LEADING ARTICLE

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Clinical Potential of Matrix Metalloprotease Inhibitors in Cancer Therapy

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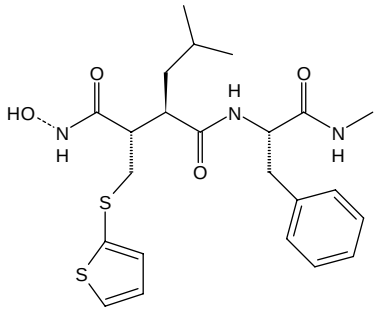
Abstract

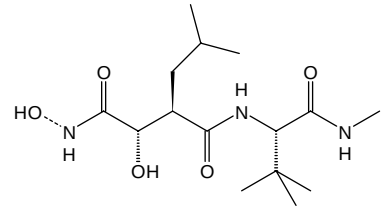
Matrix metalloproteases (MMP) are a family of enzymes that contribute to the degradation of the extracellular matrix. The destruction of the extracellular matrix eventually leads to tumour invasion, metastasis and angiogenesis. Realising this mechanism of action, there is tremendous potential for inhibitors of MMP in cancer therapy. Extensive preclinical data have shown that administration of matrix metalloprotease inhibitors (MMPI) to different animal models results in a reduction in primary tumour growth as well as in the number and size of metastatic lesions. Based on promising preclinical studies, synthetic MMPI have been developed and taken into clinical trials. These include marimastat, BAY-129566, CGS-27023A, prinomastat (AG-3340), BMS-275291 and metastat (COL-3). These drugs are all in different stages of clinical development, ranging from phase I to III. In general, musculoskeletal problems, such as joint stiffness and pain in hands, arms and shoulders seem to affect most patients in varying degrees, depending on the dose and type of compound administered. In addition to single agent therapy, several MMPI have entered trials of combination therapy. The objective of combining chemotherapy with an MMPI is to potentiate tumour cytotoxicity as well as to reduce the size and number of metastatic lesions. Several compounds have entered phase III combination therapy trials, but it is still too early to report any data. There is ongoing research in correlating biological endpoints, such as levels of MMP and markers of angiogenesis with clinical response. As the field of MMP and their inhibitors continues to mature, its role in cancer therapeutics will be better defined.

Matrix metalloproteases (MMP) constitute a family of at least 16 zinc and calcium containing proteolytic enzymes that play an integral role in the physiology of the extracellular matrix (ECM). Within the complex milieu of the ECM, several different processes are occurring, all with the common goal of maintaining appropriate tissue function and homeostasis. Because of the pivotal role the MMP play in this system of checks and balances, intensive focus in recent years in this field has led investigators to the identify the importance of several enzymes in cancer progression. One of the roles MMP play results in the degradation of the basement membrane and in the remodelling of the ECM. In pathological processes such as cancer, specific MMP may be recruited to permit primary tumour growth and metastatic disease. Activation of specific MMP has been implicated in both tissue invasiveness, metastases and angiogenesis.

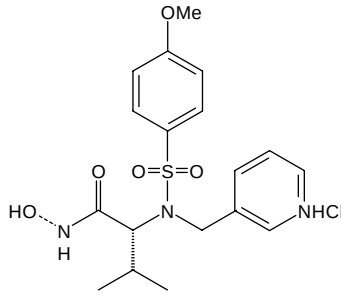
Several matrix metalloprotease inhibitors (MMPI)



