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Matrix Metalloproteinase Inhibitors: Current Developments and Future Perspectives

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ABSTRACT

Malignant tumors are characterized by invasive growth and metastasis. To facilitate this invasive behavior, the enzymatic breakdown of the extracellular matrix (ECM) is a prerequisite. Many human tumors are characterized by locally increased concentrations of matrix metalloproteinases (MMPs), enzymes that are able to degrade this ECM. A vast number of matrix metalloproteinase inhibitors (MMPIs) have been developed in recent years and after extensive preclinical testing, the results of the first clinical studies with several of these compounds have recently been presented. In this review we will describe some of the basic concepts of the degradation of the ECM, with special emphasis on the role of MMPs in the progression of cancer. Furthermore we will review the results of preclinical and clinical studies with MMPIs and discuss their future perspective. ***The Oncologist*** *2001;6:415-427*

INTRODUCTION

The importance of proteinases in tumor invasion was first recognized in 1925 when *Fischer* found that a lytic substance from sarcoma cells could degrade the fibrin stroma. Later it was found that the serine proteinase plasminogen activator (PA) played an important role in activating plasminogen to plasmin. Apart from PAs, proteinases such as serine, cysteine, and metalloproteinases have been associated with cancer [1]. It is important to realize that high levels of extracellular proteolytic activity are not restricted to the malignant phenotype, but are also seen in a number of physiological processes such as embryo implantation, wound healing, and angiogenesis. A common feature in these processes is the breaching of histological barriers with the degradation of the extracellular matrix (ECM) composed of basement membrane and extracellular stroma.

Matrix metalloproteinases (MMPs) are enzymes able to degrade most components of the ECM such as collagens, laminins, fibronectins, elastins, and the protein core of proteoglycans. At this moment more than 20 different MMPs have been identified and classified. They show a consistent sequence homology and in general share a pre-domain, which is a signal peptide for secretion, a pro-domain, important for maintaining latency, a catalytic domain with a highly conserved zinc-binding site, and a hemopexin-like domain. Most MMPs are secreted in the latent form, however, a few MMPs have a transmembrane domain and remain attached to the cell membrane (membrane-type MMPs or MT-MMPs). Based on sequence homology and substrate specificity, MMPs can be classified in five subgroups (Table 1). The classification is somewhat arbitrary, since the true physiological substrates are a matter of debate. In situ hybridization techniques showed that most MMPs are not produced by tumor cells but by adjacent stromal cells. It is suggested that tumor cells produce a stimulatory factor (extracellular matrix metalloproteinase inducer, EMMPRIN) that induces stromal fibroblasts to produce MMPs [2, 3]. In addition, various growth factors, hormones, oncogenes, and tumor promoters are thought to play important roles in the regulation of MMP gene transcription. After translation, the MMPs are secreted in a latent form. Following proteolytic cleavage of the NH2 terminal pro-domain by other MMPs or other proteases, activated MMPs are being formed. Inhibition of MMPs is obtained through protease inhibitors such as α2-macroglobulin and by a group of specific tissue inhibitors of metalloproteinases (TIMPs). It is thought that an imbalance between the activation and inhibition of MMP activity in favor of the MMP activity plays an important role in the pathophysiology

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Table 1. The matrix metalloproteinase family

Group	Descriptive name	*n*	Principal substrate
Collagenases	Interstitial collagenase	MMP-1	Fibrillar collagen types I, II, III
	Neutrophil collagenase	MMP-8	Fibrillar collagen types I, II, III
	Collagenase-3	MMP-13	Fibrillar collagen types I, II, III
	Collagenase-4	MMP-18	
Stromelysins	Stromelysin-1	MMP-3	Proteoglycans, laminin, fibronectin, nonfibrillar collagen
	Stromelysin-2	MMP-10	Proteoglycans, laminin, fibronectin, nonfibrillar collagen
	Matrilysin	MMP-7	Proteoglycans, laminin, fibronectin, nonfibrillar collagen
Gelatinases	Gelatinase A (72 kDa)	MMP-2	Gelatins, nonfibrillar collagen types IV, V
	Gelatinase B (92 kDa)	MMP-9	Gelatins, nonfibrillar collagen types IV, V
Membrane type	MT1-MMP	MMP-14	Progelatinase A, procollagenase-3
	MT2-MMP	MMP-15	Progelatinase A
	MT3-MMP	MMP-16	Progelatinase A
	MT4-MMP	MMP-17	
	MT5-MMP	MMP-21	
Others	Stromelysin-3	MMP-11	Serine protease inhibitor
	Metalloelastase	MMP-12	Elastin, nonfibrillar collagen
	Enamelysin	MMP-20	
		MMP-19	
		MMP-23	
		MMP-24	

of cancer by facilitating the invasion of tumor cells through the ECM. In addition, it is suggested that MMPs affect growth of primary tumors and metastases by stimulating release and activation of latent growth factors from the ECM such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor [4]. Furthermore, degradation of the ECM is also an essential step in the process of angiogenesis, which is required for tumor growth beyond a few millimeters in size and for metastasis [5].

There is extensive literature demonstrating the association of MMP activity and tumor progression. With regard to this association, several generalizations can be made [6, 7]:

- MMPs are detected in a large variety of tumors.
- The number of different MMP family members detected tends to increase with progression of the tumor.
- The relative levels of any individual MMP family member tend to increase with increasing tumor stage.
- MMPs can be made either by tumor cells themselves or as a host response to the tumor.
- The MMPs most frequently encountered are MMP-2, MMP-3, MMP-7, MMP-9, MMP-11, MMP-13, and MMP-14.

Evidence for the role of MMPs in the process of metastasis comes from several experiments using animal tumor models. Intraperitoneal injection of recombinant TIMP-1, a naturally occurring MMP inhibitor (MMPI), reduced lung colonization of intravenously injected B16F10 melanoma cells [8]. Transfecting an expression vector encoding for MMP-9 into a tumorigenic but non-metastatic rat embryo cell line resulted in increased metastatic capacity [9]. In addition, preclinical studies using synthetic MMPIs support the important role of MMP activity in the process of tumor progression, as discussed below.

Since there is a correlation between MMP expression in the tumor and tumor stage, it is suggested that MMP expression can be used as a diagnostic or prognostic tool. Indeed it was found that serum MMP-2 levels were increased in patients with prostate cancer compared with healthy controls or patients with benign prostate hyperplasia [10]. For colon cancer it was shown that high MMP-1 expression within the tumor correlated with hematogenous metastasis independent of other established histopathological factors [11]. This inverse relationship between increased MMP expression and clinical outcome was also found for gastric cancer (MMP-2 and MMP-9), small cell lung cancer (MMP-3, MMP-11, and MMP-14), esophageal cancer (MMP-7), and breast cancer (MMP-11) [12-15].

Basic Aspects

As the inverse relationship between MMP expression and clinical outcome in cancer became more and more obvious, inhibiting the function of the MMP cascade became a target for the development of new anticancer drugs. In theory, therapeutic intervention in the MMP cascade is possible at the induction, production, secretion, activation, or catalytic part, but thus far, most research has focused on inhibition of the

MMP activity itself. TIMPs have been shown to have inhibitory activity in both in vitro and in vivo tumor models, but their clinical use has been hampered by low oral bioavailability. The most interesting agents are the synthetic inhibitors of the enzyme activity. The majority of these MMPIs have been developed through the application of structure-based design rather than through conventional screening technologies [16-18]. Based on the structure of the collagen molecule at the site of the initial cleavage, peptide and peptide-like compounds were developed that were able to interact with the active site of the enzyme and chelate the zinc ion at the catalytic site. The majority of these inhibitors contain a hydroxamic acid group as zinc chelator. The catalytic domains of most MMPs have a high degree of homology and therefore many of the early MMPIs exhibit a broad-spectrum inhibition profile. In order to create more specificity in binding of MMPs thought to be important in the process of tumor progression, and in order to augment oral bioavailability, research has moved to the development of peptide compounds with alternative chelators to the ubiquitous hydroxamic acid group and to nonpeptide compounds with a hydroxamate chelating group. A special group of MMPIs is formed by the tetracycline derivatives that not only inhibit MMPs by chelation of the zinc ion, but are also able to downregulate the production, inhibit the activation, and increase the degradation of MMPs [19]. MMPIs investigated in clinical trials are shown in Table 2.

