Phase I and Pharmacological Study of the Oral Matrix Metalloproteinase Inhibitor, MMI270 (CGS27023A), in Patients with Advanced Solid Cancer


ABSTRACT
This Phase I study of MMI270, an p.o. administered matrix metalloproteinase inhibitor, assessed toxicity, pharmacokinetics, and tumor response data and investigated markers of biological activity to recommend a dose for Phase II studies.

MMI270 was administered continuously at seven dose levels (50 mg once daily to 600 mg three times/day). Patients were evaluated for toxicity and tumor response, and blood and urine samples were taken for pharmacokinetics, bone resorption markers, direct targets of the inhibitor [matrix metalloproteinase-2 (MMP-2), MMP-8, and MMP-9], indirect targets [tissue inhibitor of metalloproteinase-1 (TIMP-1), TIMP-2, basic fibroblast growth factor, vascular endothelial growth factor, vascular cell adhesion molecule-1, soluble urokinase plasminogen activator receptor, and cathepsins B and H] and for a tumor necrosis factor-α cytokine release assay.

Ninety-two patients were entered. There was no myelotoxicity. Eighteen patients developed a widespread maculopapular rash, which increased in frequency and severity at doses ≥300 mg bid. Thirty-nine patients developed musculoskeletal side effects, which were related to duration of treatment, not to dose level. Pharmacokinetics were linear, and MMI270 was rapidly absorbed and eliminated with minimal accumulation on chronic dosing. Sustained plasma concentrations in excess of 4 × mean IC50 for the target enzymes were observed at dose levels ≥150 mg bid. There were no tumor regressions; however, 19 patients had stable disease for ≥90 days. There was a dose-response increase of MMP-2 and TIMP-1 with MMI270. Transient effects on the bone resorption markers were detected.

MMI270 was generally well tolerated, with adequate plasma levels for target enzyme inhibition. The two main toxicities were rash, resulting in a maximum tolerated dose of 300 mg bid and musculoskeletal side effects. Biological marker data indicate drug effects. The rise in TIMP-1 suggests that a reflex rise in inhibitors could modify the effects of MMI270. The recommended Phase II dose is 300 mg bid.

INTRODUCTION
The MMPs2 are a family of proteinases able, between them, to break down all of the components of the ECM, including the basement membrane (1). There are four subfamilies of MMPs: collagenases, gelatinases, stromelysins, and membrane-type MMPs. The first three types are secreted as proenzymes, activated by cleavage of the NH2 terminus, and have highly conserved regions particularly at the catalytic site, which encloses a zinc ion. MMP activity is closely regulated by a variety of mechanisms including transcriptional control and proteolytic activation and by natural inhibitors such as α2 macroglobulin and the specific TIMP family of proteins (reviewed in Refs. 2–5). Another important role for MMPs is increasing the bioavailability of factors bound to the cell surface and ECM (e.g., bFGF; Refs. 6, 7).

MMPs are important in malignant disease. Digestion of the ECM is necessary for tumor growth, invasion, metastasis, and angiogenesis. Excess MMP expression has been associated with malignancy and, in several tumor types, has been shown to increase along with invasive and metastatic potential (8–12); e.g., the ratio of activated:latent MMP-2 was higher in malignant breast disease than in benign breast disease and increased with tumor grade (13). Furthermore, high tumor levels of certain...
MMPs have been shown to correlate with poor prognosis in human cancers (14). In view of such findings, inhibition of MMPs has become an important target for cancer therapy.