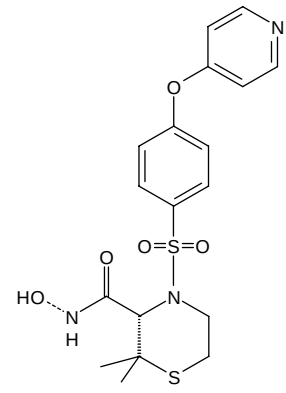
Batimastat



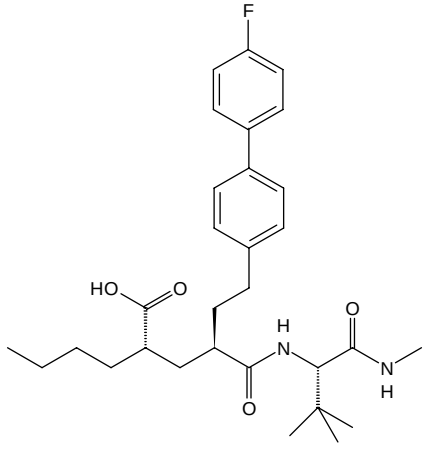
Marimastat



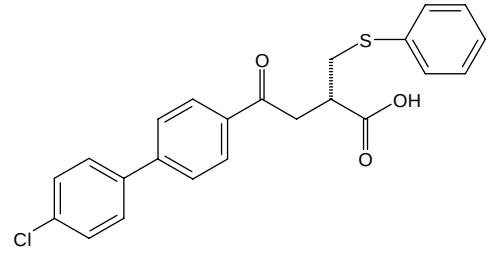
CGS-27023A



Prinomastat (AG-3340)



BMS-275291



BAY-129566

Fig. 1. Chemical structures of matrix metalloprotease inhibitors.

have been developed (fig. 1). Encouraging preclinical and early clinical data have stimulated the study of MMPI in large scale clinical trials evaluating the safety and efficacy of these novel compounds in patients with different types of cancer.

1. Rationale for the Use of Matrix Metalloprotease Inhibitors (MMPI)

Malignant cells injure patients by local tumour growth and by metastasis to different sites in the body. Unfortunately, many patients present for the first time with disease that has already metastasised. The metastatic process usually commences with damage to the basement membrane and malignant cells traversing through the basement membrane and the stroma of the ECM, entering the general circulation, penetrating between endothelial lining cells at another tissue site, invading through the basement membrane once again and proliferating as a new mass, and recruiting new blood vessels to support further growth. The interstitial stroma consists of various proteins: collagens, proteoglycans, gelatins, laminin and fibronectin. The enzymes involved in degrading the previously mentioned enzymes include serine proteases, cysteine proteases, aspartyl proteases and MMP.[1] It has become clear that the interstitial stroma is not a passive structural support, but rather, a metabolically active tissue.

There are 3 distinct classes of MMP by target: collagenases, stromelysins and gelatinases. MMP are secreted into the ECM in a proenzyme form, which requires activation by other enzymes. One class of activators is the enzyme membrane type-matrix metalloprotease (MT-MMP).[2] MT-MMP have a transmembrane domain which is essential in activating the proMMP.

To date, there are 16 known distinct members of the MMP family. All members share a highly conserved zinc binding catalytic domain and a specific sequence in the prodomain integral to maintaining a latent state in the ECM.[3] They differ in substrate specificity, in inhibitor binding and in matrix binding. Table I lists the various members of the MMP family by class, number and substrate specificity. There is a considerable amount of overlap between substrate specificity and some substrates are still unknown.

The process of ECM remodelling is under complex control. One of the mechanisms to counteract the activity of MMPs is a natural tissue inhibitor, known as tissue inhibitor of metalloproteases (TIMP). The TIMP family consists of 4 known enzymes, all possessing 12 conserved cysteine residues.[4] TIMP are not proenzymes and therefore do not require activation by another enzyme. Inhibition of MMP occurs by covalent binding at specific domains. The complex balance of effects for MMP, MT-MMP and TIMP is altered in disease states and provides a novel therapeutic target.

Gross[5] in 1962 first implicated MMP in the dissolution of the tadpole tail. Since then, the role of MMP in non-malignant human diseases, such as rheumatoid arthritis and osteoarthritis, has been studied with increasing focus. MMP as a whole may be essential for normal growth and development. For example, MMP-9 is secreted by osteoclasts and is believed to play a role in normal bone growth and resorption.[6] Other physiological roles established for MMP include ovulation and trophoblast implantation. However, in patients with rheumatoid arthritis, MMP-3 and MMP-9 have been isolated in the synovium.[7,8] Interestingly, other diseases including inflammatory bowel disease, chronic liver disease, abdominal aortic aneurysm, bronchiectasis and wound healing have all shown elements of MMP overexpression in the affected sites.[9-13] MMP overexpression resulting in the disorganisation of tissue function may play an important part in the pathogenesis of these disease states.

