Cancer Chemother Pharmacol (2002) 50: 473–478
DOI 10.1007/s00280-002-0515-6

ORIGINAL ARTICLE

**Mirjam Crul · Laurens V. Beerepoot · Ellen Stokvis
Johannes S. P. Vermaat · Hilde Rosing · Jos H. Beijnen
Emile E. Voest · Jan H. M. Schellens**

Clinical pharmacokinetics, pharmacodynamics and metabolism of the novel matrix metalloproteinase inhibitor ABT-518

Received: 10 April 2002 / Accepted: 3 August 2002 / Published online: 25 October 2002

Abstract *Purpose*: To investigate the pharmacokinetics, pharmacodynamics and metabolism of the novel matrix metalloproteinase (MMP) inhibitor ABT-518. *Methods*: Plasma and urine samples were obtained from six patients included in a phase I trial in which ABT-518 was given once daily via the oral route. Samples were analyzed by LC-MS/MS, ELISA and immunocapture assay. The pharmacokinetics of the parent compound and of detectable metabolites were calculated. *Results*: After a single dose of ABT-518 peak plasma levels were reached within 4–8 h. ABT-518 had an estimated clearance (Cl/F) of approximately 3 l/h, an estimated volume of distribution (V/F) of over 70 l and a terminal half-life ($T_{1/2}$) of 20 h. At least six different metabolites were formed. Pharmacodynamic analysis for angiogenic growth factors (bFGF and VEGF) showed plasma and urine levels in the picogram range and for total MMP-9 and MMP-2 or MMP-9 activity showed plasma and urine levels in the nanogram range. *Conclusions*: The MMP inhibitor ABT-518 is extensively metabolized in humans. No significant correlations between pharmacokinetics and pharmacodynamics could be established.

M. Crul (✉) · E. Stokvis · H. Rosing · J. H. Beijnen
Slotervaart Hospital, Department of Pharmacy
and Pharmacology, Louwesweg 6, 1066
EC Amsterdam, The Netherlands
E-mail: apmcr@slz.nl
Tel.: +31-20-5124481
Fax: +31-20-5124753

L. V. Beerepoot · J. S. P. Vermaat · E. E. Voest
University Medical Center Utrecht,
Department of Medical Oncology, Heidelberglaan 100,
3584 CX Utrecht, The Netherlands

M. Crul · J. H. Beijnen · J. H. M. Schellens
The Netherlands Cancer Institute,
Department of Medical Oncology, Plesmanlaan 121,
1066 CX Amsterdam, The Netherlands

J. H. Beijnen · J. H. M. Schellens
Division of Drug Toxicology, Faculty of Pharmacy,
Utrecht University, Sorbonnelaan 16,
3584 CA Utrecht, The Netherlands

Keywords ABT-518 · Matrix metalloproteinases

Abbreviations *bFGF:* basic fibroblast growth factor · *LC-MS/MS:* liquid chromatography-mass spectrometry/mass spectrometry · *MMP:* matrix metalloproteinase · *VEGF:* vascular endothelial growth factor

Introduction

Matrix metalloproteinases (MMPs) are a class of at least 18 enzymes capable of degrading the basement membrane and the extracellular matrix. They function in various physiological and pathological processes such as embryonic development, wound healing, bone remodeling, and ovulation. Normally, the activity of MMPs is highly controlled by transcriptional regulation, by control of their secretion or by control of their activation. Moreover, naturally occurring inhibitors are present in plasma and most tissues [4, 6, 14]. In tumors, however, MMPs are frequently overexpressed, mainly because of an increased production by stromal cells [5]. This facilitates local invasion of the tumor, metastasis and angiogenesis [2, 9, 12]. High MMP levels are associated with an aggressive phenotype and bad prognosis [7, 10].

Over the last decade, a number of MMP inhibitors have been developed for the treatment of cancer. The first compounds were nonselective inhibitors of several MMPs, but clinical testing revealed that this type of inhibitor caused significant adverse effects, especially joint toxicity [3, 15]. It was hypothesized that by selectively targeting MMP-2 and MMP-9, the MMPs that play the most dominant role in tumors, these side effects could be circumvented. ABT-518, a biaryl ether retrohydroxamate, is such a selective MMP inhibitor that can be given via the oral route. Its affinity for MMP-2 and MMP-9 is 200-fold greater than for any of the other currently known MMPs. It inhibits MMP activity by binding reversibly to the zinc atom in the active site of the enzyme. ABT-518 has demonstrated strong

cytostatic activity in preclinical experiments, both in vitro and in vivo [1].