MMI270, N-hydroxy-2(R)-(4-methoxysulfonyl)[3-picolyl]-amino)-3-methylbutanamide hydrochloride monohydrate, is a novel synthetic hydroxamic acid derivative (Fig. 1) able to competitively bind the Zn$^{2+}$ ion in the active site of a wide range of MMPs, inhibiting their activity at nm concentrations in vitro (Table 1). MMI270 did not show antiproliferative activity against tumor cell lines in vitro; however, in rat tumour models of breast and endometrial cancer, it significantly reduced the tumor burden compared with controls and enhanced the activity of cytotoxic and hormonal agents (15). MMI270 also demonstrated antitumor effects in vivo and antiangiogenic effects in vitro. Very low drug doses were required for antitumor effects, calculated to be adequate to a dose of 25 mg qd in humans. In a rat aorta model of angiogenesis, there was a dose-dependent reduction in blood vessel formation, with an IC$_{30}$ for the assay of 0.2 µM. Preclinical toxicology data demonstrated no acute toxicity in rodents, but the drug caused emesis in dogs. Chronic administration in rodents and dogs was associated with an increase in bone density and minor abnormalities of serum potassium and calcium. Pharmacokinetic studies showed the drug was rapidly absorbed from the gut and eliminated from plasma, suggesting that a multiple dosing might be required for inhibition of MMPs over a 24-h period. Bioavailability after oral administration in rats was 44%. MMI270 (0.1 and 10 µg/ml) is 57–80% protein bound to albumen and α1 acid glycoprotein. The rank order of protein binding was humans > rats > dogs. The drug appeared to be extensively metabolized before excretion, mainly in feces.

This study describes the first Phase I and pharmacological study of MMI270 in patients with advanced malignancy. The primary aims of the study were to evaluate toxicity and pharmacokinetics. Secondary aims were to measure tumor response data and investigate various markers of biological activity for drug-related change. These included bone resorption markers, direct and indirect targets of the inhibitor, and a cytokine release assay.

**PATIENTS AND METHODS**

**Eligibility.** The study was open to patients with advanced solid malignancies who had failed previous therapy and/or for whom there were no conventional treatments. Patients had to be over 21 years old with a WHO performance status of 0–2 and a life expectancy of greater than 3 months. They were required to have adequate bone marrow (hemoglobin ≥ 9 g/dl; leukocytes ≥ 4 × 10$^9$/liter; platelets ≥ 100 × 10$^9$/liter), renal and hepatic function (serum creatinine and bilirubin ≤ 1.25 times the upper limit of normal, aspartate amino transferase and alanine amino transferase ≤ 3 times the upper limit of normal). All of the patients had recovered from the acute toxic effects of previous treatment and had not received radiotherapy within 2 weeks, chemotherapy within 4 weeks (42 days for mitomycin C or nitrosoureas), or experimental treatment within 30 days of trial entry. Pregnant women, nursing mothers, and patients not using adequate contraception were excluded, as were patients with clinical evidence of cerebral metastases. Other exclusion criteria included active infection, clinically significant abnormal baseline electrocardiograms, previous exposure to MMI270, ongoing treatment with anticancer agents, and patients with a history of noncompliance to medical regimens. All of the patients gave written informed consent. The trial was approved by the local research ethics committees of participating centers.

**Dose and Dose Escalation.** MMI270 was supplied by Novartis Pharma AG (Basel, Switzerland) in size 0 capsules and swallowed with 250 ml of water. Capsules contained 25, 100, 200, and 300 mg of drug to be made up to the required dose. In view of the rapid elimination of the drug seen in preclinical studies, the drug was given in divided doses on a continuous daily basis. The starting dose, 300 mg/day, was equivalent to 0.04 times the toxic-dose low in mature dogs. Dose escalation was predetermined: 150 mg bid, 300 mg bid, 300 mg tid, and 600 mg tid, the latter being the highest dose, which in practical terms could be administered p.o. because of the required number of capsules. Compliance was assessed both using patient diaries and from returned drug bottles.

**Study Design.** Cohort size was planned to be 20 patients at the highest dose level and 10 patients at lower dose levels, recruited into three cancer centers. This was with the aim of maintaining at least three patients on trial for greater than 8 weeks, which was achieved.

