Development of Matrix Metalloproteinase Inhibitors in Cancer Therapy

Manuel Hidalgo, S. Gail Eckhardt

The matrix metalloproteinases (MMPs) are a family of zinc-dependent proteinases involved in the degradation of the extracellular matrix. The MMPs have been implicated in the processes of tumor growth, invasion, and metastasis; are frequently overexpressed in malignant tumors; and have been associated with an aggressive malignant phenotype and adverse prognosis in patients with cancer. A number of MMP inhibitors are being developed for the treatment of cancer. The most extensively studied class of MMP inhibitors includes collagen peptidomimetics and nonpeptidimimetic inhibitors of the MMP active site, tetracycline derivatives, and bisphosphonates. The hydroxamate peptidomimetic inhibitor batimastat and its orally bioavailable analogue marimastat, which bind covalently to the zinc atom at the MMP-active site, were the first MMP inhibitors to be studied in detail. Marimastat is currently being studied in randomized clinical trials. The nonpeptidic MMP inhibitors were synthesized in an attempt to improve the oral bioavailability and pharmaceutical properties of the peptidic inhibitors. Several members of this class of compounds are undergoing evaluation in phase III clinical trials. The tetracyclines and, particularly, the nonantibiotic chemically modified tetracyclines, interfere with several aspects of MMP expression and activation and inhibit tumor growth and metastases in preclinical models. A representative agent of this class, Col-3, is currently undergoing phase I clinical trials. The development of the MMP inhibitors, like that of other targeted and predominantly antiproliferative compounds, poses a challenge because the paradigms that have governed the design of clinical oncology trials may not be relevant to this new class of agents. The anticipated need for long-term administration of these drugs, together with their cytostatic mechanism of action, will require novel clinical trial design strategies. [J Natl Cancer Inst 2001;93:178–93]

During the last few decades, the discovery of new anticancer agents has been based on the development of compounds that interfere with nonspecific intracellular processes (i.e., nucleotide turnover, DNA synthesis and replication, and microtubular functions). More recently, new understanding of tumor cell biology has permitted the identification of cellular processes that are specifically disrupted when the growth and metastasis of malignant tumors without altering normal tissues and, therefore, to cause minimal toxicity. Many such agents are currently undergoing clinical evaluation.

Potential targets for new drugs include the matrix metalloproteinases (MMPs), a group of proteinases that have physiologic roles in degrading and remodeling the extracellular membrane. The MMPs are overexpressed in a variety of malignant tumor types, and their overexpression is associated with tumor aggressiveness and metastatic potential (1–4). The specific alteration of the MMPs observed in malignant tissues and their participation in some of the major oncogenic mechanisms have fueled interest in the design and evaluation of MMP inhibitors as anticancer agents (5–7).

In this review, we discuss the rationale for targeting the MMPs therapeutically and provide a comprehensive summary of the MMP inhibitors that are currently in clinical trials.

MMPs: Definition, Function, and Regulation

The MMPs are a family of zinc-dependent neutral endopeptidases that are collectively capable of degrading essentially all of the components of the extracellular matrix (1–4). The MMPs were originally described as the enzymes responsible for dissolution of the tadpole tail, and subsequent studies have indicated that these proteases, which are synthesized by connective tissue cells, are important for the remodeling of the extracellular matrix that accompanies physiologic processes, such as uterine involution, bone resorption, and wound healing (1–4).

The human MMP gene family consists of at least 18 structurally related members that fall into five classes according to their primary structure and substrate specificity: collagenases (MMP-1, MMP-8, and MMP-13), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-7, MMP-10, MMP-11, and MMP-12), membrane type (MT)-MMPs (MT1-MMP, MT2-MMP, MT3-MMP, and MT4-MMP), and nonclassified MMPs (Table 1) (4). The general structure of the MMPs includes a signal peptide, a propeptide domain, a catalytic domain, a catalytic domain with a highly conserved zinc-binding site, and a haemopexin-like domain that is linked to the catalytic domain by a hinge region (Fig. 1). In addition, MMP-2 and MMP-9 contain fibronectin type II inserts within the catalytic domain, and MT-MMPs contain a transmembrane domain at the C-terminal end of the hae-

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mopexin-like domain. The haemopexin domain is absent in the smallest MMP, matrilysin (MMP-7) (2,3).