In neoplasia, MMP are involved in both primary tumour growth and in metastatic tumour development. In particular, overexpression of MMP-2 and MMP-9 has been evaluated extensively in different tumours such as breast, colon, gastric, head and neck, prostate and lung cancer. In colorectal cancer, MMP-2 (both in pro and active forms) and MMP-9 are overexpressed compared with normal mucosa.[14] The active form of MMP-2 seems to

Table I. Classification of matrix metalloproteases (MMP)

MMP Class	MMP Number	Substrate Specificity
Collagenases		
Interstitial collagenase	MMP-1	Fibrillar collagens
Neutrophil collagenase	MMP-8	Fibrillar collagens
Collagenase-3	MMP-13	Fibrillar collagens
Stromelysins		
Stromelysin-1	MMP-3	Proteoglycans, ECM, glycoproteins, type IV collagens, gelatins
Stromelysin-2	MMP-10	Proteoglycans, ECM, glycoproteins, type IV collagens, gelatins
Stromelysin-3	MMP-11	Laminin and fibronectin
Gelatinases		
Gelatinase-A	MMP-2	Gelatins, type IV & type I collagens
Gelatinase-B	MMP-9	Gelatins, type IV & type I collagens
Membrane type (MT-MMP)		
MT-1 MMP	MMP-14	Gelatinase A, fibrillar
MT-2-MMP	MMP-15	Unknown
MT-3-MMP	MMP-16	Gelatinase A
MT-4-MMP	MMP-17	Unknown
Others		
Matrilysin	MMP-7	Proteoglycans, ECM, glycoproteins, type IV collagens, gelatins, elastin
Metalloelastase	MMP-12	Elastin
MMP-18	MMP-18	Unknown
MMP-19	MMP-19	Unknown

ECM = extracellular matrix.

only be overexpressed in cancer tissue, not normal colon mucosal tissue. Similarly, in gastric cancer, active MMP-2 and MMP-9 were found to be expressed in 59% of cancer tissue compared with 23% of normal tissue.[15] In pancreatic cancer, MMP-1, MMP-2 and MMP-9 were studied, but only MMP-2 and MMP-9 are found to be overexpressed (in 75% of the tumours).[16]

For gastrointestinal tumours in general, MMP-2 and MMP-9 overexpression appear to correlate with tumour stage, tumour aggressiveness and poor prognoses. Similar results have been published in studies of cervical, bladder and lung tumours.[17-19] Sato et al.[20] have shown expression of MT-MMP in lung, colon, head and neck tumours. Since the identification of MT-MMP and TIMP, studies evaluating the interplay of all 3 families in cancer growth and metastases have been undertaken.

Although there is clear overexpression of MMP in tumours, there is still a great variability in the levels of overexpression of MMP in different tumour types. Fiebig et al.[21] studied the expression patterns of MMP-2, MMP-3 and MMP-9 in panel of 47 human tumour xenografts. There was MMP-2 overexpression mostly in soft tissue sarcomas (100%), melanomas (84%), testicular carcinoma (53%) and bladder (26%). MMP-3 and MMP-9 were weakly expressed. Such a report is suggestive that MMP-2 is a reasonable therapeutic target in the above mentioned tumour types. However, so far there have been no studies correlating patients with stable disease with the profile of enzymes in their tumours. Such studies clearly need to be performed in the future to provide a more select group of patients along with better potential therapeutic targets.

In addition to the variability of expression in the tumour types, there are also differences in enzyme expression in primary tumours compared with their metastatic lesions.

2. Evidence from Preclinical Studies of MMPI

Evidence for the role of MMP inhibitors in primary tumour growth has been provided by preclinical data involving the synthetic MMPI batimastat. Batimastat is a broad spectrum MMPI with activity against MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9. Because of poor oral bioavailability, batimastat has been administered intraperitoneally and intrapleurally in several different models. In a xenograft model of human ovarian cancer, nude mice with intraperitoneally implanted ovarian cancer cells received either batimistat or placebo.[22] Significantly less tumour in the ascitic fluid and a significant increase in survival were seen in the batimistat-treated mice compared with the control group. Batimistat was also tested in a xenograft model of human colorectal cancer cells.[23] There was a 50% reduction in primary tumour growth in the treatment group (which had implanted tumour in the intestinal wall) compared with the control group. This reduction was associated with a significant increase in survival. In a murine melanoma model, batimastat produced a 33% reduction of tumour growth when treatment was initiated 11 to 19 days after tumour inoculation.[24] A reduction in primary tumour growth with administration of intraperitoneal batimastat has been shown in several other models, such as a xenograft model of breast cancer.[25] Batimastat in combination with docetaxel was found to potentiate the antitumour activity in a mouse forestomach carcinoma model.[26] The docetaxel plus batimastat combination showed synergy in the inhibition of primary tumour growth and lung metastases compared with docetaxel or batimastat alone.