We initiated a phase I clinical trial of ABT-518 in patients with advanced solid malignancies. After the first six patients had been included, the sponsor decided not to complete this first trial with the compound. The stock of the drug was made available to the National Cancer Institute and may be used for future studies, but as far as we know no concrete plans have been established yet. We report here on the pharmacokinetic and pharmacodynamic findings obtained from all patients who participated in this trial. Although the data are derived from a relatively small number of patients, we believe they are of interest because of the novelty of the compound and the assessment of extensive biotransformation of ABT-518. Furthermore, we applied a promising method of assessing the biological effects of MMP inhibitors using ELISA and immunocapture assays. These data may be useful for other investigators who plan to proceed with the clinical development of ABT-518 or related compounds.

Patients and methods

Patients and treatment

Patients were included at two participating institutions: the Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital in Amsterdam, and the University Medical Center of Utrecht University, Utrecht, The Netherlands. Patients were eligible if they had a histologically confirmed diagnosis of a solid malignant tumor not amenable to established forms of therapy. Other eligibility criteria included a good performance status (Karnofsky performance score ≥70%), anticipated life expectancy of at least 3 months and age ≥18 years. Previous anticancer chemotherapy or radiation therapy had to be discontinued for at least 4 weeks before entry into the study. All patients had to have acceptable bone marrow, liver and renal function: ANC $>1500/\mu l$ and platelets $>100{,}000/\mu l$; serum bilirubin not more than the normal upper limit, ASAT and ALAT not more than 2.5 times the normal upper limits and estimated creatinine clearance >50 ml/min. The Medical Ethics Committees of the cooperating hospitals approved the study protocol, and all patients gave written informed consent.

Patients received 25-mg ABT-518 capsules supplied by Abbott Laboratories (Abbott Park, Ill.). They were given once daily with a meal, in doses escalating from 25 mg. On days 2 and 3 no drug was administered to allow adequate pharmacokinetic sampling during the elimination phase. From day 4 onwards, dosing was continuous. Three patients were to be included per dose level, and up to three extra in the case of dose-limiting toxicity.

Pharmacokinetics

On days 1 and 22 pharmacokinetic samples were obtained before dosing, and 15 and 30 min, and 1, 2, 4, 8, 12 and 24 h after administration. Following day 1, samples at 48 and 72 h were drawn as well. Additionally, predose samples were taken on days 5, 8, 15, 22 and 29, weekly between day 29 and month 2, and monthly thereafter. Heparinized blood was collected and each 5-ml sample was placed on ice immediately and centrifuged within 1 h of collection (5 min, at approximately 1200 *g* or 3000 rpm). Plasma was collected, transferred to polyethylene containers and stored at –20°C for subsequent drug analysis.

A validated HPLC-MS/MS method was used for bioanalysis of ABT-518 and its metabolites [13]. In brief, plasma samples were diluted with phosphate buffer (pH 6) and a solid-phase extraction was performed on Varian Bond Elut Phenyl columns. ABT-518 and metabolites were eluted with methanol and diluted 1:1 (v/v) with water. After filtration, 50 µl of sample was injected onto a Zorbax Extend C18 analytical column (150×2.1 mm ID, particle size 5 µm) protected with an in-line filter, using a mixture of aqueous ammonium hydroxide and methanol as the mobile phase at a flow rate of 200 µl/min. One-fourth of the LC eluent was let into the Turbo-ionspray source. ABT-518 and its metabolites were detected specifically by multiple reaction monitoring in the triple quadrupole mass analyzer. The validated concentration ranges were 25–1000 ng/ml for A-302873 and 10–1000 ng/ml for ABT-518 and all other metabolites.

The following pharmacokinetic parameters were determined for ABT-518: maximal plasma concentration (C_{max}), time to maximal plasma concentration (T_{max}), terminal half-life ($T_{1/2}$), area under the plasma concentration-time curve (AUC) and estimated clearance (Cl/F) and volume of distribution (V/F). For the latter four, noncompartmental analysis was used. For the metabolites of ABT-518, C_{max} and AUC were determined if possible. All analyses were performed using the WinNonlin software package (version 3.0, Pharsight Corporation, Mountain View, Calif.).