MMPs are highly regulated at the levels of both gene expression and protein activation. Transcriptional regulation of MMP genes is mediated by an AP-1 regulatory element in their proximal promoter regions (8). In general, the MMP genes are not expressed constitutively in vivo, and the basal production of MMPs in cell cultures is low (2,9). MMP gene transcription is induced by a variety of extracellular stimuli, such as cytokines (interleukin [IL] 4 and IL-10), growth factors (EGF, transforming growth factor-α [TGF-α], basic fibroelastic growth factor [bFGF], and TGF-β-1), and cell–cell or cell–matrix interactions (10–20). Binding of these stimulatory ligands to their receptors triggers a cascade of intracellular reactions that are mediated through at least three different classes of mitogen-activated protein (MAP) kinases: extracellular signal-regulated kinase, stress-activated protein kinase/Jun N-terminal kinases, and p38 (21). Activation of these kinases culminates in the activation of a nuclear AP-1 transcription factor, which binds to the AP-1 cis element and activates the transcription of the corresponding MMP gene (22).

Most MMPs are secreted as latent precursors (zymogens) that are proteolytically activated in the extracellular space. The pro-MMPs are retained in their inactive form by an interaction between a cysteine residue located in the propeptide portion of the molecule with the catalytic zinc atom, blocking the access of substrates to the catalytic pocket of the enzyme. Partial proteolytic cleavage of the propeptide dissociates the covalent bond between the cysteine residue and the catalytic site and exposes the catalytic site to the substrate. MMPs are activated in an orderly fashion, with one activated MMP cleaving and activating the next in a complex and only partially deciphered network of proteases in the extracellular space (23). For example, one of the best characterized activation pathways involves the interaction between the MT-MMPs and MMP-2. MT1-MMP is activated prior to secretion by Golgi-associated furin-like proteases and then becomes anchored to the extracellular membrane, where it binds and activates MMP-2. The activation of MMP-2 by membrane-associated MT-MMP also acts as a mechanism for directing the proteolytic activity of the enzyme to focal areas in the extracellular space (22,23).

The proteolytic activity of MMPs is inhibited by nonspecific protease inhibitors, such as α2-macroglobulin and α1-antitrypsin, and by the specific tissue inhibitors of the metalloproteinases (TIMPs) (2). The TIMPs are a family of four structurally related proteins (TIMP-1, -2, -3, and -4), which exert a dual control on the MMPs by inhibiting both the active form of the MMPs and their activation process. The TIMPs inhibit the enzymatic activity of all members of the MMP family (with the exception of MT1-MMP, which is inhibited by TIMP-2 and -3 but not by TIMP-1) by forming noncovalent stoichiometric complexes with the active zinc-binding site of the MMPs (24–27). In addition, the TIMPs inhibit the catalytic activation of many pro-MMPs, with various members of the TIMP family having preferential inhibitory capabilities against the different pro-MMPs. For example, TIMP-1 forms preferential complexes with pro-MMP-9, whereas TIMP-2 and TIMP-4 exhibit higher affinity for pro-MMP-2 (24,25).

The TIMPs are produced by a wide variety of cell types. However, whereas cultured cells express TIMP-2 constitutively, the expression of TIMP-1 is predominantly regulated at the level of transcription by various growth factors, including TGF-β, EGF, tumor necrosis factor (TNF)-α, cytokines (IL-1, IL-6, and IL-10), retinoids, and glucocorticoids (13,28–30). The TIMPs participate in complex biologic functions that extend beyond

![Figure 1](image.png)