After recruitment of the first 60 patients, the trial was expanded to include an additional 22 patients at two lower doses of 50 mg qd and 75 mg bid. Dose escalation was based on satisfactory safety data from the previous level. Toxicity was assessed using the National Cancer Institute/NIH Common Toxicity Criteria. Dose-limiting toxicity was defined as at least three patients experiencing grade 3 hematological toxicity or at least two patients experiencing grade 4 hematological toxicity or grade 3 nonhematological toxicity (excluding nausea, vomiting, and alopecia). The maximum tolerated dose was defined as the dose level below that at which dose-limiting toxicity was observed.
Pretreatment Evaluation. Pretreatment evaluation included full history, physical examination, and assessment of performance status. In addition, a complete blood count, clotting screen, renal, bone, and liver biochemical profiles, tumor markers, an electrocardiogram, urinalysis, radiological assessment of disease, and a pregnancy test, if appropriate, were performed. One cycle of treatment lasted 28 days, and assessment of disease was repeated after each cycle, according to WHO criteria (16). Patients were eligible to continue to additional cycles provided they did not have evidence of progressive disease or unacceptable toxicity.

Pharmacokinetics. Patients received only the morning dose of MMI270 during the pharmacokinetic sampling on days 1 and 28 of cycle 1. Heparinized blood specimens were collected before drug administration and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after receiving the dose of drug. The blood specimens were centrifuged at 2500 rpm for 15 min at room temperature, and the resulting plasma samples were transferred by pipette into screw-cap plastic tubes. Samples were immediately frozen to −20°C and maintained frozen until analysis. MMI270 was determined in plasma by a validated high-performance liquid chromatography method. After thawing, the study and quality control samples were homogenized by shaking on a vibration shaker for a few seconds. Samples were diluted as necessary with blank human plasma. A 50-μl aliquot of the internal standard working solution (CGS 26835) was added to the study and quality control samples. Aliquots of 1.0 ml were transferred into extraction tubes and analyzed.

MMI270 and the internal standard (CGS 26835) were extracted from human plasma with ether:methylene chloride (2:1) after acidification of the plasma with 0.1 M potassium phosphate, monobasic. The organic layer was transferred and evaporated to dryness under nitrogen, and the residue was reconstituted in 200 μl of high-performance liquid chromatography mobile phase. Chromatographic separation of the compounds was achieved using a 5-μm Zorbax SB-C18 analytical column (4.6 mm internal diameter (ID) × 150 mm) with acetic acid (pH 3.00):acetonitrile with 9% methanol (80:20; v/v) as the mobile phase at a flow rate of 1 ml/min. The analytes were monitored using UV detection at a wavelength of 242 nm.

Calibration curves (y = mx + b), represented by the plots of the peak area ratios (y) of MMI270 to the internal standard versus the concentrations (x) of the calibration samples, were generated using weighted (1/x²) linear least-squares regression as the mathematical model. Concentrations in quality control and study samples were calculated from the resulting peak area ratios and interpolation from the regression equations of the respective calibration curves. Turbochrom II 2700 (Version 4.0 and 4.1) software from PE Nelson was used. Concentrations of MMI270 are expressed as the free base.

Specificity of the method in blank human plasma and in the predose samples on day 1 was demonstrated by the lack of interfering peaks at the retention times of MMI270 and CGS 26835. The method was linear over the concentration range of 50.8 to 5080 nm for MMI270, with a lower limit of quantification of 50.8 nm. Pharmacokinetic analysis was done by non-compartmental methods using WinNonlin Professional (version 1.5) software (Scientific Consulting, Inc.). Calculated parameters included Cmax, Tmax, terminal elimination t1/2, and AUC.
because of toxicity, and 2 patients withdrew consent, of whom, 1 lost motivation to continue with treatment and the other felt her condition to be deteriorating and opted for more local palliative care. Five patients came off study because of early progressive disease; one patient had a rising activated partial thromboplastin time and was withdrawn and one patient had a pulmonary embolus, not thought to be related to the study drug. Eighty-four % of patients, excluding those who came off the study before the end of the first cycle, were compliant with their medication (measured as taking greater than 80% of their expected total dose of trial drug during the first 28 days). There was no relationship between duration of treatment and dose level.