Pharmacodynamics

Pharmacodynamic plasma and midstream urine samples were collected prior to dosing on days 1 and 22, and after 2 months of treatment. Plasma samples for bFGF and VEGF were collected in citrated tubes. Plasma samples for MMP-2 and MMP-9 activity were collected in heparinized tubes. All plasma samples were centrifuged at 4°C at 800 g for 15 min. Plasma was collected, transferred to polyethylene cups and stored at ≤ –70°C until analysis. Urine samples (for MMP-2 and MMP-9 activity, total MMP-9, VEGF and bFGF levels) were collected in sterile containers and stored at 4°C. They were centrifuged at 200 g for 3 min, transferred to separate tubes and frozen at ≤ –70°C until analysis.

Quantification of bFGF and VEGF was done using ELISA (R&D Systems, Minneapolis, Minn.), and an ELISA (Amersham Pharmacia Biotech, Little Chalfont, UK) was also used for total MMP-9. For MMP-9 activity assessment an immunocapture activity assay was used (Amersham Pharmacia Biotech). In this assay, MMP was bound to a microtiter plate coated with anti-MMP-9. Latent MMP-9 was activated using APMA (*p*-aminophenylmercuric acetate). An artificial pro detection enzyme was activated by total active MMP-9 and was detected using a chromogenic substrate using a spectrophotometer. MMP-2 activity was quantified using the same technique as for MMP-9 activity (Amersham Pharmacia Biotech).

Results

Six patients were entered into the study, all females, with a median age of 54 years (range 40–70 years). They all had a Karnofsky performance status $>80\%$. Tumor types were non-small-cell lung, ovarian, colon, head and neck and renal cell cancer, and melanoma, and all patients had received previous chemotherapy. The first four patients received ABT-518 at a dose of 25 mg daily, and the next two at 50 mg daily. One patient at the lowest dose level went off study during the first month of treatment, and the other five patients received 1–2 months of treatment. Pharmacokinetic and pharmacodynamic samples for day 1 were available for all patients included, and samples for day 22 as well as

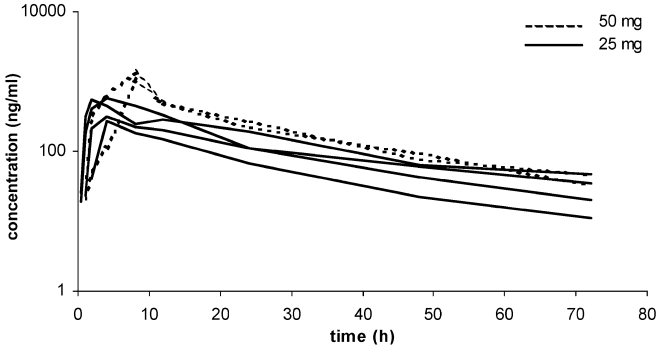


Fig. 1 Plasma concentration time curves of ABT-518 after oral administration on day 1

weekly predose samples were obtained from five patients. Pharmacodynamic samples from five patients were obtained on day 21. After 2 months, plasma from two patients and urine from three patients were available for analysis.

Pharmacokinetics of ABT-518

Plasma concentration-time curves of ABT-518 are shown in Fig. 1, and the obtained pharmacokinetic parameters per dose level are given in Table 1. As can be seen, at a dose of 50 mg, plasma levels were increased as compared to the levels at a dose of 25 mg. Peak plasma levels were reached within 4–8 h, and the terminal half life of ABT-518 was approximately 20 h. The weekly measured trough levels ranged from 13 to 677 ng/ml, which is believed to be within the in vitro bioactive range. Visual inspection of these trough levels indicated that a steady state was reached within the first weeks of treatment. No clear differences could be discerned in the pharmacokinetics between day 1 and day 22 (Table 1).