**Table 1. Matrix metalloproteinase (MMP) family**

<table>
<thead>
<tr>
<th>Group</th>
<th>MMP</th>
<th>Substrate(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Collagenases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interstitial collagenase</td>
<td>MMP-1</td>
<td>Collagen types I, II, III, VII, and X</td>
</tr>
<tr>
<td>Neutrophil collagenase</td>
<td>MMP-8</td>
<td>Collagen types I, II, III, VII, and X</td>
</tr>
<tr>
<td>Collagenase-3</td>
<td>MMP-13</td>
<td>Collagen types I, II, III, VII, and X</td>
</tr>
<tr>
<td><strong>Gelatinases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatinase A</td>
<td>MMP-2</td>
<td>Gelatin types I, IV, V, and X; laminin V</td>
</tr>
<tr>
<td>Gelatinase B</td>
<td>MMP-9</td>
<td>Gelatin types I, IV, V, and X; laminin V</td>
</tr>
<tr>
<td><strong>Stromelysins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stromelysin-1</td>
<td>MMP-3</td>
<td>Collagen types III, IV, IX, and X; gelatin; pro-MMP-1; laminin; and proteoglycan</td>
</tr>
<tr>
<td>Stromelysin-2</td>
<td>MMP-7</td>
<td>Gelatin, fibronectin and pro-MMP-1</td>
</tr>
<tr>
<td>Stromelysin-3</td>
<td>MMP-11</td>
<td>Collagen types III, IV, IX, and X; gelatin, pro-MMP-1; laminin; and proteoglycan</td>
</tr>
<tr>
<td><strong>Membrane-type (MT) MMPs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT1-MMP</td>
<td>MMP-14</td>
<td>Pro-MMP-2, gelatin, and collagens</td>
</tr>
<tr>
<td>MT2-MMP</td>
<td>MMP-15</td>
<td>Pro-MMP-2</td>
</tr>
<tr>
<td>MT3-MMP</td>
<td>MMP-16</td>
<td>Pro-MMP-2</td>
</tr>
<tr>
<td>MT4-MMP</td>
<td>MMP-17</td>
<td>Not known</td>
</tr>
<tr>
<td><strong>Nonclassified MMPs</strong></td>
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<td></td>
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<tr>
<td>RASI-1</td>
<td>MMP-18/19</td>
<td>Not known</td>
</tr>
<tr>
<td>Enamelysin</td>
<td>MMP-20</td>
<td>Not known</td>
</tr>
<tr>
<td></td>
<td>MMP-23/24</td>
<td>Not known</td>
</tr>
</tbody>
</table>

**Fig. 1.** General structure of the matrix metalloproteinases (MMPs). The general structure of the MMPs includes a signal peptide, a propeptide domain, a catalytic domain with a highly conserved zinc-binding site, and a haemopexin-like domain linked to the catalytic domain by a hinge region. MMP-2 and MMP-9 contain fibronectin type II inserts within the catalytic domain. The membrane-type MMPs (MT-MMPs) contain a transmembrane domain at the C-terminal end of the haemopexin-like domain. The haemopexin domain is absent in the smallest MMP, matrilysin (MMP-7). [Modified with permission from (2)].
their roles as inhibitors of MMP activity. For example, they induce changes in cell morphology, stimulate growth of several cell types, promote steroidogenesis and germ cell development, and inhibit angiogenesis (31–34). Paradoxically, TIMP-2 is involved in the activation of MMP-2 by acting as an adaptor molecule between pro-MMP-2 and MT1-MMP (35).

**ROLE OF MMPs IN TUMOR GROWTH, INVASION, AND METASTASIS**

Tumor growth, invasion, and metastasis are a multistep and complex process that includes cell division and proliferation, proteolytic digestion of the extracellular matrix, cell migration through basement membranes to reach the circulatory system, and remigration and growth of tumors at the metastatic sites (4,36,37).

The proposed role of MMPs in this process is based on *in vitro* and *in vivo* preclinical studies as well as on studies of clinical specimens. MMPs degrade the basement membrane and extracellular matrix, thus facilitating the invasion of malignant cells through connective tissues and blood vessel walls and resulting in the establishment of metastases (1). In rat embryo fibroblasts, overexpression of MMP-9 degraded the matrix, resulting in enhanced metastatic potential (38,39). In addition, transfection of MMP genes into malignant human tumor cell lines, such as the MYV3L rat bladder carcinoma, Madison 109 mouse lung carcinoma, and DU-145 human prostate carcinoma, increased the number and size of pulmonary metastasis in *in vivo* studies (40–42). Tumors in knockout mice lacking specific MMPs exhibit reduced tumorigenesis, angiogenesis, and tumor progression (43–45). The degradation of the extracellular matrix by MMPs not only facilitates metastasis but also promotes tumor growth by increasing the bioavailability of growth factors that reside in the extracellular matrix and are released during extracellular matrix degradation (14,46,47).