Preclinical Experience

MMPIs have been extensively studied in numerous tumor models. The first and most extensively studied MMPI is batimastat, a low molecular weight broad spectrum MMPI with a hydroxamate group as a zinc chelator. In in vitro experiments no cytotoxic activity was found [20-22], whereas in studies with various human xenograft models a significant reduction of tumor growth rate was seen when batimastat was administered shortly after tumor inoculation [20, 23, 24]. Administration shortly after tumor inoculation in pancreatic, orthotopic colon, and orthotopic liver tumor models showed reduced growth of the primary tumor, a reduction in the onset of distant metastases, and even prolongation of survival [22, 25, 26].

Although a significant reduction in tumor growth was seen when batimastat was administered shortly after tumor inoculation, treatment in a more advanced tumor stage did not result in a significant growth reduction in a B16-BL6 murine melanoma tumor model [23]. The issue of the growth inhibitory effect of MMPIs in the minimal residual disease state is particularly addressed in the studies mimicking the adjuvant setting. Using orthotopic human breast cancer models (MDA-MB-435 and HOSP.1P) it was shown that administration of batimastat shortly after resection of the tumor significantly inhibited local regrowth, decreased the number and volume of pulmonary metastases, and improved survival [27, 28]. While treatment with batimastat for a short period of time did not result in reduction of regional lymph node metastases, prolonged treatment did. It was suggested that batimastat was not able to prevent invasion of lymphatic channels (which lack a basement membrane), but that prolonged treatment was able to inhibit subsequent growth of nodal metastases [28].

AG3340 is a selective, nonpeptide inhibitor of MMP-2, MMP-3, MMP-9, MMP-13, and MMP-14. Activity has been explored in a wide range of human tumor xenograft models [29]. Oral AG3340 given twice daily, started shortly after tumor implantation, resulted in a profound delay of tumor growth in a human colon, an androgen independent human prostate, and a human non-small cell lung cancer (NSCLC) tumor model. A similar inhibition of growth was seen when AG3340 was initiated after growth of established tumors of a human breast cancer xenograft (MDA-MB-435). AG3340 was the first MMPI tested in a human glioma tumor model (U87), where it was administered intraperitoneally starting 3 weeks after subcutaneous tumor implantation. It caused

Table 2. Matrix metalloproteinase inhibitors in clinical development

Agent	Class	MMP Inhibition	Remarks
Batimastat	Peptido-mimetic	MMP-1, 2, 3, 7, 9	Only parenterally available
Marimastat	Peptido-mimetic	MMP-1, 2, 3, 7, 9, 12	
AG3340 (prinomastat)	Nonpeptido-mimetic	MMP-2, 3, 9, 13, 14	
BAY 12-9566	Nonpeptido-mimetic	MMP-2, 3, 9	
MMI270	Nonpeptido-mimetic	MMP-1, 2, 3, 9, 13, 14	
COL-3 (metastat)	Tetracycline derivative	MMP-2, 9	Multiple mechanisms of action
BMS-275291	Nonpeptido-mimetic	MMP-2, 9	
CP-471,358	Nonpeptido-mimetic	MMP-2, 3, 8, 9, 12, 13, 14	
AE-941(neovastat)	Shark cartilage extract	MMP-1, 2, 7, 9, 12, 13	Multiple mechanisms of action

profound inhibition of tumor growth, decreasing tumor size by 78% compared with controls after 31 days, resulting in a two to three times increased survival [30].

Apart from the role of MMPIs as inhibitors of the remodeling of the ECM surrounding the tumor, there is also evidence that MMPIs inhibit tumor-induced angiogenesis. Analyzing angiogenesis using antibodies to CD-31, an endothelial marker that is almost exclusively expressed on newly formed vessels, revealed that AG3340 decreased angiogenesis in three of the four tumor models studied [29]. Furthermore, using murine endothelioma cells transformed by a polyoma middle-T oncogene, which forms tumors that are constituted of recruited host cells for more than 95%, it was shown that batimastat was able to induce a significant growth reduction [21].

MMPIs have been tested in combination with cytotoxic chemotherapy. In a murine Lewis lung cancer model CT1746, an inhibitor of MMP-2 and MMP-9, combined with either cisplatin or cyclophosphamide was significantly more active than single agent therapy in delaying local tumor growth and reducing number and size of pulmonary metastases [31]. The effect was most obvious when CT1746 was started shortly after tumor implantation, again suggesting that MMPIs are more active when administered under conditions of low tumor volume. AG3340 was studied in combination with carboplatin or paclitaxel using a lung colonization model after i.v. injection of B16-F10 melanoma cells in mice [32]. Neither AG3340 nor carboplatin started 1 day after injection of tumor cells decreased the number of lung lesions (>1 mm^3) significantly. However, the combination produced a significant decrease in the number of lung lesions. AG3340 and paclitaxel, at single agent doses not able to reduce the number of lung lesions, in combination caused a significant decrease in the number of lung lesions. In an MV522 NSCLC model paclitaxel given at suboptimal dose was able to potentiate the activity of AG3340 resulting in enhanced tumor growth inhibition [33]. Finally in a human gastric KKLS tumor model, AG3340 not active as a single agent potentiated the activity of paclitaxel [29].

Giavazzi et al. studied the effects of batimastat in combination with cisplatin in two human ovarian carcinoma xenografts (HOC22 and HOC8) inoculated in the peritoneal cavity of nude mice [34]. In the HOC22 model the early treatment with a combination of batimastat and cisplatin significantly prolonged survival compared with either single agent. In the HOC8 model, only moderately sensitive to cisplatin and not responsive to batimastat, the combination therapy resulted in a prolonged disease-free survival. When treatment was started in the advanced or late stage, monotherapy with cisplatin or batimastat was not effective in the HOC22 model, but the combination resulted in an increased survival.

MMPIs in Clinical Trials

Phase I Studies

Several MMPIs have been tested in phase I/II trials. These studies are summarized in Table 3. Batimastat showed a poor oral bioavailability and also could not be given intravenously due to its limited solubility. Therefore clinical studies were performed using intraperitoneal or intrapleural administration [35-38]. Following intraperitoneal administration, rapid systemic absorption was seen with serum levels exceeding concentrations causing 50% inhibition (IC_{50}) of major MMPs for prolonged periods of time. Side effects considered to be drug related included abdominal discomfort, nausea, vomiting, and fever. Although response is difficult to assess in patients with ascites, patient benefits consisting of decreases in weight, abdominal girth, or frequency of drainage were observed [36, 37]. In a study performed in patients with malignant pleural effusions, batimastat was administered in the pleural cavity following pleural drainage [38]. Peak plasma levels were detected between 4 hours and 1 week after administration and in patients with doses ≥60 mg/m^2, plasma levels were detectable for 9 to 12 weeks. Side effects were comparable to those previously mentioned, with the exception of nonsymptomatic elevation of liver enzymes occurring in 44% of the patients. There were no clear relationships between the elevated liver enzymes and the batimastat dose or the peak plasma levels. Peak values of liver enzymes were generally seen in the second week and in some patients elevations persisted for up to 5 months after batimastat administration. A partial response, with a significant reduction in the need for pleural reaspiration, was achieved in 7 of 16 evaluable patients. The reason for this activity is not clear and it cannot be ruled out that batimistat acted simply as a sclerosing agent, especially since no experimental data existed on MMP inhibition in vivo. Because batimastat could only be administered intraperitoneally or intrapleurally, further clinical development was halted.