Metabolism of ABT-518

Six metabolites of ABT-518 were detectable plasma. The time of appearance, maximal plasma concentrations and AUC_{0-24h} on day 22 per patient are given in Table 2. On day 1, two metabolites, the active diol (A-302873) and the amine ketal (A-347542) were detectable. All other metabolites appeared only after several days of treatment. Whether this was a consequence of their late formation or of the fact that ABT-518 was only administered at low dose levels so that metabolites could have been present below the level of detection, remains to be clarified. In general, ABT-518 metabolism can start at two sites in the molecule: either at the nitrogen atom, yielding a ketal group, or at the heterocyclic group, which leads to the formation of an active diol. A proposed metabolic pathway for ABT-518 is depicted in Fig. 2. The metabolite that reaches the highest levels is the amine ketal, A-347542. As can be seen in Table 2, there was a considerable difference between patients. For example, the methyl sulfone metabolite was detectable in only one patient at the higher dose level, whereas other metabolites, such as the diols, were detectable in all. Moreover, the time after which the metabolites appeared was highly variable.

Pharmacodynamics of ABT-518

VEGF and bFGF were detectable in plasma and urine samples of all patients (Table 3). MMP-2 and MMP-9 activity was detectable in plasma samples from almost all patients, whereas low MMP-2 and low MMP-9 activity in urine was detectable in samples from six and three patients, respectively. Patient 3 showed a large increase in MMP-9 level, which occurred during rapidly progressive lung metastases from renal cell carcinoma. No significant associations could be established between the pharmacokinetics of ABT-518 and any of the monitored parameters.

Discussion

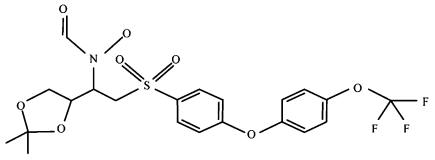
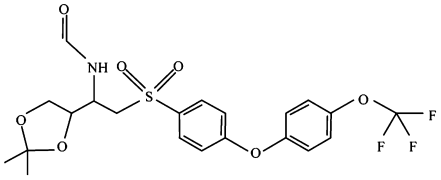
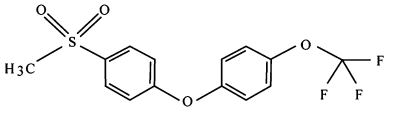
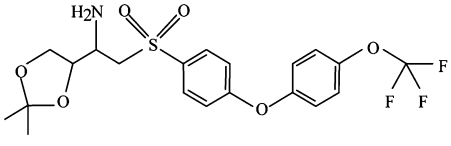
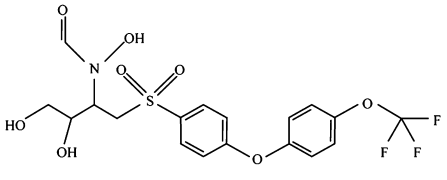
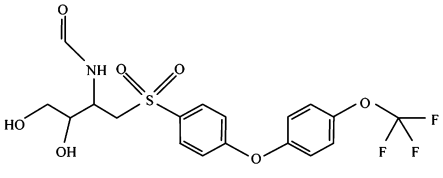
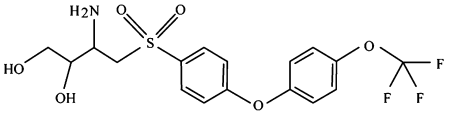
The pharmacokinetics of the novel compound ABT-518 were evaluated at two dose levels, 25 and 50 mg/day. Unfortunately, the trial was discontinued prematurely because of a decision made by the sponsor. Therefore, our data set remained limited to only six patients and no

Table 1 Pharmacokinetic parameters of ABT-518 per dose level (mean ± SD if $n \geq 3$, individual parameters if $n = 2$) calculated on day 1 and on day 22

	n	T_{max} (h)	C_{max} (ng/ml)	AUC_{0-24h} (ng·h/ml)	AUC_{0-inf} (ng·h/ml)	$T_{1/2}$ (h)	V/F (l)	Cl/F (l/h)
Day 1								
25 mg	4	3.5 ± 1.0	432 ± 421	5531 ± 3649	9371 ± 5423	22.0 ± 4.3	93 ± 26	3.1 ± 1.1
50 mg	2	8.0 and 8.0	1040 and 1340	10281 and 13032	17053 and 19259	17.1 and 17.5	73 and 65	2.9 and 2.6
Day 22								
25 mg	3	2.3 ± 1.5	726 ± 453	7339 ± 4425	–[a]	–[a]	42 ± 29	–[a]
50 mg	2	8.0 and 8.1	1010 and 893	18450 and 12862	–[a]	–[a]	37 and 52	–[a]