MMP expression, although low or undetectable in most normal tissues, is substantially increased in the majority of malignant tumors. Numerous studies (36,48–54) in a variety of tumor types, including lung, colon, breast, and pancreatic carcinomas, demonstrate overexpression of MMPs in malignant tissues in comparison to adjacent normal tissues. In addition, the plasma and urine levels of MMPs are elevated in patients with cancer compared with healthy subjects (55). The MMPs in tumor tissues are produced not only by malignant tumors but also by stromal fibroblasts and inflammatory cells (56). These cells may produce cytokines and proteins that induce the production of MMPs by surrounding cells, creating extracellular networks of MMP secretion and activation (57–59). In addition, parallel analyses of tissue samples spanning the process from normal tissue to tumor formation have demonstrated that overexpression of MMPs is a feature of progression to the malignant phenotype (55,60–62). Furthermore, analyses of cellular components derived from primary tumor tissues or their corresponding lymph node metastases demonstrated increased expression of MMPs in the metastatic tissue, indicating that MMP expression is a component of the metastatic process (51,63,64). In addition to the well-documented overproduction and activation of MMPs in malignant tissue, there is now ample clinical evidence that overproduction of these molecules confers a poor prognosis in patients with a variety of malignancies (65–67).

Whether specific members of the MMP family are associated with oncogenesis is a matter of debate and varies among the tumor types and stage of lesions studied. Some of this variability can be attributed to the different experimental conditions and techniques used in different studies. In general, the gelatinases (MMP-2 and MMP-9) have been most consistently detected in malignant tissues and associated with tumor aggressiveness, metastatic potential, and a poor prognosis. More recently, matrilysin (MMP-7) has been the focus of attention because its preferential expression in early-stage tumors and premalignant lesions may make it a suitable target for chemopreventative strategies (43,68).

The role of the MMP family in tumor development is further complicated by the balance of these proteins in relation to TIMPs (25). The TIMPs have various biologic functions that are, in general, antioncogenic, and the expression of the TIMPs has been associated with less aggressive tumor behavior and favorable prognosis in patients with cancer (25). For example, the exposure of mouse fibroblasts to TIMP-1 and -2 *in vitro* inhibited oncogenic transformation by oncogenic viruses, whereas the administration of recombinant TIMP-1 to mice injected with B16F10 melanoma cells reduced the number of pulmonary metastases (69,70). Furthermore, transgenic mice overexpressing TIMPs displayed resistance to the establishment of intravenously injected malignant cells; conversely, exposure of mouse fibroblasts to TIMP-1 antisense oligonucleotides resulted in the formation of metastatic tumors in nude mice given an injection of malignant cells (71). Of interest, overexpression of TIMP-3 induces apoptosis in various types of malignant cells, suggesting that TIMPs may play a role in tumor cell death (25). Thus, the role of TIMPs *in vivo* is complex, and the expectation that malignant tumors have increased MMP expression accompanied by decreased TIMP expression is probably too simplistic (72–79).

**INHIBITION OF MMPs AS ANTICANCER THERAPY**

If, as the preclinical and clinical data suggest, MMPs play a pivotal role in the process of malignant progression, then pharmacologic inhibition of MMP activity could markedly inhibit the invasiveness of primary and metastatic tumors and, therefore, be of therapeutic benefit to patients with cancer. MMP expression and activation involve multiple steps—transcription of MMP genes, secretion of the zymogen into the extracellular matrix, and activation of the zymogen—several of which are amenable to pharmacologic intervention (Fig. 2). For example, inhibition of signal transduction transmitted through the MAP kinases markedly inhibits the expression of MMPs and the invasive potential of cancer cell lines (13,80,81). Cancer cell lines treated with specific inhibitors of the MAP kinases, such as PD 98059 and SB 203560, or other nonspecific tyrosine kinase inhibitors, such as PD 166285, have reduced expression of MMP *in vitro* (13,80,81).

A second approach to the inhibition of MMP expression is via the use of specific antisense oligonucleotides. This strategy has been studied in preclinical models of colon cancer using an antisense phosphorothioate oligodeoxyribonucleotide to MMP-7, an MMP that has been implicated in the progression of colon cancer in experimental models (82). When nude mice bearing the human WiDr colon cancer cell line xenograft were treated with this compound, basement membrane penetration was inhibited and the development of liver metastases was suppressed. A potential advantage of antisense strategies is their selectivity
for a specific MMP subtype, thereby potentially resulting in fewer systemic toxic effects.