Marimastat (BB-2516) was the first oral MMPI tested in clinical trials. It is a low molecular weight peptido-mimetic agent with a hydroxamate group closely related to batimastat. Marimastat is a potent and reversible MMPI with IC_{50}s in the nanomolar range against MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, and MMP-12. The first phase I study was performed with healthy volunteers [39]. Marimastat was rapidly absorbed and well tolerated, with pharmacokinetic data indicating that a total daily dose of 50 to 100 mg can achieve trough levels exceeding 40 ng/ml which is six times the IC_{50} for the major MMPs. Since it was anticipated that no tumor regressions

Table 3. Clinical phase I/II studies of MMPIs: side effects at recommended dose levels

MMPI [reference]	Schedule*	Tumor type	Patient *n*	Recommended dose	Side effects at recommended dose
Batimastat [35]	Intraperitoneal	All	9	1,200 mg/m^2	Abdominal pain
Batimastat [36]	Intraperitoneal	All	9	600 mg/m^2	Mild abdominal pain
Batimastat [37]	Intraperitoneal	All	23		
Batimastat [38]	Intrapleural	All	18	300 mg/m^2	Fever, elevated liver enzymes
Marimastat [40]	5 mg od-50 mg bid	Colon	70	20 mg od-25 mg bid	Musculoskeletal
Marimastat [41]	5 mg od-50 mg bid	Ovary	66	10-25 mg bid	Musculoskeletal
Marimastat [42]	10 mg od-75 mg bid	Pancreas	64	5-25 mg bid	Musculoskeletal
Marimastat [43]	5 mg od-50 mg bid	Colon	61		
Marimastat [44]	2 mg od-50 mg bid	Prostate	88		
Marimastat [45]	5 mg od-75 mg bid	Ovary	66		
Marimastat [47]	25-100 mg bid	NSCLC	12		
Marimastat [48]	25 mg od-50 mg bid	Gastric	35	25 mg od	Musculoskeletal
Marimastat [82]	25 mg	Pancreas	34		
Marimastat [83]	10-100 mg bid	Melanoma	26		
AG3340 [49]	2-100 mg bid	All	45		
BAY 12-9566 [55]	100 mg od-800 mg bid	All	26	800 mg bid	Mild thrombocytopenia, transaminase elevation
BAY 12-9566 [56]	400 mg od-800 mg bid	All	13	800 mg bid	
BAY 12-9566 [57]	100 mg od-800 mg bid	All	29	800 mg bid	Mild thrombocytopenia, transaminase elevation
BAY 12-9566 [59]	100 mg od-800 mg bid	All	21	800 mg bid	Mild thrombocytopenia, transaminase elevation, hypophosphatemia
MMI270 [62]	150 mg bid-600 mg tds	All	92	300 mg bid	Maculopapular skin rash, musculoskeletal
COL-3 [19]	36-98 mg/m^2/day	All	35	36 mg/m^2	Cutaneous phototoxicity
COL-3 [63]	36-98 mg/m^2/day	All	26		
BMS-275291 [66]	150-1,200 mg od	Healthy males	40		
BMS-275291 [68]	600-2,400 mg od	All	44	1,200 mg od	No DLTs
CP-471,358	Ongoing	All			

*All oral intake, except for batimastat. Abbreviations: od = once daily; bid = twice daily; tds = three times daily.

would be seen and that chronic administration would be necessary to exert optimal antitumor activity, a number of phase I/II studies were initiated where early information about activity was based on the rate of rise of serum tumor marker levels [40-45]. A combined analysis of these studies including 415 patients with advanced colorectal, ovarian, pancreatic, and prostate cancer using the serum tumor markers carcinoembryonic antigen (CEA), CA-125, CA 19-9, and prostate specific antigen (PSA), respectively, was published [46]. All patients studied had serum tumor marker levels rising by 25% or more above prespecified levels in a predefined period of 4 or 12 weeks. Marimastat doses studied varied from 2 mg once daily to 75 mg twice daily. Pharmacokinetic analysis showed that mean trough levels increased almost linearly with dose and that these levels for a given dose were substantially higher compared with healthy volunteers with mean trough levels greater than 40 ng/ml observed at total daily doses of 20 mg and above. The principal toxicity of marimastat was found to be reversible musculoskeletal events (myalgia, arthralgia, and tendinitis, predominantly in the upper limbs) with frequency and severity increasing with higher doses. Musculoskeletal events severe enough to reduce the dose occurred mostly after the first month of treatment and particularly at doses of 25 mg twice daily or higher, resulting in dose modification or withdrawal in more than one-third of the patients. Other infrequent severe side effects involved the gastrointestinal system and a few episodes of elevated liver enzymes. Evaluation of serum tumor marker levels following 4-12 weeks of treatment showed that the proportion of patients showing a rise in their tumor marker at the end of the study period of <0% or from 0%-25% increased with dose and was significant only in patients receiving doses of 20 mg daily or higher. This single evaluation point led to major discussion and is likely to overestimate clinical potential of the agent. Because the rate of rise in tumor marker levels is not yet validated as a marker of tumor response, the authors compared the survival of the patients with a tumor marker rise of <0% or from 0%-25% ("responders") with the patients with a tumor marker rise >25% ("non-responders"), and found that survival was significantly different in favor of the responders, thereby suggesting that these marker level changes could be a valid surrogate endpoint. However, due to the limited number of patients and the

larger number of variables, this suggestion will have to be further tested in large-sized trials. Based on the combined analysis of the biological activity, the pharmacokinetic data, and the dose-related musculoskeletal pain, the recommended dose range for further studies was 10-25 mg twice daily.

A study in patients with advanced lung cancer using marimastat at three different dose levels (25 mg, 50 mg, and 100 mg all twice daily) was performed [47]. Dose-limiting toxicity consisting of inflammatory polyarthritis, which occurred within 3 weeks from the start of treatment, was seen in the first three patients in the 100 mg twice daily group. The next three patients at this dose level received prophylactic nonsteroidal anti-inflammatory drugs, but these drugs could not prevent the development of the inflammatory polyarthritis. In the 50 mg group similar though less severe toxicity was seen. Two out of three patients in this group did not complete the 8-week study period because of early progression, so no reliable recommendations could be made about the optimal dose for further studies.

Trying to find evidence of biological activity based on endoscopic appearance and tumor histology, marimastat was also studied in patients with advanced gastric or gastroesophageal cancer [48]. Initially 50 mg twice daily was used based on data of the healthy volunteers study. After five out of six patients developed side effects (gastrointestinal or musculoskeletal), and based on pharmacokinetic data from this and other studies, it was decided to continue the study using a lower dose of 25 mg once daily in 29 additional patients. Again the principal side effect was related to the musculoskeletal system. Eventually 37% of the patients experienced this reversible side effect, the frequency of which increased following prolonged therapy. Additionally, in four patients using marimastat for more than 3 months, a subcutaneous skin thickening of the palmar surface of the hands resembling Dupuytren's disease developed. These side effects were also reversible to a large extent. Activity of the drug, studied by endoscopic changes of the tumor with respect to hemorrhage, fibrous cover, and tumor size, showed a definite increase in fibrous cover in three of six patients in the 50 mg twice daily group and 7 of 29 of the patients in the 25 mg once daily group. Although in three patients an endoscopic reduction in tumor size was suggested, this should be interpreted with caution given the difficulties of endoscopic response assessment. Microscopic assessment of tumor tissue samples did not show major histological changes after 28 days of treatment in all but two patients where an increase in fibrous stroma was seen.

AG3340 is a selective inhibitor of MMP-2, MMP-3, MMP-9, MMP-13, and MMP-14, but not MMP-1 (collagenase-1) thought to be associated with the joint-related toxicities. In a phase I study doses from 2 to 100 mg orally twice daily were studied [49]. Reversible joint-related complaints typically beginning in the shoulders, knees, or hands occurred in a dose- and time-dependent manner. Symptoms were manageable with a drug holiday of 2-4 weeks and a subsequent dose reduction. Drug holidays were necessary in a significant number of patients using doses of 25 mg twice daily and higher for more than 4 weeks. Preliminary data showed that AG3340 was rapidly absorbed and pharmacokinetics were linear with a plasma half-life ($t_{1/2}$) of 2-3 hours. Plasma levels reached were in the active dose range determined in preclinical tumor models [50].