[a]could not be calculated on day 22 due to the restricted sampling period of 24 h

Table 2 Pharmacokinetics of ABT-518 metabolites per patient

metabolite	patient	1002 (25 mg)	1003 (25 mg)	1004 (25 mg)	1101 (50 mg)	1102 (50 mg)	
29151	Cmax (ng/ml)	869	219	1090	1080	893	
parent	Tmax (day)	2	4	1.02	8	8.08	
	AUC day 22 (ng*h/ml)	8412	2476	11133	18450	12862	
34845	detectable after day	21	-	-	21	8	
formamide	Cmax (ng/ml)	-	-	-	35.2	51.3	
ketal	Tmax (day)	last (56)	-	-	last (63)	22	
	AUC day 22 (ng*h/ml)	-	-	-	291.6	1114.7	
34705	detectable after day	-	-	-	29	-	
methyl	Cmax (ng/ml)	-	-	-	28.5	-	
sulfone	Tmax (day)	-	-	-	27.9	-	
	AUC day 22 (ng*h/ml)	-	-	-	-	-	
34754	detectable after day	4	4	1	1	1	
amine	Cmax (ng/ml)	72.8	196	160	255	475	
ketal	Tmax (day)	last (56)	last (56)	15	35	22	
	AUC day 22 (ng*h/ml)	1360	1267.5	3549	4903.9	61303	
30287	detectable after day	1	1	1	1	1	
active	Cmax (ng/ml)	45.5	25.5	34.6	136	105	
diol	Tmax (h)	±3	8	8	8	8	
	AUC day 22 (ng*h/ml)	-	-	-	1912.6	-	
34481	detectable after day	28	28	7	14	7	
formamide	Cmax (ng/ml)	15.6	12.6	60.9	36	52.7	
diol	Tmax (day)	last (56)	29	35	last (63)	22	
	AUC day 22 (ng*h/ml)	-	-	454.6	347.1	1181	
34481	detectable after day	14	7	7	4	7	
amide	Cmax (ng/ml)	21.5	43.9	63.5	64.3	112	
diol	Tmax (day)	last (56)	last (56)	35	29	22	
	AUC day 22 (ng*h/ml)	343.3	318	982.8	1389	2362	