**Toxicity.** There was no hematological toxicity. There were two main nonhematological toxicities. The first was rash, observed in 18 patients. Their clinical details are shown in Table 4. Fourteen of the patients developing rash were on dose levels greater than 400 mg tid. The rash was maculopapular with a symmetrical distribution generally affecting the trunk and arms more than the neck and legs and sometimes sparing the face. A typical rash is shown in Fig. 2A. One patient had a skin biopsy. A perivascular monocytic infiltrate in the dermis consistent with a toxic drug reaction. Sixteen of the 18 patients developed rash within 1 month of starting dosing. Four patients came off the study because of the rash. Two patients were rechallenged with MMI270, and in both cases the rash recurred. Treatment of the rash with antihistamines, steroids, antifungal creams, and aqueous creams did not appear to enhance resolution, which resolved in all of the patients, usually by 6 weeks.

The second major toxicity was musculoskeletal. Arthralgia and/or myalgia was observed in 39 patients (Table 4). Typically, this began after at least 1 month of treatment and often started with finger or shoulder stiffness, which were the most commonly affected joints. Involvement of the wrists, elbows, knees, neck, and back was also observed. Symptoms worsened with ongoing treatment and were sometimes associated with a reduction in the range of movement in the joint. One patient, who continued on the drug for over 7 months, lost abduction in his shoulders above 80 degrees. This patient and two others also developed Dupytren’s contractures after 8, 12, and 17 weeks on MMI270, which persisted for the duration of the trial. Nonsteroidal anti-inflammatory drugs, steroids, physiotherapy, complementary therapy, and periods of time off the drug all gave symptom relief to some patients. These measures were not used prophylactically. Three patients discontinued treatment because of arthralgia, and an additional three patients stopped the drug temporarily; however, the majority of patients’ symptoms settled over time after stopping the drug. The frequency and severity of musculoskeletal toxicity did not appear to be dose related or to be reduced with qd dosing. Once a patient had developed arthralgia, it was persistent, and temporary or permanent stoppage of MMI270 appeared to be the most effective management.

Fifteen patients reported mild to moderate nausea, six with associated vomiting (Table 4). There was no renal or hepatic toxicity. The eighth patient coming off study because of toxicity had an unexplained fever, in view of which it was determined that stopping the drug was appropriate. One additional patient developed an unexplained pyrexia, which settled without intervention.

**Response.** There were no tumor responses, and neither were there any significant reductions in tumor markers. Stable disease lasting ≥ 90 days was seen in 19 patients (Table 3). These patients had a wide variety of primary tumor types, and there was no apparent relationship to dose level (Tables 2 and 3).

**Pharmacokinetics.** Blood for pharmacokinetic analysis was taken from between 5 and 10 patients at dose levels 150 mg bid to 600 mg tid on days 1 and 28. At the two lowest dose levels, plasma concentrations were below the limit of detection.
of the assay used. Plasma MMI270 concentration showed a rapid increase after dosing, followed by a rapid decrease (Fig. 3A), indicating that MMI270 is rapidly absorbed from the gastrointestinal tract and rapidly eliminated from plasma. On day 28 for all of the patients, the median $T_{\text{max}}$ value was 0.58 h (range, 0.3–3 h), and the median $t_{1/2}$ was 1.6 h (range, 0.6–7.6 h). Because of the relatively short $t_{1/2}$, there was little or no accumulation of the drug on bid or tid dosing.

The pharmacokinetics of MMI270 demonstrated a linear relationship between AUC values and the dose administered ($r = 0.59; P < 0.001$). However, there was marked inter-patient variability. $C_{\text{max}}$ also increased with dose (Fig. 3A and Table 5). Two patients in the 600-mg bid dose cohort had unusually high $C_{\text{max}}$ values on day 1, which did not recur on day 28. The reason for this is unclear.