At present, inhibition of the function of MMPs in the extracellular matrix is being most actively pursued for anticancer therapy. The naturally occurring inhibitors of MMP activity (TIMPs) were the first compounds to be considered for clinical development. Theoretically, the ability of TIMPs to potently and specifically inhibit the activity of several MMPs could result in a beneficial therapeutic effect (83,84). However, the lack of effective methods of systemic gene delivery has limited the clinical utility of this approach, whereas the development of synthetic inhibitors of MMPs has been actively pursued and widely tested in clinical trials (Table 2) (5–7,85). Inhibitors of MMPs fall into three pharmacologic categories: 1) collagen peptidomimetics and nonpeptidomimetics, 2) tetracycline derivatives, and 3) bisphosphonates.

**Peptidomimetic MMP Inhibitors**

These compounds are pseudopeptide derivatives that have been synthesized to mimic the structure of collagen at the site where MMP binds to cleave it. The inhibitor binds reversibly at the active site of the MMP in a stereospecific manner and chelates the zinc atom on the enzyme activation site (86). Several zinc-binding groups have been tested for their ability to competitively inhibit MMP by binding at the active site; these groups include carboxylates, aminocarboxylates, sulfhydryls, derivatives of phosphoric acid, and hydroxamates. Most MMP inhibitors in clinical development are hydroxamate derivatives.

**Batimastat.** Batimastat, the first MMP inhibitor evaluated in cancer patients, is a nonorally bioavailable low-molecular-weight hydroxamate. This compound is potent but relatively nonselective, with IC_{50} values of less than 10 ng/mL for MMP-1, -2, -3, -7, and -9 inhibition. In vitro, batimastat had cytostatic effects against a variety of cancer cell lines and was not cytotoxic (7). Batimastat also induced significant antiproliferative effects in several preclinical models of cancer, including inhibition of tumor growth in orthotopic tumor xenografts model, inhibition of metastasis in experimental metastasis models, and suppression of ascites formation in ovarian cancer models (87–96). Early-stage tumors were more sensitive to batimastat than tumors at later stages of development. The drug also potentiated the growth-suppressive activity of cisplatin and demonstrated synergistic antitumor effects with docetaxel and captopril (97,98).

Because of its poor solubility, batimastat was administered intraperitoneally and intrapleurally for evaluation in clinical trials in cancer patients (99–101). Table 3 summarizes the principal results from clinical trials with this agent. Batimastat is relatively well tolerated, except in patients without malignant ascites, in whom intraperitoneal administration of the agent resulted in significant abdominal pain (99). Systemic toxicity has been mild and of no clinical consequences. Plasma levels of batimastat peaked 24–48 hours after intracavitary administration and were characterized by a prolonged elimination half-life of approximately 3–4 weeks (99–101). Batimastat treatment also resulted in beneficial effects in the majority of patients with pleural effusion who were treated with the drug, as measured by dyspnea scores and a reduction in the requirement for thoracentesis (100).

**Marimastat.** Marimastat is a synthetic low-molecular-weight MMP inhibitor that, in contrast to batimastat, is orally bioavailable, with an absolute bioavailability of 20%–50% in preclinical studies. The compound is a nonselective, synthetic, low-molecular-weight hydroxamate. Marimastat is a synthetic nonpeptide inhibitor of MMP-1, -2, -3, -7, and -9 (5–7,99). In vitro, marimastat had cytostatic effects against a variety of cancer cell lines and was not cytotoxic (7). Marimastat also induced significant antiproliferative effects in several preclinical models of cancer, including inhibition of tumor growth in orthotopic tumor xenografts model, inhibition of metastasis in experimental metastasis models, and suppression of ascites formation in ovarian cancer models (87–96). Early-stage tumors were more sensitive to marimastat than tumors at later stages of development. The drug also potentiated the growth-suppressive activity of cisplatin and demonstrated synergistic antitumor effects with docetaxel and captopril (97,98).