The role of MMPI in the metastatic process was initially thought to be only the inhibition of tumour extravasation. For example, if a cancer cell overexpresses certain degradative MMP, then an inhibitor would stop that process and therefore stop tumour extravasation. However, intravital videomicroscopy has clarified this process in real time. Mouse melanoma cells genetically engineered to overexpress TIMP-1 were injected into chick embryos.[27] The expected result from overexpression of TIMP-1 was defective extravasation. However, the study showed successful extravasation of the cancer cells. Unexpectedly, there was reduced growth in the new target tissue. This finding shed new light on one mechanism of metastatic tumour reduction by MMPI.

These new discoveries led to further studies where the number and size of the metastatic lesions as well as primary tumour growth were evaluated. BAY-129566 is a second generation, oral, biphenyl MMPI, selective for MMP-2, MMP-3, MMP-9 and MMP-13. Flynn et al.[28] administered oral doses of BAY-129566 to SCID (severe combined immunodeficiency) mice with surgically attached HCT-116 human colon tumour. Not only was primary tumour size reduced by 35% in the treated group, but there was a 50% reduction in the overall incidence of metastasis, with a 60% decrease in liver metastases and a 46% decrease in pancreatic metastases. In addition, the actual size of the metastatic tumour lesions were smaller in the treated group than the control group. This reduction may reflect the additional mechanism of inhibition of angiogenesis in metastatic tumour sites.

A new macrocyclic inhibitor (A-177430) was shown to decrease primary tumour growth and metastases not only by inhibiting angiogenesis, but also by promoting apoptosis in a model of rate prostate cancer.[29] These results are encouraging, especially in terms of enhancing cytotoxicity of the primary tumour by combining MMPI with chemotherapeutic agents.

Based on data supporting overexpression of MMP in different cancer tissues and preclinical studies showing reduction of primary tumour growth and distant metastatic disease in various tumours with synthetic MMPI either via apoptosis or antiangiogenic mechanisms, new synthetic MMPI have been developed and taken into a clinical trials.

3. Study End-Points

In general, these MMPI can be administered to mice at very high doses before significant toxicity or lethality is seen (>100 to 500 mg/kg). They are not mutagenic in Ames tests. If doses of these agents are escalated, they will affect larger families of proteases, eventually including tumour necrosis factor (TNF)α converting enzyme (TACE) and presumably provoking inflammatory responses. One example that may be clinically important is the enzyme which processes TNFα, a member of the adamalysin family. The shedding of other cell surface molecules, including TNF receptors, interleukin receptors, FAS ligand, transforming growth factor (TGF)α and L-selectin may also be processed by similar metalloproteases. Since shedding of these receptors may down-regulate the activity of the membrane bound protein, inhibiting the metalloproteases responsible for shedding may circumvent complex systems controlling the inflammatory response. The ideal drug exposure to provide relatively selective MMP inhibition is undefined, but pharmacokinetically targeted trials to achieve steady state or trough plasma concentrations of free drug that are 1 to 10 times greater than the IC50 for the targeted enzymes have been undertaken.[30]

Phase I trials included analysis of tumour markers, particularly prostate specific antigen (PSA) in prostate trials. Although a reduced rate of rise in PSA levels to <25% over 4 weeks in 50 to 70% of patients with dosages ranging from 5 to 25mg twice daily were thought to suggest benefit, others have questioned this approach.[31] Other tumour markers such as CA-125, CA 19-9 are utilised as secondary biomarkers to predict clinical activity.