BAY 12-9566 is an orally bioavailable biphenyl compound with inhibitory activity against MMP-2, MMP-3, and MMP-9. In preclinical studies, growth inhibitory activity and reduction in the number of metastases were shown in various tumor models, with elevation of transaminase levels and mild depression of erythropoiesis as the principal toxic effects in animals [51-54]. Four phase I studies including a total of 89 patients have been performed [55-59]. Dose levels studied ranged from 100-1,600 mg/day. The main dose-related toxicities were mild anemia and thrombocytopenia, elevated transaminase levels, and occasionally reversible bilirubin elevations. Other toxicities were mild nausea and vomiting, fatigue, and headache. Musculoskeletal effects did not occur. Pharmacokinetic analysis showed a rapid absorption and a less-than-proportionate increase in plasma steady-state levels (C_{ss}) with doses exceeding 100 mg/day suggesting saturable drug absorption. Since C_{ss} levels seemed to reach a plateau at the higher dose levels (C_{ss} 122 μg/ml at doses of 1,600 mg/day), which exceeded biologically active concentrations by at least two or three orders of magnitude, no further dose escalation was performed and therefore the maximum tolerated dose could not be determined. Despite achieving relevant plasma concentrations, no consistent effects were found on plasma levels of MMP-2 and MMP-9. For TIMP-2 levels a small increase was found in the higher dose range [59]. Also, for other surrogate markers, like plasma levels of VEGF, bFGF, and urinary pyridinoline and deoxypyridoline cross-links, no obvious relationship with dosing was found [56]. With regard to efficacy, no responses were reported, but about one-third of the patients remained in the study for more than 3 months and about 6% of patients were studied for more than 1 year. Based on the results of these four phase I studies, the recommended dose for further studies is 800 mg twice daily.

MMI270 (previously CGS27023A) is an orally bioavailable broad spectrum synthetic hydroxamic acid derivative able to inhibit a wide range of MMPs at nanomolar concentrations in vitro (MMP-1, MMP-2, MMP-3, MMP-9, and MMP-13). Reduced tumor growth in a breast and endometrial rat tumor model and inhibition of hematogenic metastasis of B16 melanoma cells in an experimental and spontaneous

metastasis model were seen [60, 61]. In a phase I study with doses ranging from 50 mg once daily to 600 mg three times daily the main toxicities were a self-limiting maculopapular rash at higher dose levels and mild to moderate myalgia and arthralgia that were not dose related [62]. The recommended phase II dose was determined to be 300 mg twice daily, as at higher dose levels a marked increase in both incidence and severity of rash were seen. Pharmacokinetic analysis showed a rapid absorption and rapid elimination from the plasma with a $t_{1/2}$ of 1.6 hours. At the recommended dose the plasma levels of MMI270 were ≥5 times IC_{50} of the target MMPs for more than 10 hours a day.

COL-3 is an orally available tetracycline analogue. Unlike the other MMPIs, tetracycline derivatives not only inhibit collagenase activity but also downregulate its production, inhibit its activation, and increase the degradation of the proenzyme. A phase I study was performed with doses escalating from 36 mg to 98 mg/m^2/day [19]. Cutaneous phototoxicity was dose limiting and occurred already at the first dose level. With sun avoidance, protective clothing, and the prophylactic use of sunblock, the maximum tolerated dose (MTD) was 70 mg/m^2/day. Three out of 35 patients developed a drug-induced systemic lupus erythematosus with arthralgia and fever. In four patients there was unexplained anemia, while bone marrow examinations in three of these patients revealed ringed sideroblasts. Other toxicities included fatigue, anorexia, nausea, vomiting, and elevated liver enzymes. Pharmacokinetic analysis revealed that peak plasma levels (C_{max}) were reached after a median of 6 hours and that in the higher dose ranges the increase in C_{max} was not dose proportional suggesting a saturable absorption. The median single-dose $t_{1/2}$ of 56 hours could potentially lead to accumulation. No information was given about trough levels throughout the study. Three patients, all with a nonepithelial malignancy, had stable disease for more than 6 months. The authors recommend a dose of 36 mg/m^2 for further studies and higher doses when diligent sun precautions are used. In another study with COL-3, preliminary results indicate an MTD of 50 mg/m^2/day, with photosensitivity of the skin and asthenia as principal toxicities [63]. In addition, it was found that plasma MMP-2 and MMP-9 levels were considerably decreased in a number of patients, possibly reflecting a decreased production, since in peripheral blood mononuclear cells the expression of MMP-9 was also decreased. The recommended doses of both studies yielded COL-3 plasma concentrations within the dose that in preclinical models resulted in growth inhibition of primary and metastatic tumors [64]. At this moment a phase I/II study with COL-3 is ongoing in patients with high grade gliomas.

BMS-275291 is a novel orally available nonhydroxamate MMPI with potent inhibitory activity against MMP-2 and MMP-9, which in an animal model did not cause joint-related toxicity [65]. In a double-blind placebo-controlled study with healthy volunteers using doses from 150 to 1,200 mg once daily for 14 days, the agent was very well tolerated and no dose-limiting toxicity was found [66]. A phase I study was performed in 44 patients with advanced cancer with doses from 600 to 2,400 mg once daily [67, 68]. Again the agent was well tolerated and a maximum tolerated dose was not reached. Grade 1 and 2 arthralgia and myalgia were seen in a significant number of patients, but no frank arthritis and only one case of grade 2 tenosynovitis was observed. Based on pharmacokinetic data, showing trough levels at steady state at least 20-fold the IC_{50} values for MMP-2 and MMP-9 at a dose of 1,200 mg once daily, this dose was recommended for further clinical studies.

Phase I Studies of MMPIs in Combination with Chemotherapy

As MMPIs should be regarded as cytostatic drugs that inhibit tumor growth but do not induce tumor regressions, it is theoretically attractive to combine MMPIs with cytotoxic regimens to augment their effectiveness. In preclinical models MMPIs were shown to have synergistic activity with cytotoxic regimens [27, 31-34]. Several phase I studies have been performed combining a wide range of commonly used cytotoxic regimens with several MMPIs (Table 4). Marimastat was tested in a number of phase I studies using doses varying from 2-20 mg twice daily, which is within the range of the recommended dose for further evaluation determined in single-agent studies. In general, the combinations were well tolerated without indication of additional toxicity. Of some concern are pharmacokinetic data from a study combining carboplatin and paclitaxel with marimastat 10 or 20 mg twice daily, which show trough levels of marimastat of 19.2 and 61 ng/ml that are substantially lower than in the single-agent studies and which for the 10 mg twice daily group are below the target trough levels of 40 ng/ml [73].

AG3340 25 mg twice daily was tested in combination with carboplatin/paclitaxel in patients with advanced tumors and with mitoxantrone/prednisone in patients with hormone refractory prostate cancer [75, 76]. In both studies the combination seemed safe and well tolerated, but no pharmacokinetic data were given.

BAY 12-9566 was tested in a number of phase I studies in combination with carboplatin/paclitaxel, 5-fluorouracil (5-FU)/folinic acid, carboplatin/etoposide, and doxorubicin/docetaxel [77-80]. Preliminary data suggest that, in general, toxicity of these combinations is acceptable and that no significant pharmacological interactions occur. In the study with 5-FU 350 mg/m^2 and folinic acid 20 mg/m^2 × 5 days q 28 days with BAY 12-9566 starting on day 13, 400 mg twice daily was

Table 4. Phase I/II studies combining MMPIs with cytotoxic drugs

MMPI [reference]	Schedule of MMPI*	Cytotoxic regimen	Tumor type	Patient *n*
Marimastat [69]	10 mg bid	Doxorubicin/cyclophosphamide	Breast	9
Marimastat [70]	5-10 mg bid	5-Fluorouracil continuous/bolus	All	13
Marimastat [71]	2-20 mg bid	Carboplatin	Ovarian	31
Marimastat [72]	5-20 mg bid	Gemcitabine	Pancreatic	31
Marimastat [73]	10-20 mg bid	Paclitaxel/carboplatin	NSCLC	22
Marimastat [74]	20 mg bid	Doxorubicin/docetaxel	Breast	11
AG3340 [75]	25 mg bid	Paclitaxel/carboplatin	All	15
AG3340 [76]	25 mg bid	Mitoxantrone/prednisone	Prostate	15
BAY 12-9566 [77]	800 mg bid	Paclitaxel/carboplatin		19
BAY 12-9566 [78]	400-800 mg bid	5-Fluorouracil/folinic acid		17
BAY 12-9566 [79]	400 mg od-400 mg bid	Doxorubicin/docetaxel	All	7
BAY 12-9566 [80]	800 mg bid	Carboplatin/etoposide	All	8
MMI270 [81]	150 mg tds-300 mg bid	5-Fluorouracil/folinic acid	Colorectal	18

*Oral intake for all MMPIs. Abbreviations: od = once daily; bid = twice daily; tds = three times daily.

well tolerated, while 800 mg twice daily, the recommended dose in single-agent studies, was not feasible due to occurrence of grade 2/3 thrombocytopenia.