In an attempt to consider whether pharmacologically relevant drug levels were being achieved, a plasma concentration of 200 nM was used. This level is $\approx 4$ times the $IC_{50}$ for the MMP enzymes and, therefore, should provide near complete inhibition. The percentage of time during the course of the dosing interval that MMI270 concentrations exceeded 200 nM was determined for dose levels 150 mg bid to 600 mg tid (Fig. 3B). Pharmacologically relevant plasma levels were achieved at these dose levels. For dose levels 600 mg bid to 600 mg tid, MMI270 concentrations exceeded 200 nM an average of 65–75% of time during the dosing interval and, for the lower two dose levels, the average was about 35%. For inhibition of the target enzymes, 600 mg tid produced plasma levels $\approx 4 \times IC_{50}$ for a mean of 18/24 h.

**Bone Resorption Markers and Angiogenic Factors.** The percentage change from baseline of the ratios of the bone resorption markers pyridinoline and deoxypyridinoline to paired serum creatinine values were compared with the corresponding pharmacokinetic values: $C_{\text{max}}$ and AUC. At day 14, the bone resorption marker ratios tended to fall with $C_{\text{max}}$ and AUC, but this relationship was not seen at day 28 (Table 6).

There was a significant correlation for the percentage increase of MMP-2 and TIMP-1 protein levels after one cycle of treatment, compared to baseline, with AUC (Fig. 4, A and B) and $C_{\text{max}}$ (Table 6) and also with each other (correlation of 0.4; $P = 0.007$; Fig. 4C). These remain significant when the outlying data point is removed. Significant positive correlations were also observed between TIMP-2 with AUC, MMP-9 with $C_{\text{max}}$, and bFGF with AUC (Table 6). There were no other significant changes observed in the levels of MMP-8, MMP-9, TIMP-2, the ratio of MMP-2 to TIMP-2, bFGF, VEGF, VCAM-1, suPAR, CATB, or CATH with either of the above calculated pharmacokinetic parameters.

**Cytokine Release Assay.** The cytokine release assay measured the effect of MMI270 on release of TNF-α from ex vivo stimulated peripheral blood cells. Before treatment, TNF-α release from stimulated whole blood cultures ranged from 50–1250 pg/ml. TNF-α levels in the unstimulated controls (controls) were usually below detection limits of assay, i.e., 20 pg/ml. The

### Table 4 Toxicity data

<table>
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<th>Musculoskeletal</th>
<th>Nausea</th>
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Fig. 2 A, typical rash observed after MMI270. B, histology of a skin biopsy from a patient with rash.
Fig. 3  
A, time course of mean MMI270 plasma concentration on days 1 and 28.  
B, percentage time MMI270 concentration is above 200 nM for dose levels 150 mg bid to 600 mg tid.
mean reduction in TNF-α release was 25.9% at 4 h, 212.9% at 24 h, 27.3% at 7 days, and 25.9% at 28 days, respectively, when results from all of the patients were combined. Although there was some inhibition of TNF-α release during treatment, the results did not reach significance; nor was there any significant difference between the results from patients receiving low doses (≤300 mg bid) or high doses (>300 mg bid) of the drug.

**DISCUSSION**

MMPs are among a number of antiangiogenic agents currently undergoing clinical trials. The difficulties in adapting traditional Phase I study design appropriately for investigation of such drugs has been discussed (25–27). Biological modifiers, such as the MMPIs, may not have a serious dose-limiting toxicity. Therefore, the concept of the maximum tolerated dose indicating the appropriate level for Phase II dosing may be inappropriate. MMPIs are likely to be most useful when prescribed over prolonged periods of time because their mode of action is prevention of invasion and metastasis. For this reason, it was desirable to collect data on toxicity associated with chronic dosing. To achieve this, a minimum of 10 patients were recruited at each dose level to improve the chance of some patients continuing the drug for at least 8 weeks. After completion of recruitment to the first five dose levels, the trial was further expanded with two new lower dose levels. This was as a result of additional preclinical data, which indicated that MMI270 had antimitastatic effects in mouse models at markedly lower concentrations than determined previously, together with the rationale that the longer term toxicities, which emerged during the trial, might be reduced with lower doses or a qd schedule.

