% of the dose appears in the feces as this

metabolite. The N-demethylated compound is the only major metabolite formed by any species. Hepatic microsomes from rat, dog and human form the N-desmethyl metabolite from the parent compound at about the same rate, 1 to 3 pmol/min/mg. Cytochrome P450 CYP1A2 is possibly responsible for most of this reaction. Less than 3% of the dose appears as unidentified polar metabolites. At 24 hours, dogs eliminate less of a topotecan dose in both the urine and the feces, but the proportion in each is about the same. Again, the N-desmethyl compound is the only major identified metabolite. Topotecan does not induce any major hepatic cytochrome P450 isozymes nor does it inhibit any major cytochrome P450 activities *in vitro*.

One toxicology study submitted is adequate to qualify the impurity, ——————, in drug product batches of topotecan at a specification limit of—%. Two other studies that compared the toxicity of different batches of topotecan do not clearly demonstrate toxicological equivalence among those batches.

Topotecan is one of the first topoisomerase I inhibitor available as a cancer chemotherapeutic drug. Its limiting toxicities are reasonably well characterized in mice, rats, rabbits and dogs, and these toxicities are those usually associated with a potent cytotoxin.

W. David McGuinn, Jr., M.S., Ph.D., D.A.B.T.
September 20, 2007
Revised October 5, 2007

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

William McGuinn
10/8/2007 02:02:36 PM
PHARMACOLOGIST

Leigh Verbois
10/9/2007 09:07:25 AM
PHARMACOLOGIST

MEMORANDUM

Date: October 4th, 2007
From: S. Leigh Verbois, Ph.D.
Acting Pharmacology/Toxicology Team Leader
Division of Drug Oncology Products
To: File for NDA #20-981
Oral HYCAMTIN (topotecan)
Re: Approvability of Pharmacology and Toxicology

Non-clinical studies that investigated the pharmacology and toxicology of topotecan provided to support the NDA for Oral HYCAMTIN the treatment of relapsed small cell lung cancer, were reviewed in detail by Dr. W. David McGuinn, Jr., M.S., Ph.D., D.A.B.T. The supporting information included studies of IV and/or oral topotecan that investigated the drug's pharmacology, pharmacokinetic and ADME, safety pharmacology, general toxicology (mouse, rat and dog), genetic toxicity (*in vivo* and *in vitro*), and reproductive toxicity in both rats and rabbits. The studies cited in the review by Dr. McGuinn consist primarily of original research conducted by the applicant.

The general toxicology studies submitted to the NDA demonstrate that topotecan is a topoisomerase I inhibitor which causes well defined toxicities, including dose limiting myeolosuppression, thrombocytopenia and anemia in all species. Toxicology studies (up to 2 five day cycles) were conducted with multiple batches of topotecan in order to qualify impurities in the to-be-marketed formulation. One toxicology study submitted is adequate to qualify the impurity. [redacted] in drug product batches of topotecan at a specification limit of [redacted]%. Two other studies that compared the toxicity of different batches of topotecan do not clearly demonstrate toxicological equivalence among those batches. However these studies will not impact the approvability of Oral HYCAMTIN for the proposed indication. Topotecan is genotoxic to mammalian cells both *in vivo* and *in vitro*. The sponsor did not conduct carcinogenicity studies with topotecan.

b(4)

Topotecan is a teratogen and a fetotoxin at doses equal to or less than the proposed clinical dose in rats and rabbits. IV topotecan is associated with fetal resorption, post-implantation mortality, and fetal malformations of the eye, brain, skull and vertebrae. In lactation studies, rats excrete topotecan into breast milk at concentrations up to 48 fold higher than those in plasma. Additionally, dosing with topotecan appears to damage the testes in dogs and impair fertility in female rats.

Recommendations: I concur with Dr. McGuinn's conclusion that pharmacology and toxicology data support the approval of NDA 20-981, Oral HYCAMTIN. There are no outstanding nonclinical issues related to the approval of Oral HYCAMTIN.

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/s/

Leigh Verbois
10/9/2007 09:25:54 AM
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