MMI270 was also tested in combination with 5-FU/folinic acid administered according to the Gramont scheme [81]. MMI270 was added from the second cycle onward. At 300 mg twice daily, preliminary pharmacokinetic analysis did not indicate a marked effect of MMI270 on 5-FU levels. The toxicity related to MMI270 was comparable with the toxicity seen in the single-agent study.

Phase II/III Studies with MMPIs

Two randomized phase II studies with marimastat have been performed in patients with pancreatic carcinoma and malignant melanoma, respectively, but mature results from these studies have not yet been published [82, 83]. A randomized phase II study in patients with glioblastoma multiforme comparing oral temozolomide from day 1-5 every 28 days plus AG3340 or placebo daily until unacceptable toxicity or disease progression is ongoing.

Randomized phase III studies with MMPIs have been performed in a range of tumor types and a range of strategies [7]. In general, phase III study strategies include those comparing an MMPI with conventional cytotoxic chemotherapy, those comparing chemotherapy with an MMPI versus chemotherapy alone, and those comparing an MMPI with placebo in patients with minimal residual disease.

In 369 patients with inoperable gastric cancer, marimastat 10 mg twice daily was compared with placebo [84]. Pretreatment with chemotherapy was allowed if patients had responded or had stable disease. Progression-free survival was significantly increased in the patients using marimastat, but overall survival was not improved. In subgroups of patients with prior chemotherapy and of patients without distant metastases, overall survival was significantly better in the marimastat-treated group. About 10% of the patients in the marimastat group stopped their treatment due to side effects, mostly musculoskeletal complaints. This study is currently the only one suggesting a benefit of an MMPI, but one must realize that this suggestion is only based on subgroup analysis in small cohorts of patients.

In patients with advanced pancreatic cancer, marimastat (5, 10, or 25 mg twice daily) was tested as first-line treatment and compared with gemcitabine 1,000 mg/m^2 weekly for 7 out of 8 weeks [85]. Time to progression and overall survival were significantly better in the gemcitabine group, with no major differences in the marimastat subgroups. Therefore, there is no reason to suggest that the difference was caused by subtherapeutic marimastat dose levels. Preliminary data from a randomized trial testing the addition of marimastat 20 mg twice daily to gemcitabine in 239 patients with advanced pancreatic cancer without prior chemotherapy did not show an advantage of the combination in terms of survival, time to progression, and quality of life. A study comparing marimastat with placebo in an adjuvant setting in patients after surgery for pancreatic cancer is currently ongoing. A randomized study comparing marimastat 10 mg twice daily with placebo in patients with glioblastoma multiforme or gliosarcoma following completion of surgery and radiotherapy did not show a survival benefit for the marimastat group [86]. In addition, studies with marimastat are being performed in other tumor types with minimal disease, for example, non-small cell lung cancer (NSCLC) stage IIIA/IIIB with minimal disease after optimal cytoreductive treatment, small cell lung cancer in partial or complete remission after first-line chemotherapy, and metastatic breast cancer with stable disease or response after first-line chemotherapy.

Two large phase III studies are currently ongoing in patients with NSCLC (686 patients) and hormone refractory prostate cancer (553 patients) studying the addition of AG3340 (5, 10, or 15 mg bid) or placebo to a regimen of carboplatin/paclitaxel or mitoxantrone/prednisone respectively [87, 88]. Interim results of both studies, including the majority of the included patients, thus far revealed no differences in response rate, progression-free survival, or overall survival in the treatment arms.

BAY 12-9566 was tested in several phase III trials in which this MMPI was compared with placebo in patients with small cell lung cancer, NSCLC, and ovarian cancer with partial or complete remission after primary treatment. An interim analysis of the study in patients with small cell lung cancer showed inferior survival in the patients treated with BAY 12-9566 [89]. In another phase III trial BAY 12-9566 was compared with gemcitabine in patients with advanced pancreatic carcinoma [90]. An interim analysis, after including 277 patients, showed inferior progression-free survival and overall survival in the BAY 12-9566 group, after which the accrual has been closed. Based on these negative results, clinical development of BAY 12-9566 has been suspended.

Several other compounds like BMS-275291 and AE-941 have entered phase III trials, but it is too early to report on any data.

Discussion

The important role of MMPs in the process of tumor growth and metastasis has led to the development of specific inhibitors of these enzymes. Several of these inhibitors have entered clinical trials, and results of these studies have recently been presented. Results from preclinical studies and the currently available data from clinical studies make clear that MMPIs will have to be regarded as antiproliferative instead of cytotoxic agents. The development of clinical trials that can optimally assess the role of these new agents forms a major challenge for oncologists, similar to the situation of angiogenesis inhibitors, farnesyl transferase inhibitors, and tyrosine kinase inhibitors [91]. In contrast to cytotoxic agents, where phase I studies are being performed to define dose-limiting toxicities (DLTs) and to determine the recommended dose for phase II studies, defining the recommended dose for antiproliferative or cytostatic agents is more complicated because often DLTs do not occur. As cytostatic agents will have to be administered for prolonged periods of time in order to exert optimal antitumor activity, knowledge of toxicities following this prolonged administration is important for defining an optimal dose. Furthermore, as some cytostatic agents are completely devoid of side effects, it might not even be possible at all to define one single recommended phase II dose, and instead the optimal biological effective dose must be defined based on other endpoints. Examples of these endpoints are threshold plasma levels known to inhibit tumor growth in preclinical models, threshold plasma levels exceeding IC_{50} of target MMPs, or inhibition of target enzymes within tumors. The last option is often practically impossible since this requires multiple tumor biopsies. Measurement of MMP levels in plasma and other body fluids can give insight into the activity of the MMPI, but thus far such correlative studies have been disappointing [47, 59]. Perhaps this is reflecting the fact that MMPIs in general inhibit enzyme activity rather than their secretion. Measurement of surrogate markers of target inhibition can also give insight into biological activity, for example, changes in tumor marker levels (CEA, CA 15.3, CA 19.9, and PSA) or changes in blood flow assessed by positron emission tomography scanning or dynamic magnetic resonance imaging. However, these methods have not yet been validated.

Classic single-agent phase II studies using tumor regression as an endpoint of activity will almost certainly lead to underestimation of potential antitumor activity of cytostatic agents. Therefore, in order to select agents for further testing in large randomized phase III trials, it may make sense to perform properly designed phase II trials that should preferably be randomized [92]. In these studies tumor regression should be replaced by surrogate endpoints of antitumor activity, for example, time to progression, tumor marker inhibition, and survival rate at a certain predefined timepoint. In order to avoid a bias in patient selection, the study design should be randomized, for example, using the "randomized discontinuation" design, in which all included patients are being treated with the cytostatic agent for a predefined period of time. Patients not showing disease progression during or at the end of this period could then be randomized to either continue treatment or to receive no drug or placebo. Although these trials can never be powered to detect significant differences, performing them could prevent too early rejection of a potentially active agent or prevent performing large, time-consuming phase III trials with inactive agents. Until now, no results of such randomized phase II trials with MMPIs have been published.