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**Table 2. Matrix metalloproteinase (MMP) inhibitors in clinical development**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Class</th>
<th>Specificity</th>
<th>Company</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG3340</td>
<td>Nonpeptidomimetic inhibitor</td>
<td>MMP-2 and -3</td>
<td>Agouron Pharmaceuticals (San Diego, CA)</td>
<td>Phase II/III</td>
</tr>
<tr>
<td>BAY 12-9566</td>
<td>Nonpeptidomimetic inhibitor</td>
<td>MMP-2 and -3</td>
<td>Bayer Corporation (Pittsburgh, PA)</td>
<td>Development halted</td>
</tr>
<tr>
<td>BMS-275291</td>
<td>Nonpeptidomimetic inhibitor</td>
<td>MMP-2 and -9</td>
<td>Bristol-Myers Squibb (New York, NY)</td>
<td>Phase I</td>
</tr>
<tr>
<td>CGS 27023A</td>
<td>Nonpeptidomimetic inhibitor</td>
<td>Broad spectrum</td>
<td>Novartis Pharmaceuticals (East Hanover, NJ)</td>
<td>Phase I</td>
</tr>
<tr>
<td>Batimastat</td>
<td>Peptidomimetic inhibitor</td>
<td>MMP-1, -2, -3, -7, and -9</td>
<td>British Biotech (Oxford, U.K.)</td>
<td>No further development</td>
</tr>
<tr>
<td>Col-3 (metastat)</td>
<td>Chemically modified tetracycline</td>
<td>MMP-2 and -9</td>
<td>Collagenex (Newtown, PA)</td>
<td>Phase I</td>
</tr>
</tbody>
</table>
studies. The drug contains a collagen-mimicking hydroxamate structure that chelates the zinc ion at the active site of MMPs. Like batimastat, marimastat is relatively nonspecific, inhibiting the activity of MMP-1, -2, -3, -7, and -9 with IC_{50} values of 2.5, 3, 115, 8, and 1.5 ng/mL, respectively (6). Preclinical studies (6) of marimastat against lung and breast cancer experimental metastases models demonstrated a reduction in the number and size of metastatic foci in treated versus control animals. In preclinical toxicology studies (102) at doses of 100–500 mg/kg per day, the agent induced gastrointestinal toxicity and weight loss, as well as hemorrhage, fibrosis, inflammation, and necrosis at peripheral ankle and knee tissues.

The initial phase I studies of marimastat were performed in healthy volunteers. Single oral doses of up to 800 mg were well tolerated and did not result in appreciable toxicity. The pharmacokinetics of marimastat were linear, with dose-proportional increases in plasma concentrations and in area under the concentration-versus-time curve (AUC). Peak plasma concentrations were detectable within 1.5–3 hours after oral administration, and the estimated elimination half-life range was 8–10 hours. Continuous administration of oral doses of 50–200 mg twice a day for 6 consecutive days did not result in plasma accumulation. At the highest doses, minor, transient elevations in liver function tests were observed in some individuals without clinical sequelae (103).

Marimastat has subsequently been evaluated in several phase I–II studies administered on a continuous oral schedule at doses of 2–100 mg twice a day (Table 4), either alone or in combination with chemotherapy (102,104–112). The principal toxic effect of marimastat in these studies was the appearance of a dose-limiting inflammatory polyarthritis that typically appeared during the first month of treatment and persisted for longer than 8 weeks after treatment was discontinued (102). The occurrence of this musculoskeletal toxic effect was related to marimastat dose, substantially limiting administration of the agent at doses more than 10 mg twice a day (111). Pharmacologic studies demonstrated that marimastat is well absorbed from the gastrointestinal tract and exhibits a linear pharmacokinetic behavior. Doses exceeding 10 mg twice a day resulted in minimum plasma concentrations that were sixfold greater than those required for MMP inhibition in vitro. However, recent preliminary data indicate that, at doses of 5–10 mg twice a day, plasma concentrations of marimastat ranged from 7 to 13 ng/mL, well below the target range for biologic activity (40–200 ng/mL), and that doses of 25–50 mg twice a day may be required to achieve biologically relevant concentrations (112,113). However, the relationship between plasma concentrations and in vitro IC_{50} values for MMPs must be interpreted with caution because total plasma concentrations reflect the total amount of drug (both protein bound and non-protein bound) in plasma and not the unbound—that is, active—fraction. In addition, it is not known how these plasma (i.e., circulating) drug concentrations correspond to inhibitory effects on MMPs at the tumor tissue.