In this Phase I study, four separate approaches were used to assess the effects of MMI270. First, patients underwent traditional assessment of toxicity. Second, pharmacokinetic monitoring allowed the dose level to be related to drug plasma levels and, thereby, to biological effects seen in preclinical studies. Third, direct measurement of the affected target enzymes was attempted, and, finally, indirect measures of biological activity were also studied.

There were two main toxicities associated with MMI270, the first of which was rash. Eighteen patients developed a rash, 16 within the first month of treatment. The rash was generally mild but required cessation of treatment in four patients in the highest three dose levels. It was not a typical allergic reaction because most patients who developed rash could continue on treatment with the gradual disappearance of the skin reaction. The maximum tolerated dose was determined to be 300 mg bid, because at all of the dose levels higher than this, there was a marked increase in both the incidence and severity of rash. Rash was determined to be the dose-limiting toxicity because no

| Table 5 | Mean C_max/4h on days 1 and 28 for dose levels 150 mg bid to 600 mg tid |
|---------|------------------|------------------|------------------|------------------|------------------|
| Dose level | 150 mg bid | 300 mg bid | 600 mg bid | 400 mg tid | 600 mg tid |
| Day 1     |              |              |              |              |
| No.       | 8             | 10            | 9             | 9             | 9             |
| Mean      | 2394          | 5743          | 23272         | 7624          | 11129         |
| SD        | 1171          | 4797          | 14784         | 5621          | 5305          |
| Day 28    |              |              |              |              |
| No.       | 8             | 7             | 7             | 7             | 7             |
| Mean      | 3332          | 6691          | 11018         | 11488         | 11220         |
| SD        | 2104          | 5491          | 6171          | 9985          | 9081          |

| Table 6 | Significant Pearson’s correlations of the biological markers to pharmacological endpoints* |
|---------|---------------------------------|--------|-----------------|-----------------|-----------------|
|         | C_max at day 14 | AUC    | PYCRE at day 14 | DEOCRE at day 14 |
| n       | r      | P    | n   | r     | P    |
| MMP-2   |        |      |     |       |      |
| C_max   | 32     | -0.48 | 0.01 | 32   | -0.43 | 0.02 |
| AUC     | 32     | -0.36 | 0.04 | 32   | -0.28 | 0.12 |
| TIMP-1  |        |      |     |       |      |
| C_max   | 25     | 0.47  | 0.02 | 25   | 0.22  | 0.28 |
| AUC     | 26     | 0.64  | 0.0004| 26   | 0.57  | 0.002|
| MMP-9   |        |      |     |       |      |
| C_max   | 25     | 0.40  | 0.048|

* PYCRE and DEOCRE are the percentage changes from baseline of the ratios of the bone resorption markers pyridinoline and deoxypyridinoline to paired serum creatinine values.

* n, number studied; r, correlation.
patients developed dose-limiting musculoskeletal toxicity during the assessment phase (first two cycles) of the trial. With prolonged exposure to drug, dose-limiting musculoskeletal toxicity was observed at several dose levels, including 75 mg bid; however, this toxicity was related in frequency and severity to duration of treatment rather than to dose level and, therefore, was not used to determine the maximum tolerated dose. Musculoskeletal toxicity was the other significant side effect. Forty percent of the patients experienced symptoms ranging from general myalgia/arthralgia to severe tendonitis with limitation of range of movement in the affected joints. Although three patients discontinued MMI270 as a result of musculoskeletal side effects, 36 patients were able to continue on the trial. None of a variety of pharmacological and nondrug therapies appeared particularly effective in reducing these symptoms, although some patients found temporary relief.