As mentioned, the results of the few randomized phase III trials with MMPIs that have been presented so far are disappointing. However, in view of the mechanisms of action of MMPIs one can argue whether the patient population studied is the most likely to benefit from growth inhibitory and antiinvasive agents such as MMPIs. Usually patients in these kinds of studies have a large tumor load, often with multiple metastases. Therefore, a more realistic approach should be to perform studies with MMPIs in patients in whom the tumor load has been optimally reduced either following surgery or optimal cytoreductive, cytotoxic treatment. Such an adjuvant

study using marimastat in optimally operated pancreatic carcinoma is currently ongoing.

In these situations, once again, one has to bear in mind, however, that toxicity occurring after prolonged periods of drug administration becomes important and thus, even toxicity regarded as mild in studies with only short-lasting drug administration can turn into a serious problem following prolonged treatment.

One of the most intriguing toxicities related to treatment with MMPIs is musculoskeletal toxicity. The clinical spectrum varies from mild myalgia and arthralgia to frank tendonitis and arthritis. This toxicity occurs in almost all broad-spectrum MMPIs especially after a prolonged period of treatment, with symptoms occurring earlier and being more severe at the higher dose ranges, although in the study with MMI270 musculoskeletal complaints were dose independent [62]. It is suggested that inhibition of MMP-1 is associated with the joint-related problems and therefore MMPIs that do not inhibit MMP-1 have been developed. BAY 12-9566 is such an MMPI, and in clinical studies with this compound indeed no musculoskeletal side effects were seen, whereas with AG3340, which only inhibits MMP-1 in the nanomolar range compared with inhibition of other MMPs in the picomolar range, musculoskeletal toxicity was only seen at the higher dose levels. The exact role of MMP-1 in the pathogenesis of the musculoskeletal side effects is still a matter of debate. Another possible explanation for the differences in musculoskeletal side effects reported could be the differences in inhibition of tumor necrosis factor-α (TNF-α)-converting enzyme (TACE), an enzyme belonging to the reprolysin family of Zn^{2+} metalloproteinases. TACE acts as a sheddase and is held responsible for the release of soluble TNF-α from its membrane-bound precursors, while TNF-α is associated with inflammatory arthritis [93]. In an animal tendinitis model it was found that a broad spectrum MMPI with anti-sheddase activity was active in a mouse B16 melanoma model without inducing a tendinitis, while a comparable broad-spectrum MMPI without anti-sheddase activity was also active in the cancer model but did induce development of tendinitis [94]. In the same experiments it was shown that small spectrum MMPIs did not cause development of tendinitis but were not active either in reducing tumor growth. Although these findings may not be generalized, these data show that changes in MMP specificity can influence antitumor effects and toxicity profile and that, therefore, further research is needed to characterize the exact role of individual MMPs in different tumor types.

Conclusion

The recognition of the concept of MMPs being involved in the process of tumor growth and metastasis and the subsequent development of a large number of agents able to inhibit the enzyme activity has led to the evaluation of several of these new agents in early clinical trials and randomized clinical trials for which the first results are now available. The initial enthusiasm on the possible use of MMPIs in the treatment of cancer has clearly been dampened. We seem to be in a period of considerable concern whether a balance between required activity and avoidance of toxicity, based on focused targeting of specific MMPIs, can ever be achieved. We believe that the concept of MMPIs is too intriguing to completely reject their development, but that at the current stage focus should again be on preclinical research.

References

1 Mignatti P, Rifkin DB. Biology and biochemistry of proteinases in tumor invasion. Physiol Rev 1993;73:161-195.

2 Guo H, Zucker S, Gordon MK et al. Stimulation of matrix metalloproteinase production by recombinant extracellular matrix metalloproteinase inducer from transfected Chinese hamster ovary cells. J Biol Chem 1997;272:24-27.

3 Van den Oord JJ, Paemen L, Opdenakker G et al. Expression of gelatinase B and the extracellular matrix metalloproteinase inducer EMMPRIN in benign and malignant pigment cell lesions of the skin. Am J Pathol 1997;151:665-670.

4 Noël A, Gilles C, Bajou K et al. Emerging roles for proteinases in cancer. Invasion Metastasis 1997;17:221-239.

5 Stetler-Stevenson WG. Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. J Clin Invent 1999;103:1237-1241.

6 Chambers AF, Matrisian LM. Changing views of the role of matrix metalloproteinases in metastasis. J Natl Cancer Inst 1997;89:1260-1270.

7 Nelson AR, Fingleton B, Rothenberg ML et al. Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol 2000;18:1135-1149.

8 Schultz RM, Silberman S, Persky B et al. Inhibition by human recombinant tissue inhibitor of metalloproteinases of human amnion invasion and lung colonization by murine B16-F10 melanoma cells. Cancer Res 1988;48:5539-5545.

9 Bernhard EJ, Gruber SB, Muschel RJ. 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 USA 1994;91:4293-4297.

10 Gohji K, Fujimoto N, Hara I et al. Serum matrix metalloproteinase-2 and its density in men with prostate cancer as a new predictor of disease extension. Int J Cancer 1998;79:96-101.

11 Sunami E, Tsuno N, Osada T et al. MMP-1 is a prognostic marker for hematogenous metastasis of colorectal cancer. ***The Oncologist*** 2000;5:108-114.

12 Sier CFM, Kubben FJGM, Ganesh S et al. Tissue levels of matrix metalloproteinases MMP-2 and MMP-9 are related to the overall survival of patients with gastric carcinoma. Br J Cancer 1996;74:413-417.

13 Michael M, Babic B, Tsao M et al. Prognostic significance of metalloproteinases and their tissue inhibitors in patients with small cell lung cancer. Proc Am Soc Clin Oncol 1998;17:563a.

14 Yamamoto H, Adachi Y, Itoh F et al. Association of matrilysin expression with recurrence and poor prognosis in human esophageal squamous cell carcinoma. Cancer Res 1999;59:3313-3316.

15 Ahmad A, Hanby A, Dublin E et al. Stromelysin 3: an independent prognostic factor for relapse-free survival in node-positive breast cancer and demonstration of novel breast carcinoma cell expression. Am J Pathol 1998;152:721-728.

16 De B, Natchus MG, Cheng M et al. The next generation of MMP inhibitors. Design and synthesis. Ann N Y Acad Sci 1999;878:40-60.

17 Skotnicki JS, Zask A, Nelson FC et al. Design and synthetic considerations of matrix metalloproteinase inhibitors. Ann N Y Acad Sci 1999;878:61-72.

18 Brown PD. Matrix metalloproteinase inhibitors. Breast Cancer Res Treat 1998;52:125-136.

19 Rudek MA, Figg WD, Dyer V et al. Phase I clinical trial of oral COL-3, a matrix metalloproteinase inhibitor, in patients with refractory metastatic cancer. J Clin Oncol 2001;19:584-592.

20 Davies B, Brown PD, East N et al. A synthetic matrix metalloproteinase inhibitor decreases tumor burden and prolongs survival of mice bearing human ovarian carcinoma xenografts. Cancer Res 1993;53:2087-2091.

21 Taraboletti G, Garofalo A, Belotti D et al. Inhibition of angiogenesis and murine hemangioma growth by batimastat, a synthetic inhibitor of matrix metalloproteinases. J Natl Cancer Inst 1995;87:293-298.

22 Zervos EE, Norman JG, Gower WR et al. Matrix metalloproteinase inhibition attenuates human pancreatic cancer growth in vitro and decreases mortality and tumorigenesis in vivo. J Surg Res 1997;69:367-371.

23 Chirivi RGS, Garofalo A, Crimmin MJ et al. Inhibition of the metastatic spread and growth of B16-BL6 murine melanoma by a synthetic matrix metalloproteinase inhibitor. Int J Cancer 1994;58:460-464.

24 Low JA, Johnson MD, Bone EA et al. The matrix metalloproteinase inhibitor batimastat (BB-94) retards human breast cancer solid tumor growth but not ascites formation in nude mice. Clin Cancer Res 1996;2:1207-1214.

25 Wang X, Fu X, Brown PD et al. Matrix metalloproteinase inhibitor BB-94 (batimastat) inhibits human colon tumor growth and spread in a patient-like orthotopic model in nude mice. Cancer Res 1994;54:4726-4728.