The early efficacy trials of marimastat represented a departure from the standard response-based trials of cytotoxic agents. Because the preclinical information on this compound suggested predominantly cytostatic effects, the studies were performed in patients with rapidly rising serologic tumor markers (i.e., elevation >25% in the 4 weeks before study entry). Biologic response was assessed according to the rate of rise of the tumor marker before and after initiating therapy. For example, a complete biologic effect was deemed to be present if the tumor marker did not rise above the pretreatment value, whereas a partial biologic effect was demonstrated by a less than 25% increase over pretreatment values during the first 4 weeks after treatment.

With the use of this study design, a total of 415 patients with tumors of the colon, pancreas, ovary, and prostate have been treated with marimastat in different phase II trials (104,105,114). Unfortunately, compliance in these studies was poor, with only 54% of the patients assessable for a response to therapy. Nevertheless, the combined results of these trials demonstrated that marimastat exerted a dose-related inhibitory effect on the rate of tumor marker elevation, with maximal effects at doses greater than 20 mg twice a day. These results should be interpreted with caution, however, because the relationship between the biologic responses as defined in this study and conventional endpoints, such as progression-free and overall survival, have not been defined. As is often seen in conventional response-based studies, longer survival was observed in patients who achieved a partial or complete biologic response to therapy (114).

Marimastat is currently undergoing phase III clinical trials, both alone and in combination with classic chemotherapy, in
<table>
<thead>
<tr>
<th>Investigator(s) (reference No.)</th>
<th>Regimen</th>
<th>Dose</th>
<th>Tumor type</th>
<th>No. of pts</th>
<th>MTD</th>
<th>DLT</th>
<th>Activity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primrose et al. (104)</td>
<td>Single agent</td>
<td>5, 10, and 25 mg od 10, 25, and 50 bid</td>
<td>Colon</td>
<td>70</td>
<td>20 mg od 25 mg bid</td>
<td>Musculoskeletal</td>
<td>27 of 55 (49%)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Greater BR in pts treated with higher doses and the twice-a-day schedule; mean C&lt;sub&gt;min&lt;/sub&gt; concentrations ranged from 12.6 to 286.2 ng/mL.&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rosemurgy et al. (105)</td>
<td>Single agent</td>
<td>10 and 25 mg od 5, 10, 25, 50, and 75 mg bid</td>
<td>Pancreas</td>
<td>64</td>
<td>5, 10, and 25 mg bid</td>
<td>Musculoskeletal</td>
<td>5.3-mo median Sv 21% 1-yr Sv</td>
<td>Doses of 5, 10, and 25 mg twice a day achieved better BR; mean C&lt;sub&gt;min&lt;/sub&gt; &gt;30 ng/mL at all dose levels;&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wojtowicz-Praga et al. (102)</td>
<td>Single agent</td>
<td>25, 50, and 100 mg bid</td>
<td>Lung</td>
<td>12</td>
<td>50 mg bid</td>
<td>Musculoskeletal</td>
<td>NA</td>
<td>Assessment of MMP-2 and -9 levels by zymography were uninformative; at the MTD, peak plasma concentration of marimastat averaged 196 ng/mL.&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bourthe et al. (106)</td>
<td>Single agent</td>
<td>10 and 100 mg bid</td>
<td>Melanoma</td>
<td>26</td>
<td>10 mg bid</td>
<td>Musculoskeletal</td>
<td>1/19 PR</td>
<td></td>
</tr>
<tr>
<td>Carmichael et al. (107)</td>
<td>Gemcitabine§</td>
<td>5, 10, 15, and 20 mg bid</td>
<td>Pancreas</td>
<td>31</td>
<td>NA</td>
<td>NA</td>
<td>2 of 11 PR; 9 pts had reduction in CA 19-9</td>
<td></td>
</tr>
<tr>
<td>O’Reilly et al. (108)</td>
<td>5-FU&lt;sup&gt;</td>
<td></td>
<td>&lt;/sup&gt;</td>
<td>5 and 10 mg bid</td>
<td>All</td>
<td>13</td>
<td>10 mg bid</td>
<td>NA</td>
</tr>
<tr>
<td>Adams and Thomas (109)</td>
<td>Carboplatin¶</td>
<td>2, 5, 10, 15, and 20 mg bid</td>
<td>Ovarian</td>
<td>31</td>
<td>NA</td>
<td>NA</td>
<td>8 of 25 responses (3 CR); 14 pts had CA125 reductions</td>
<td></td>
</tr>
<tr>
<td>Anderson et al. (110)</td>
<td>Paclitaxel/carboplatin#</td>
<td>10 and 20 mg bid</td>
<td>NSCLC</td>
<td>22</td>
<td>NA</td>
<td>NA</td>
<td>11 of 22 PR</td>
<td></td>
</tr>
<tr>
<td>Eisenberger et al. (112)</td>
<td>Single</td>
<td>2, 5, 10, 25, and 50 mg bid 2, 5, 10, and 25 mg od</td>
<td>Prostate</td>
<td>88</td>
<td>NA</td>
<td>NA</td>
<td>No responses</td>
<td></td>
</tr>
<tr>
<td>Miller et al. (113)</td>
<td>Single</td>
<td>5–10 mg bid for 12 mo</td>
<td>Breast</td>
<td>63</td>
<td>NA</td>
<td>NA</td>
<td>Adjuvant trial</td>
<td></td>
</tr>
</tbody>
</table>