Other studies correlating a biological effect of a drug with clinical response have been reported. Bazzett et al.[32] measured urinary levels of MMP in patients with endometrial and ovarian carcinoma. By using gelatin zymography, the study showed that all advanced stages of endometrial carcinoma were positive for MMP-2 and MMP-9 while only half of advanced staged ovarian carcinoma were positive. Similarly, Koshiba et al.[33] also used gelatin zymography, and reported that pancreatic carcinoma tissue specimens had significantly higher MMP-2 level in more advanced stage tumours (T3) compared with earlier stage tumours (T1). The MMP-2 level was also higher in patients with nodal or distant metastatic disease compared with those without. However, gelatin zymography is a relatively new technique and needs to be further developed. Another potential biochemical marker correlates bone turnover with extent of metastatic bone disease. Markers such as serum bone-specific alkaline phosphatase (B-AP), serum levels of the collagen cross-link associated C-telopeptide (ICTP), and urine concentration of the pyridinium cross-link pyridinoline (PYD) are suggestive of extent of bone metastases.[34] Such markers have not been evaluated in patients receiving MMPI, but early studies to immunolocalise MMP in bone metastases have been performed. Lhotak et al.[35] reported that tumour cells from breast carcinoma indeed strongly expressed MMP-1. This is not surprising in light of the musculoskeletal adverse effects seen with MMPI that selectively inhibit MMP-1. More studies evaluating biological correlates to clinical response clearly need to be done.

4. MMPI in Clinical Development

Table II lists MMPI that have entered clinical trials. The chemical structures of the various compounds are illustrated in figure 1. Oral batimastat did not undergo further evaluation because marimastat had better characteristics for oral administration. However, intrapleural batimastat was administered to 18 patients with malignant pleural effusions in single doses ranging from 15 to 300 mg/m^2.[36] Only 8 patients did not require further thoracentesis, comparable with the efficacy of bleomycin or tetracycline. Prolonged plasma exposure persisted for up to 12 weeks after peak concentrations of 20 to 200 μg/L were measured; patients receiving 300 mg/m^2 intrapleurally maintained plasma concentrations above 25 μg/L through the 12th week. Fever for 1 to 2 days after treatment was common, 8 patients had elevated liver function tests, and 5 patients had intrapleural pain.

Table II. Matrix metalloproteases (MMP) inhibitors in clinical trials

Drug	Company	Trial	Disease	Mechanism
Batimastat	British Biotech	Discontinued phase I	Advanced solid tumours	Broad spectrum
Marimastat	British Biotech	Phase III	Pancreas,[a] NSCL, breast, brain, ovarian[a]	Broad spectrum
BAY-129566	Bayer	Discontinued phase III	Pancreas,[a] NSCL[a]	MMP-2, 3, 9, 13
Prinomastat (AG-3340)	Agouron	Phase III	Prostate,[a] NSCL[a]	MMP-2, 3, 9, 14
CGS-27023A	Novartis	Phase I	Advanced solid tumours	Broad spectrum
BMS-275291 (D2163)	Bristol-Myers Squibb, Celltech Group	Phase I	Advance solid tumours	MMP-1, 2, 9
Metastat (COL-3)	CollaGenex	Phase I/II	Advanced solid tumors	Broad spectrum

a Given in combination with cytotoxic chemotherapy.

NSCL = non-small cell lung cancer.

4.1 Marimastat

4.1.1. Single Agent Use

Marimastat has entered phase II trials in several diseases. Phase I trials established the feasibility of extended administration. However, an unusual joint problem limited the ability to administer marimastat for extended periods at higher doses. At oral doses of 10mg twice daily for 3 to 5 months, there was a 30% incidence of musculoskeletal problems.[37] The musculoskeletal problems, which also appear to be associated with long term administration of other hydroxamic acid based MMPI, included joint stiffness and pain in the hands, arms and shoulder, attributed to tendonitis. These joint complications are dealt with by discontinuing the drug for a period of time and reinitiating treatment at a reduced dose. Preclinical models of tendonitis have been developed in marmosets, where batimastat and marimastat do cause changes.[38] Such changes are not observed with the more selective MMPI. Although hypotheses have been raised regarding the mechanism of this toxicity, there are no experimental data addressing the potential contributions of inhibition of MMP-1, TACE, or other potential targets that might alter remodelling of damage to actively used tendons or later immunological responses to such damage. Although CGS-27023A and marimastat do inhibit several sheddases, this occurs at micromolar concentrations, much higher than those required to inhibit the targeted enzymes.