26 Bu W, Tang ZY, Sun FX et al. Effects of matrix metalloproteinase inhibitor BB-94 on liver cancer growth and metastasis in a patient-like orthotopic model LCI-D20. Hepatogastroenterology 1998;45:1056-1061.

27 Sledge Jr GW, Qulali M, Goulet R et al. Effect of matrix metalloproteinase inhibitor batimastat on breast cancer regrowth and metastasis in athymic mice. J Natl Cancer Inst 1995;87:1546-1550.

28 Eccles SA, Box GM, Court WJ et al. Control of lymphatic and hematogenous metastasis of a rat mammary carcinoma by the matrix metalloproteinase inhibitor batimastat (BB-94). Cancer Res 1996;56:2815-2822.

29 Shalinsky DR, Brekken J, Zou H et al. Broad antitumor and antiangiogenic activities of AG3340, a potent and selective MMP inhibitor undergoing advanced oncology clinical trials. Ann N Y Acad Sci 1999;878:236-270.

30 Price A, Shi Q, Morris D et al. Marked inhibition of tumor growth in a malignant glioma tumor model by a novel synthetic matrix metalloproteinase inhibitor AG3340. Clin Cancer Res 1999;5:845-854.

31 Anderson IC, Shipp MA, Docherty AJP et al. Combination therapy including a gelatinase inhibitor and cytotoxic agent reduces local invasion and metastasis of murine Lewis lung carcinoma. Cancer Res 1996;56:715-718.

32 Neri A, Goggin B, Kolis S et al. Pharmacokinetics and efficacy of a novel matrix metalloproteinase inhibitor, AG3340, in single agent and combination therapy against B16-F10 melanoma tumors developing in the lung after IV-tail vein implantation in C57BL/6 mice. Proc Am Assoc Cancer Res 1998;39:302a.

33 Shalinsky DR, Brekken J, Zou H et al. Marked antiangiogenic and antitumor efficacy of AG3340 in chemoresistant human non-small cell lung cancer tumors: single agent and combination chemotherapy studies. Clin Cancer Res 1999;5:1905-1917.

34 Giavazzi R, Garofalo A, Ferri C et al. Batimastat, a synthetic inhibitor of matrix metalloproteinases, potentiates the antitumor activity of cisplatin in ovarian carcinoma xenografts. Clin Cancer Res 1998;4:985-992.

35 Wojtowicz-Praga S, Low J, Marshall J et al. Phase I trial of a novel matrix metalloproteinase inhibitor batimastat (BB-94) in patients with advanced cancer. Invest New Drugs 1996;14:193-202.

36 Parsons SL, Watson SA, Steele RJC. Phase I/II trial of batimastat, a matrix metalloproteinase inhibitor, in patients with malignant ascites. Eur J Surg Oncol 1997;23:526-531.

37 Beattie GJ, Smyth JF. Phase I study of intraperitoneal metalloproteinase inhibitor BB94 in patients with malignant ascites. Clin Cancer Res 1998;4:1899-1902.

38 Macaulay VM, O'Byrne KJ, Saunders MP et al. Phase I study of intrapleural batimastat (BB-94), a matrix metalloproteinase inhibitor, in the treatment of malignant pleural effusions. Clin Cancer Res 1999;5:513-520.

39 Steward WP. Marimastat (BB2516): current status of development. Cancer Chemother Pharmacol 1999;43(suppl):S56-S60.

40 Primrose JN, Bleiberg H, Daniel F et al. Marimastat in recurrent colorectal cancer: exploratory evaluation of biological activity by measurement of carcinoembryonic antigen. Br J Cancer 1999;79:509-514.

41 Poole C, Adams M, Barley V et al. A dose-finding study of marimastat, an oral matrix metalloproteinase inhibitor, in patients with advanced ovarian cancer. Ann Oncol 1996;7(suppl 5):68.

42 Rosemurgy A, Harris J, Langleben A et al. Marimastat in patients with advanced pancreatic cancer: a dose-finding study. Am J Clin Oncol 1999;22:247-252.

43 Zaknoen S, Wolff R, Cox J et al. Marimastat in advanced progressive colorectal cancer—a dose finding study. Proc Am Soc Clin Oncol 1997;16:273a.

44 Boasberg P, Harbaugh B, Eisenberger M et al. Marimastat in patients with hormone refractory prostate cancer: a dose finding study. Proc Am Soc Clin Oncol 1997;16:316a.

45 Malfetano J, Teng N, Barter J et al. Marimastat in patients with advanced cancer of the ovary—a dose finding study. Proc Am Soc Clin Oncol 1997;16:373a.

46 Nemunaitis J, Poole C, Primrose J et al. Combined analysis of studies of the effects of the matrix metalloproteinase inhibitor marimastat on serum tumor markers in advanced cancer: selection of a biologically active and tolerable dose for longer-term studies. Clin Cancer Res 1998;4:1101-1109.

47 Wojtowicz-Praga S, Torri J, Johnson M et al. Phase I trial of marimastat, a novel matrix metalloproteinase inhibitor, administered orally to patients with advanced lung cancer. J Clin Oncol 1998;16:2150-2156.

48 Tierney GM, Griffin NR, Stuart RC et al. A pilot study of the safety and effects of the matrix metalloproteinase inhibitor marimastat in gastric cancer. Eur J Cancer 1999;35:563-568.

49 Hande K, Wilding G, Ripple G et al. A phase I study of AG3340, a matrix metalloproteinase inhibitor, in patients having advanced cancer. Ann Oncol 1998;9(suppl 2):74.

50 Pithavala Y, Shalinsky D, Wilding G et al. Comparison of preclinical efficacy and associated plasma concentrations of AG3340, a matrix metalloproteinase inhibitor, with plasma concentrations achieved clinically. Proc Am Soc Clin Oncol 1999;18:223a.

51 Nozaki S, Sissons S, Casazza AM et al. Inhibition of human breast cancer regrowth and pulmonary metastases by BAY 12-9566 in athymic mice. Proc Am Assoc Cancer Res 1998;39:301.

52 Flynn C, Bull C, Matherne C et al. Anti-invasive and anti-metastatic activity of the novel MMP inhibitor BAY 12-9566 in subcutaneous and orthotopic models using the human colon carcinoma, HCT 116. Ann Oncol 1998;9(suppl 2):75.

53 Hibner B, Bull C, Flynn C et al. Activity of the matrix metalloproteinase inhibitor BAY 12-9566 against murine subcutaneous and metastatic in vivo models. Ann Oncol 1998;9(suppl 2):75.

54 Clemens GR, Detzer K, Bomhard E et al. Pre-clinical drug safety profile for the anti-metastatic matrix metalloproteinase inhibitory agent BAY 12-9566. Ann Oncol 1998;9(suppl 2):74.

55 Grochow L, O'Reilly S, Humphrey R et al. Phase I pharmacokinetic study of the matrix metalloproteinase inhibitor BAY 12-9566. Proc Am Soc Clin Oncol 1998;17:213a.

56 Erlichman C, Adjei AA, Alberts SR et al. Phase I study of the matrix metalloproteinase inhibitor, BAY 12-9566. Ann Oncol 2001;12:389-395.

57 Hirte H, Goel R, Major P et al. A Phase I dose escalating study of matrix metalloproteinase inhibitor BAY 12-9566 administered orally in patients with advanced solid tumours. Ann Oncol 2000;11:1579-1584.

58 Lathia C, Seymour L, Grochow L et al. BAY 12-9566, a selective, non-peptidic biphenyl inhibitor of matrix metalloproteinases: summary of phase I clinical and pharmacokinetic results. Proc AACR-NCI-EORTC Int Conf 1999:3.

59 Rowinsky EK, Humphrey R, Hammond LA et al. Phase I and pharmacological study of the specific matrix metalloproteinase inhibitor BAY 12-9566 on a protracted oral daily dosing schedule in patients with solid malignancies. J Clin Oncol 2000;18:178-186.

60 Wood JM, Müller M, Schnell C et al. CGS27023A, a potent and orally active matrix metalloproteinase inhibitor with antitumor activity. Proc Am Assoc Cancer Res 1998;39:83a.