Batimastat (also known as BB-94) was the first MMPI to be assessed in clinical trials; however, although it showed some activity, it is not p.o. bioavailable and is insoluble, limiting its use (28-31). Marimastat was the first p.o. bioavailable MMPI and has been extensively evaluated, currently in a series of Phase III studies. In Phase I studies (32), marimastat showed biological activity in patients with advanced malignancy, as measured by the effects on levels of tumor markers. In the Phase II studies of marimastat (reviewed in Ref. 27), the major toxicity was musculoskeletal effects, similar to those described in this trial. It is probable that musculoskeletal side effects are a feature of this class of broad spectrum MMPs. Fourteen of the 19 patients with prolonged stable disease developed arthralgia, in keeping with the observation that this side effect was related to the duration of treatment and, therefore, possibly to drug activity. Notably, BAY12-9566, an oral MMPI, which selectively targets MMP-2, MMP-9, and MMP-3 but not MMP-1, did not cause musculoskeletal side effects in Phase I studies (33-36); however, it was not clinically active either. An explanation for the musculoskeletal side effects could be that broad spectrum inhibitors may also affect the reprodysin family of Zn2+ metalloproteinases (37). It is a member of this family of enzymes that has been found to hydrolyze pro-TNF-α, TNF-α convertase. TNF-α release is known to be blocked by some MMPs (38). Our results on stimulated white cells analyzed for TNF-α release suggest this enzyme was not inhibited by MMI270 ( unlike marimastat), and, hence, inhibition of this enzyme is not the explanation for musculoskeletal side effects.

Pharmacokinetic monitoring showed the drug to be rapidly absorbed and eliminated with a maximal concentration after a median of 35 min and a median half-life of 1.6 h. There was minimal accumulation of drug over time. The AUC and Cmax of MMI270 both increased approximately proportionally with increasing dose; however, marked inter-patient variability was noted. From preclinical data, it was expected that plasma concentrations greater than 200 nM would result in full inhibition of the target enzymes. Thus, all of the five higher dose levels resulted in biologically relevant plasma levels. The 600 mg tid level resulted in active plasma levels for a mean of 18/24 h, implying that higher doses would be unlikely to cause significant additional enzyme block. Therefore, in this trial, pharmacokinetic monitoring was used to correlate human dosing with levels required in xenograft models for biological effect and, thus, determine the maximum dose level. A concurrent study examined the effect of food intake on the pharmacokinetics of MMI270 (39). Although Cmax was significantly reduced after food intake and Tmax was delayed, the mean AUC was not significantly affected, and there were no recommendations that MMI270 should be taken in either the fasted or fed state.

MMI270 inhibits a wide range of MMPs at nM concentrat-

Fig. 4 A, percentage change from baseline at day 28 of MMP-2 levels against AUC. B, percentage change from baseline at day 28 of TIMP-1 levels against AUC. C, percentage change from baseline at day 28 of MMP-2 against TIMP-1 after one cycle of treatment with MMI270. The correlation coefficient between TIMP-1 and MMP-2 is $r = 0.04; P = 0.007$. 

3 P. Thavasu and F. Balkwill, unpublished data.
tions. Total serum protein levels of three of these, MMP-2, MMP-8, and MMP-9, together with their natural inhibitors, TIMP-1 and TIMP-2, were measured to investigate whether a direct effect on the target enzymes of the drug could be observed. It has been suggested that serum MMP levels may be a method of following disease progression and response to therapy in advanced cancer patients (40); however, other studies (30, 41) indicate that MMP-2 and MMP-9 are not always elevated in such patients. It may be that the ratio of active:inactive protein level is more important than the absolute values; however, only total levels were measured in this study. TIMP-1 and TIMP-2 were identified in the late 1980s (3, 42). Both are effective inhibitors of a wide range of MMPs. TIMP-1 has been demonstrated to block endothelial responses to angiogenic factors such as bFGF and to inhibit angiogenesis (43). Raised TIMP-1 mRNA levels have been demonstrated previously (44) in tumors, and this may result in the raised protein levels found in this study. The ratio of MMP-2:TIMP-2 was also calculated, because TIMPs bind stochiometrically to MMPs. In this study, there was a significant trend for MMP-2 and TIMP-1 to increase at 1 month with increasing drug concentration and with each other, in terms of the percentage change after one cycle. The natural inhibitor for MMP-2 is TIMP-2, which also increased with AUC and may indicate a trend for MMP-2. TIMP-1, and TIMP-2 to rise in parallel. Clear interpretation of these data are not possible, particularly because protein levels may not be an accurate surrogate for enzyme activity. However, it may be that MMP-2 is part of a negative feedback loop, such that increasing inhibition of the enzyme results in further production. Future studies could investigate this further with more early time points measuring MMP-2, MMP-9, TIMP-1, and TIMP-2.