Wojtowicz-Praga et al.[38] studied 12 patients with advanced lung cancer in a standard phase I study. The lowest oral dose of 25mg twice daily resulted in plasma concentrations of between 67.4 to 394.8 μg/L. The highest dose of 100mg twice daily resulted in plasma concentrations in the range of 255.9 to 746.1 μg/L. In comparison, the *in vitro* IC50 for MMP-2 is several fold lower at 1.5 to 8 μg/L. The higher plasma concentrations in this study were correlated with increasing musculoskeletal adverse effects.

A phase III trial in gastric cancer involving 369 patients randomised to either placebo or marimastat was reported as showing a statistically significant benefit of marimastat compared with placebo in terms of the secondary end-point analysis of progression-free survival.[39] This randomised trial used marimastat 10mg twice daily. Although the primary objective of improving survival rate of the trial did not achieve statistical significance at the pre-defined clinical cut-off, there appears to be a survival benefit with longer follow-up.

Another trial has been completed in patients with unresectable pancreatic cancer evaluating marimastat versus gemcitabine as first line therapy.[40] Full analysis of the data are still pending.

4.1.2 Combination Therapy

A phase I study of marimastat in combination with doxorubicin and cyclophosphamide was reported in patients with metastatic breast cancer.[41] In this setting, marimastat was administered over 1 year at a dose of 10mg twice daily. Concurrent cyclophosphamide and doxorubicin were given at standard doses every 3 weeks for a total of 6 cycles.

23 patients were enrolled with 18 of them already discontinued. The primary reason for discontinuation was disease progression with a mean of 6 months, range 2 to 10 months. 17% of patients were discontinued secondary to unacceptable musculoskeletal adverse effects. This trial reported more than the expected incidence of musculoskeletal adverse events but, overall, the toxicities experienced were believed to be no higher than expected.

Combination therapy with carboplatin was evaluated in patients with relapsed ovarian cancer.[42] In this study of 31 patients, 20 have completed 6 cycles of carboplatin [area under the plasma concentration-time curve (AUC) 6 mg/ml × min] every 21 days in combination with escalating doses of marimastat up to 20mg twice daily. A notable adverse effect attributed to higher doses of marimastat was musculoskeletal pain, which necessitated the discontinuation of the drug in 9 patients. There were 8 responses, of which 3 were complete responses as measured by computed tomography (CT) scans. The complete responses lasted for 5, 7 and 13 months, respectively. This frequency of responses is comparable with single agent carboplatin in advanced ovarian cancer. In a meta-analysis evaluating 3 trials with 385 patients receiving carboplatin, a complete response rate of 23% was achieved.[43] The time to progression was 14 months and the median survival was 22 months. A phase III randomised trial comparing chemotherapy with or without marimastat is ongoing.

In pancreatic cancer, combination therapy with gemcitabine was used in patients with unresectable disease.[44] Gemcitabine at standard doses of 1000 mg/m^2 weekly for 3 out of 4 weeks was administered along with escalating doses of marimastat (up to 20mg twice daily). The toxicities were primarily attributed to gemcitabine, including grade 4 bilirubin elevation and grade 3 myelosuppression. Two of the 11 patients in the study had a response and 6 had stable disease.

In metastatic or locally advanced inoperable non-small cell lung cancer, marimastat was given in combination with paclitaxel and carboplatin.[45] The combination of the two chemotherapeutic agents with marimastat was reported to be well tolerated. There were no unexpected toxicities from the combination, although further analysis of this trial is underway.

Other studies in advanced colon, melanoma and prostate cancer have been performed in a phase I setting.[46-48] A study with fluorouracil with and without leucovorin in combination with marimastat (5mg or 10mg twice daily) was performed in 13 patients with advanced solid tumours. Unfortunately, 5 of the 13 patients developed thrombotic events, including a femoral artery embolus and a fatal pulmonary embolus, although eventually these were considered not to be related to treatment.[48]

4.2 BAY-129566

The next MMPI to enter phase I clinical trials was BAY-129566, a selective inhibitor of MMP-2, MMP-3, MMP-9 and MMP-13. It is orally bioavailable with a half-life of 5 to 7 days. Seymour[49] have recently reported a summary of the 4 phase I studies performed in patients with advanced cancers. 90 patients with sarcoma, melanoma, or breast, ovarian or colorectal cancers received escalating doses of oral BAY-129566 up to 800mg twice daily. The toxicities were mild, reversible thrombocytopenia and anaemia, and mildly increased transaminase levels. No joint problems were observed. Dose escalation was limited by decreasing bioavailability: 8-fold dose increase produced only doubling in AUC or maximum plasma drug concentration (C_{max}). For example, patients receiving BAY-129566 800mg daily had an AUC of 2300 mg/dL compared with those receiving 100mg daily who had an AUC of 1161 mg/dL. Of 90 patients, 2 patients with refractory disease at the start of study had stable disease for greater than 4 weeks (4 to 41 weeks).