61 Kasaoka T, Nishiyama H, Okada M et al. Matrix metalloproteinase inhibitor CGS27023A inhibited hematogenic metastasis of B16 melanoma cells in both experimental and spontaneous metastasis models. Proc Am Assoc Cancer Res 2000;41:134a.

62 Levitt NC, Eskens FA, O'Byrne KJ et al. A phase I and pharmacological study of the oral matrix metalloproteinase inhibitor, MMI270 (CGS27023A), in patients with advanced solid cancer. Clin Cancer Res 2001;7:1912-1922.

63 Munoz-Mateu M, de Grafenried L, Eckhardt SG et al. Pharmacodynamic studies of Col-3, a novel matrix metalloproteinase inhibitor, in patients with advanced cancer. Proc Am Soc Clin Oncol 2001;20:76a.

64 Rowinsky EK, Eckhardt SG, Rizzo J et al. Protracted daily treatment with COL-3 an oral tetracycline analog, matrix metalloproteinase inhibitor, is feasible: a phase I, pharmacokinetic and biological study. Proc Am Soc Clin Oncol 2000;19:180a.

65 Ferrante K, Winograd B, Canetta R. Promising new developments in cancer chemotherapy. Cancer Chemother Pharmacol 1999;43(suppl):S61-S68.

66 Daniels R, Gupta E, Kollia G et al. Safety and pharmacokinetics of BMS-275291, a novel matrix metalloproteinase inhibitor in healthy subjects. Proc Am Soc Clin Oncol 2001;20:100a.

67 Gupta E, Huang M, Mao Y et al. Pharmacokinetic evaluation of BMS-275291, a matrix metalloproteinase inhibitor, in cancer patients. Proc Am Soc Clin Oncol 2001;20:76a.

68 Hurwitz H, Humphrey J, Williams K et al. A phase I trial of BMS-275291: a novel, non-hydroxamate, sheddase sparing matrix metalloproteinase inhibitor with no dose-limiting arthritis. Proc Am Soc Clin Oncol 2001;20:98a.

69 Gradishar W, Sparano J, Cobleigh M et al. A phase I study of marimastat in combination with doxorubicin and cyclophosphamide in patients with metastatic breast cancer. Proc Am Soc Clin Oncol 1998;17:144a.

70 O'Reilly S, Mani S, Ratain MJ et al. Schedules of 5FU and the matrix metalloproteinase inhibitor marimastat: a phase I study. Proc Am Soc Clin Oncol 1998;17:217a.

71 Adams M, Thomas H. A phase I study of the matrix metalloproteinase inhibitor, marimastat, administered concurrently

with carboplatin, to patients with relapsed ovarian cancer. Proc Am Soc Clin Oncol 1998;17:217a.

72 Carmichael J, Ledermann JA, Woll PJ et al. Phase IB study of concurrent administration of marimastat and gemcitabine in non-resectable pancreatic cancer. Proc Am Soc Clin Oncol 1998;17:232a.

73 Anderson I, Supko J, Eder J et al. Pilot pharmacokinetic study of marimastat in combination with carboplatin/paclitaxel in patients with metastatic or locally advanced inoperable non-small cell lung cancer. Proc Am Soc Clin Oncol 1999:18:187a.

74 Wolff AC, Krishnamurthi S, Sparano JA et al. A pharmacokinetic and pharmacodynamic study of doxorubicin and docetaxel combinations plus marimastat in metastatic breast cancer. Proc Am Soc Clin Oncol 2001;20:98a.

75 D'Olimpio J, Hande K, Collier M et al. Phase I study of the matrix metalloproteinase inhibitor AG3340 in combination with paclitaxel and carboplatin for the treatment of patients with advanced solid tumors. Proc Am Soc Clin Oncol 1999;18:160a

76 Wilding G, Small E, Ripple G et al. Phase I study of AG3440, a matrix metalloproteinase inhibitor, in combination with mitoxantrone/prednisone in patients having advanced prostate cancer. Ann Oncol 1998;9(suppl 2):74.

77 Tolcher A, Rowinsky EK, Rizzo J et al. A phase I and pharmacokinetic study of the oral matrix metalloproteinase inhibitor BAY 12-9566 in combination with paclitaxel and carboplatin. Proc Am Soc Clin Oncol 1999;18:160a.

78 Goel R, Chouinard E, Stewart DJ et al. Phase I/pharmacokinetic study of the matrix metalloproteinase inhibitor BAY 12-9566 in conjunction with 5-fluorouracil/folinic acid. Proc Am Soc Clin Oncol 2000;19:232a.

79 Grethlein SJ, Lathia C, Humphrey R. A phase I and pharmacokinetic study of the matrix metalloproteinase inhibitor BAY 12-9566 in combination with doxorubicin and docetaxel in patients with advanced cancer. Proc AACR-NCI-EORTC Int Conf 1999:69.

80 Alberts S, Reid J, Erlichman C et al. A phase I and pharmacokinetic study of the selective, non-peptidic inhibitor of matrix metalloproteinases BAY 12-9566 in combination with etoposide and carboplatin. Proc AACR-NCI-EORTC Int Conf 1999:69.

81 Eatock M, Cassidy J, Johnson J et al. A phase I study of the matrix metalloproteinase inhibitor MMI270 (previously termed CGS27023A) with 5FU and folinic acid. Proc Am Soc Clin Oncol 1999;18:209a.

82 Evans J, Bramhall S, Carmichael J et al. A phase II study of marimastat (BB-2516) in advanced pancreatic cancer. Ann Oncol 1996;7(suppl 5):51.

83 Bodurtha A, Eisenhauer E, Steward W et al. Phase I-II study of marimastat (BB2516) in patients with metastatic melanoma. Proc Am Soc Clin Oncol 1997;16:493a.

84 Fielding J, Scholefield J, Stuart R et al. A randomized double-bind placebo-controlled study of marimastat in patients with inoperable gastric adenocarcinoma. Proc Am Soc Clin Oncol 2000;19:240a.

85 Rosemurgy A, Buckels J, Charnley R et al. A randomized study comparing marimastat to gemcitabine as first line therapy in patients with non-resectable pancreatic cancer. Proc Am Soc Clin Oncol 1999;18:261a.

86 Phuphanich S, Levin VA, Yung W et al. A multicenter, randomized, double-blind, placebo controlled trial of marimastat in patients with glioblastoma multiforme or gliosarcoma following completion of conventional first-line treatment. Proc Am Soc Clin Oncol 2001;20:52a.

87 Smylie M, Mercier R, Aboulafia D et al. Phase III study of the matrix metalloproteinase inhibitor prinomastat in patients having advanced non-small cell lung cancer. Proc Am Soc Clin Oncol 2001;20:307a.

88 Ahmann FR, Saad F, Mercier R et al. Interim results of a phase III study of the matrix metalloproteinase inhibitor prinomastat in patients having metastatic, hormone refractory prostate cancer. Proc Am Soc Clin Oncol 2001;20:174a.

89 Hidalgo M, Eckhardt SG. Development of matrix metalloproteinase inhibitors in cancer therapy. J Natl Cancer Inst 2001;93:178-193.

90 Moore MJ, Hamm J, Eisenberg P et al. A comparison between gemcitabine and the matrix metalloproteinase inhibitor BAY 12-9566 in patients with advanced pancreatic cancer. Proc Am Soc Clin Oncol 2000;1:240a.

91 Gelmon KA, Eisenhauer EA, Harris AL et al. Anticancer agents targeting signaling molecules and cancer cell environment: challenges for drug development? J Natl Cancer Inst 1999;91:1281-1287.

92 Eskens FALM, Verweij J. Clinical studies in the development of new anticancer agents exhibiting growth inhibition in models: facing the challenge of a proper study design. Crit Rev Oncol Hematol 2000;34:83-88.

93 Black RA, Rauch CT, Kozlosky CJ et al. A metalloproteinase disintegrin that releases tumor-necrosis-α from cells. Nature 1997;385:729-733.

94 Drummond AH, Beckett P, Brown PD et al. Preclinical and clinical studies of MMP inhibitors in cancer. Ann N Y Acad Sci 1999;878:228-235.