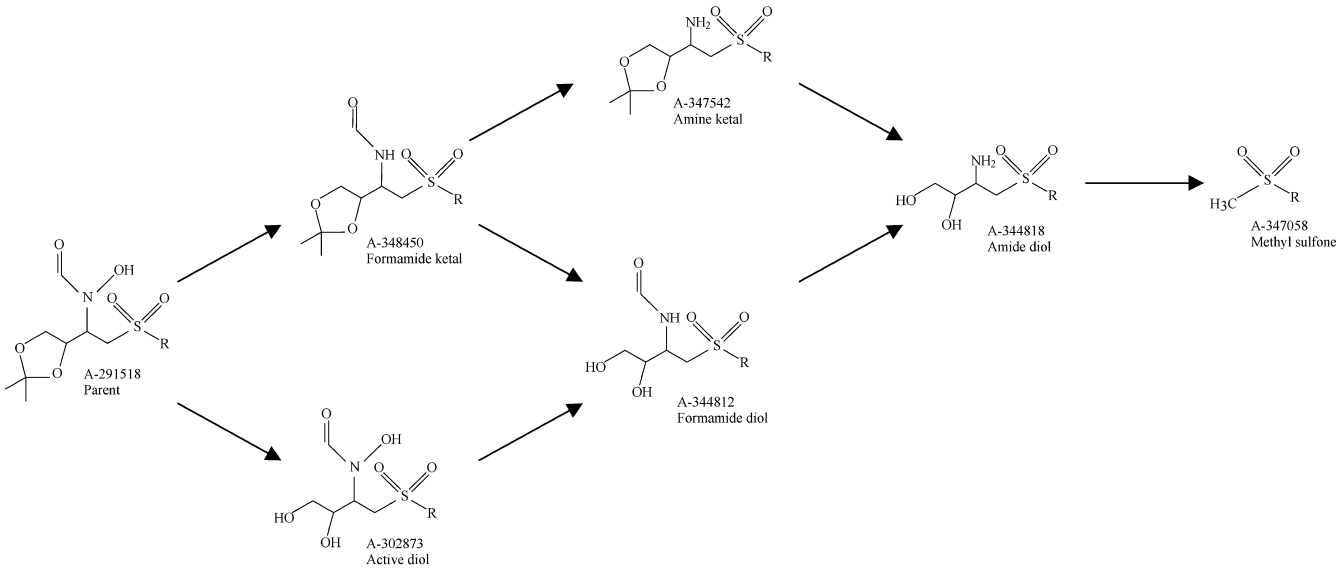


Fig. 2 Proposed metabolic pathway for ABT-518. *R* = trifluoromethoxy-phenoxy-phenyl. See Table 2 for complete structures

dose-limiting toxicity was reached. However, because of the extensive analysis of ABT-518 metabolism, and because of the possibility that this compound will be developed further in the near future, we decided to present the data obtained. The pharmacokinetics of the parent compound ABT-518 revealed that the doses used here were probably within the bioactive range: the in vitro IC_{50} of ABT-518 for MMP-2 is 0.78 n*M*, corresponding to 394 ng/ml, and for MMP-9 is 0.50 n*M*, corresponding to 253 ng/ml. Particularly at the second dose level of 50 mg daily, trough levels of ABT-518 were above these values. However, the possibility of protein binding should be taken into account. As yet, little is known about the protein binding of ABT-518 in humans, but the observation that the estimated volume of distribution greatly exceeds the volume of the plasma compartment, combined with in vitro data demonstrating >90% protein binding (Abbott Laboratories, data on file), make further investigation in this direction warranted.

The metabolism of ABT-518 appears to be quite complex. So far, six metabolites have been detected in human plasma, but the formation of other species cannot be excluded. In rat and monkey pharmacokinetic studies, five additional metabolites have been identified (Abbott Laboratories, data on file), which were undetectable in our patients. In humans, the metabolite that reached the highest concentration was the amine ketal, A-347542, indicating that the formation of the ketal metabolites prevails over the formation of the diols. Future studies are required to further elucidate the exact metabolic pathway, as well as the possible pharmacological activity of the identified metabolites.

In order to assess biological activity of this selective MMP inhibitor, assays were done to investigate the effect of ABT-518 treatment on angiogenic growth factors and on the activity and total levels of MMPs. In

Table 3 Pharmacodynamic parameters before and during treatment with ABT-518 (*m* missing, no sample received)

Patient number	Study day	VEGF (pg/ml)		bFGF (pg/ml)		MMP-2 activity (ng/ml)		MMP-9 activity (ng/ml)		MMP-9 total level (ng/ml)
		Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma
1	1	68	78	6	5	5	3	1	<0.2	<0.5
1	16	65	36	6	2	6	2	1	<0.2	<0.5
3	1	49	68	3	0.5	4	0	6	0.4	<0.5
3	21	69	144	4	1	2	3	3	2	4
3	57	82	99	3	1	4	1	3	12	34
4	1	15	98	3	0.1	6	1	1	2	1
4	21	37	77	4	2	6	<0.2	2	<0.2	<0.5
2	1	24	167	2	6	6	1	0.6	0.5	<0.5
2	22	28	39	5	1	6	<0.2	0.5	0.4	<0.5
5	1	22	115	2	1	4	0.4	0.4	<0.2	<0.5
5	22	10	57	2	4	5	<0.2	<0.2	<0.2	<0.5
5	55	m	20	m	1	5	<0.2	0.3	<0.2	<0.5
6	1	24	64	2	0.3	4	<0.2	1	<0.2	<0.5
6	22	34	22	2	0.2	6	<0.2	1	<0.2	<0.5
6	55	m	101	m	2	m	0.4	m	<0.2	<0.5

agreement with previous reports (as reviewed in reference 11), we found that an increase in VEGF plasma levels was associated with disease progression. No effects of ABT-518 on VEGF or bFGF levels in either urine or plasma were observed. The activities of MMP-2 and MMP-9 did not decrease during ABT-518 treatment. This is in accordance with the results of analysis of plasma samples from patients enrolled in a phase I study of a moderately selective MMP inhibitor [8]: no correlation was found in 29 patients between MMP-9 activity and plasma levels of the MMP inhibitor. Whether MMP activity analysis is the correct way to assess biological activity remains to be established. When ABT-518 is further explored at doses near dose-limiting toxicity, the relative decrease in MMP activity may guide investigators towards an optimal biological dose of ABT-518 below the maximum tolerated dose, at which all MMP activity is abolished.