Indirect effects of MMP inhibition were also sought. Pyridinoline and deoxypyridinoline are components of collagen cross-links found chiefly in bone and excreted in urine. If MMPs cause breakdown of the collagen components of bone, then inhibition of these enzymes might result in a fall in urinary pyridinoline and deoxypyridinoline. With MMII270, there was such a fall at 2 weeks, which was no longer observed at 4 weeks. It may be that these results reflect a transitory reduction in the release of ECM breakdown products at the start of MMP inhibition, which is then compensated for as time progresses by a rise in MMP-2 and TIMP-1. Clearly evaluation of intervening time points would help investigate this further.

VEGF, bFGF, VCAM-1, suPAR, CATB, and CATH were also measured. Both VEGF and bFGF are important promoters of tumor angiogenesis (45, 46), and raised levels have been found in cancer patients and are associated with poorer prognosis (47–52). uPA is a serine peptidase, produced by many tumor cells (53), which when bound to its receptor is able to cleave plasminogen to release plasmin, a known activator of MMPs. Elevated plasma levels of suPAR have also been shown to correlate with poor prognosis in colorectal cancer patients (22). CATB and CATH are members of a family of lysozomal proteases, able to degrade various components of the ECM and to activate uPA. VCAM-1 is able to bind the integrin α5β1 and is involved in the transmigration of leukocytes across the vascular endothelium, a process also affected by the MMPs (reviewed in Ref. 54). Because all of these molecules are involved in alterations of the microenvironment of the ECM, it is possible that inhibition of ECM breakdown might be reflected in alternations in their levels. In a similar study, however, BAY12-9566 did not affect VEGF or bFGF plasma levels (33).

Although statistically significant correlations between the percentage changes from baseline of MMP-9 with C max and bFGF with AUC were also found, it may be that they represent artifacts of the statistical analysis as opposed to true biological trends. There were no apparent correlations between other extracellular components and drug concentration; however, given the wide inter-patient variability of both measured ECM components and plasma drug concentrations, it is possible that minor levels of inhibition are occurring but are not observable.

In this study, there were no objective tumor responses; however, prolonged disease stabilization occurred in 19 of 92 patients. Although this will include patients with slow growing tumors, it may be indicative of drug activity. It has been suggested that a reduction in tumor markers can also be used as an indicator of disease response (32). These were measured where appropriate in this study; however, no significant reduction was observed. As discussed in Ref. 26, although tumor responses remain the main aim of cancer therapy, maintenance of stable disease and even delay in progression of disease would still be of clinical benefit to many patients. In the future, other methods such as magnetic resonance spectroscopy, positron emission tomography, or color Doppler ultrasound, which can be used to assess tumor metabolism and blood flow, may prove useful in monitoring patient responses to antiangiogenic agents (55).

In conclusion, MMII270 is a novel oral, broad-spectrum MMP inhibitor with antiangiogenic and antimetastatic effects in animal models. In this large Phase I study in patients with advanced malignancies, the drug was generally well tolerated with rash and musculoskeletal side effects as the main toxicities. MMII270 plasma levels were achieved at greater than four times the mean IC 50 for the target enzymes at the dose determined by conventional toxicity end points. Therefore, from this trial, it would be reasonable to consider MMII270 for additional clinical trials at a dose level of 300 mg bid, which was the maximum tolerated.

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REFERENCES


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