Tolcher et al.[50] have reported a trial combining BAY-129566 with paclitaxel and carboplatin in advanced cancers. The trial has 3 arms: paclitaxel alone, paclitaxel and carboplatin, and carboplatin alone. BAY-129566 800mg twice daily was administered 1 week after chemotherapy. A total of 19 patients were enrolled. The initial pharmacokinetic data do

not show any significant alterations in BAY-129566 C_{max}, trough or steady-state AUC with the addition of paclitaxel or paclitaxel and carboplatin. Similarly, there was no alteration in paclitaxel clearance.

However, recently, all trials utilising BAY-129566 were discontinued because of concerns about the drug's efficacy.[51]

4.3 CGS-27023A

CGS-27023A (MM-1270) is a nonselective MMPI studied in the phase I setting. Levitt et al.[52] reported the results of a study of CGS-27023A 150mg twice daily to 600mg three times daily involving 36 patients with advanced solid tumours, including colon cancer, mesothelioma and melanoma. Two significant toxicities were observed: maculopapular rash and musculoskeletal symptoms. There were no tumour responses.

Another trial combining CGS-27023A with fluorouracil and folinic acid was performed in patients with advanced colon cancer.[53] Early pharmacokinetic analysis did not show any effect of this compound on fluorouracil concentrations. Musculoskeletal symptoms were observed in 8 out of 20 patients, with a median time to onset of 9 weeks. In terms of tumour responses, there were 2 patients with partial responses and 10 patients with stable disease. The dose of 300mg twice daily in this combination appears to be well tolerated, but final analysis is still pending.

4.4 Prinomastat

Prinomastat (AG-3340) is a selective MMPI of MMP-2, MMP-3, MMP-9 and MMP-14. It is orally bioavailable but has a short half-life of 3 hours. Shalinsky et al.[30] have found that growth inhibition with prinomastat is correlated with maintenance of a minimum effective plasma concentration and not necessarily the total daily dose. Prinomastat administered every 6 hours at a dose of 6.25 mg/kg to nude mice with human colon tumours (COLO-320DM) resulted in a maximal growth inhibition of 74% ($p < 0.05$) compared with administration every 12 (12.5mg twice daily) or 24 (25mg daily) hours, where there was no growth inhibition.

The combination of prinomastat with carboplatin has been reported to increase survival in an orthotropic nude rat model of primary and metastatic human lung cancer.[54] At an oral dose of 100 mg/kg/dose of prinomastat or an intraperitoneal dose of 10 or 20 mg/kg/dose of carboplatin, neither agent improved overall survival. However, in combination, there was prolongation of overall survival; 41.6 ± 2.5 days versus 35.7 ± 2.7 days ($p < 0.03$).

Phase I trials of single agent prinomastat have been completed. 47 patients receiving dosages between 5 and 100mg twice daily were studied.[55] Three patients were reported to have minimal tumour regression. 12 patients had periods of stable disease lasting from 4 to 10 months. Arthralgia and body aches were managed with treatment breaks and reduced dosages.

A phase I study has been reported using the combination of prinomastat with paclitaxel and carboplatin in patients with advanced solid tumours.[56] 15 patients were enrolled, 7 of whom had advanced non-small cell lung cancer. Currently, toxicities appeared to be primarily from the chemotherapeutic agents. Toxicities due to prinomastat were altered taste in 1 patient and 1 patient with grade 2 myalgia which resolved after a dose reduction of prinomastat. Further follow-up is still necessary for this study.

Similarly, 15 patients with advanced prostate cancer have been enrolled in a phase I trial with prinomastat in combination with mitoxantrone and prednisone.[57] Prinomastat pharmacokinetics did not appear to be altered in the presence of prednisone or mitoxantrone. The toxicities also related to the chemotherapy agents, with the exception of joint symptoms. However, the musculoskeletal adverse effects were comparable with those of prinomastat alone. This study is continuing its accrual process.