In conclusion, we have shown that the MMP inhibitor ABT-518 is extensively metabolized in humans. The pharmacokinetics do not correlate with pharmacodynamic parameters in this patient group. We will proceed with our pharmacokinetic and pharmacodynamic studies of this agent as soon as its clinical development is continued.

References

1. Albert DH, Morgan DW, Magoc T, Tapang G, Kherzai A, Marcotte P, Elmore I, Glaser K, Pease L, Li J, Leal J, Michaelides M, Curtin M, Holms J, Wada C, Dai Y, Davidson SK (2000) Preclinical pharmacology of ABT-518, a novel and potent inhibitor of gelatinase A and B with anti-tumor activity. NCI-EORTC-AACR 11:301 (abs)
2. Bernhard EJ, Gruber SB, Muschel RJ (1994) Direct evidence linking expression of matrix metalloproteinase 9 (92-kDa gelatinase/collagenase) to the metastatic phenotype in transformed rat embryo cells. Proc Natl Acad Sci U S A 91:4293
3. Bramhall SR, Rosemurgy A, Brown PD, Bowry C, Buckels JA (2001) Marimastat as first-line therapy for patients with unresectable pancreatic cancer: a randomized trial. J Clin Oncol 19:3447
4. Brew K, Dinakarpandian D, Nagase H (2000) Tissue inhibitors of metalloproteinase: evolution, structure and function. Biochim Biophys Acta 1477:267
5. Coussens LM, Fingleton B, Matrisian LM (2002) Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 295:2387
6. Crawford HC, Matrisian LM (1996) Mechanisms controlling the transcription of matrix metalloproteinase genes in normal and neoplastic cells. Enzyme Protein 49:20
7. Duffy MJ, Maguire TM, Hill A, McDermott E, O'Higgins N (2000) Metalloproteinases: role in breast carcinogenesis, invasion and metastasis. Breast Cancer Res 2:252
8. Duivenvoorden WCM, Hirte HW, Singh G (2001) Quantification of matrix metalloproteinase activity in plasma of patients enrolled in a BAY-129566 phase I study. Int J Cancer 91:857
9. Fang J, Shing Y, Wiederschain D, Yan L, Butterfield C, Jackson G, Harper J, Tamvakopoulos G, Moses MA (2000) Matrix metalloproteinase-2 is required for the switch to the angiogenic phenotype in a tumor model. Proc Natl Acad Sci U S A 97:3884
10. Kanayama H (2001) Matrix metalloproteinases and bladder cancer. J Med Invest 48:31
11. Poon RTP, Fan ST, Wong J (2001) Clinical implications of circulating angiogenic factors in cancer patients. J Clin Oncol 19:1207
12. Ray JM, Stettler-Stevenson WG (1995) Gelatinase A activity directly modulated melanoma cell adhesion and spreading. EMBO J 14:908
13. Stokvis E, Rosing H, Crul M, Rieser MJ, Schellens JHM, Beijnen JH (2002) Quantitative analysis of the novel anticancer drug ABT-518 and six potential metabolites in human plasma using high-performance liquid chromatography coupled with turbo-ionspray tandem mass spectrometry. Proceedings of the 19th (Montreux) LC/MS Symposium, International Association of Environmental Analytical Chemistry. http://www.ia-eac.ch/lcmsbegin.htm
14. Vu TH, Werb Z (2000) Matrix metalloproteinases: effectors of development and normal physiology. Genes Dev 14:2123
15. Wojtowicz-Praga S, Torri J, Johnson M, Steen V, Marshall J, Ness E, Dickson R, Sale M, Rasmussen HS, Chiodo TA, Hawkins MJ (1998) Phase I trial of Marimastat, a novel matrix metalloproteinase inhibitor, administered orally to patients with advanced lung cancer. J Clin Oncol 16:2150