4.5 BMS-275291

BMS-275291 (D-2163) is a MMPI of intermediate selectivity that has recently entered phase I trials. The IC50s of this agent range from 10 to 25 nmol/L against MMP-1, MMP-2, MMP-8 and MMP-9; it is also potent against membrane type

metalloprotease 14 (MT-1 MMP) which may be involved in the activation of MMP-2. Like the other selective MMPI, the IC50 of BMS-275291 against TACE is in the μmol/L range. It shows activity in metastatic models consistent with that reported for other MMPI. It will be informative to see if BMS-275291 (which is inactive against sheddases but highly active for MMP-1) produces tendonitis or not.[58]

4.6 Metastat

Metastat (COL-3) is an oral analogue of tetracycline which has been synthetically modified with no antimicrobial activity. *In vitro*, there is inhibition of MMP expression in human colon cancer cell lines (COL0205) and in breast cancer cell lines (E10). It is unclear whether its inhibition of primary tumour growth and metastatic lesion growth is due to MMP-2 down-regulation alone or a combination of down-regulation and direct inhibition. In the Dunning MAT Lylu animal model, *in vivo* data show a decrease in the primary tumour growth.[59]

In a phase I study in 35 patients with advanced cancer, 4 dose levels ranging from 36 to 98 mg/m^2 resulted in the dose limiting toxicity of cutaneous photosensitivity in 69% of patients. There was no change in the plasma vascular endothelial growth factor (VEGF) levels, but the majority of patients had a modest decline in plasma MMP-2 levels. The maximum tolerated dose was 70 mg/m^2/day. However, down-regulation of MMP-2 was on average less than 30% in this study compared with in vitro data with metastat where there was a 60 to 70% down-regulation of MMP-2 expression.[59]

4.7 Other Compounds

Additional compounds with selective collagenase inhibition are being developed specifically for osteoarthritis indications. Brewster et al.[60] studied trocade (Ro-323555), an orally active collagenase selective inhibitor in the STR/ORT mouse model of arthritis. With a 10 to 50 mg/kg dose, significant inhibition of joint space narrowing and other protective effects were seen. Animal models of bone and cartilage changes suggest that in addition to their potential utility for prevention of osteoarthritis, MMPI may alter the natural history of bone metastasis. Models of forced bone metastasis suggest that MMPI may inhibit osteoclast mediated resorption of bone and decrease the structural changes in bone in the presence of metastases.[61] This change may also alter the sensitivity of diagnostic tests which depend on active bone resorption. For example, CT scans may not show lytic changes and bone scans may be negative despite soft tissue and marrow involvement with metastatic lesions. Further studies with selective MMPI in patients with bone metastases will undoubtedly be important in the future.

5. Conclusion

The MMPI currently in clinical trials have shown biological effects, including musculoskeletal and platelet changes that may reflect alteration in the balance of endogenous proteases and their natural inhibitors. Prolonged time-to-progression in early trials is suggestive of their potential therapeutic utility.

If prolongation of time-to-progression is shown in randomised trials, and if that is associated with improved survival, MMPI may have a role as single agents for patients with cancer. Based on synergistic effects on tumour growth and survival in animal models, MMPI are also being evaluated clinically in combination with chemotherapeutic agents and will be studied in combination with radiation therapy in the future.

Outcome measures will have to be redefined when assessing the utility of cytostatic drugs in early clinical trials. As new antiangiogenesis inhibitors and other agents that may have cytostatic rather than cytotoxic effect enter cancer therapeutics in the next few years, patients may benefit from accepting disease stability or increased disease free survival as measures of success, instead of focusing primarily on eradicating tumours. In the past, partial regression induced with chemotherapy, radiation therapy or surgery has not consistently translated into prolonged survival. The eventual role of inhibition of MMP is likely to be in combination with other cytoreductive or cytostatic agents in disease-controlling regimens. The optimal use of MMPI is yet to be

identified. It may be in combination therapy or in sequence after cytotoxic agents, as well as in adjuvantive therapy. Future trials will be needed to establish the proper use of these novel agents.

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