*pts = patients; MTD = maximum tolerated dose; DLT = dose-limiting toxic effects; od = once a day; bid = twice a day; BR = biologic response; Sv = survival; C<sub>min</sub> = minimum steady-state concentration; NA = not available; PR = partial response; SD = stable disease; CR = complete response; NSCLC = non-small-cell lung cancer; C<sub>ss</sub> = plasma steady-state concentration.

<sup>†</sup>Activity defined as biologic response if the tumor marker rose by less than or equal to 0% during the 28-day marimastat treatment period or partial biologic response if the tumor marker rose by greater than 0% and less than 25%.

<sup>‡</sup>IC<sub>50</sub>s for inhibition of MMP-1, -2, -3, -7, and -9 were 2.5, 3, 115, 8, and 1.5 ng/mL, respectively.

<sup>§</sup>Gemcitabine dose was 1000 mg/m<sup>2</sup> on days 1, 8, and 15 every 28 days.

<sup>||</sup>5-Fluorouracil dose was 300 mg/m<sup>2</sup> by continuous infusion or 450 mg/m<sup>2</sup> per day by intravenous bolus plus leucovorin at 20 mg/m<sup>2</sup> per day daily for 5 days every 4 weeks.

<sup>¶</sup>Carboplatin dose was targeted to an area under the concentration-versus-time curve (AUC) of 6 mg · min/mL.

<sup>#</sup>Paclitaxel dose was 175–200 mg/m<sup>2</sup> over a 3-hour infusion; carboplatin dose was targeted to an AUC of 7 mg · min/mL.
patients with various solid tumor types (115). Overall survival of patients with advanced pancreatic cancer who were treated with marimastat was not better than that of patients treated with gemcitabine (116). However, patients with advanced gastric cancer who either had not received prior therapy or were stable after initial chemotherapy demonstrated a statistically significant improvement in disease-free and overall survival after treatment with marimastat than after treatment with placebo (117). This study is the first randomized trial supporting the use of MMP inhibitors in cancer treatment.

Nonpeptidic MMP Inhibitors

Problems with the peptidic MMP inhibitors include poor oral bioavailability (except for marimastat) and a relative lack of specificity for the MMPs thought to contribute to cancer progression (103). In an effort to avoid these problems, several nonpeptidic MMP inhibitors have been rationally synthesized on the basis of the three-dimensional x-ray crystallographic conformation of the MMP active site. Several of these molecules demonstrated antitumor activity in preclinical models and were selected for clinical development (Table 2). The rational chemical design of MMP inhibitors made possible the synthesis of compounds with specific inhibitory activity against the MMP subtypes that predominate in certain diseases, such as cancer and arthritis. For example, AG3340, BAY 12–9566, and BMS-275291 were designed to be relatively selective inhibitors of MMP-2, whereas Ro 32–3555 was designed to be specific for MMP-1, which is frequently associated with osteoarticular diseases, and is thus being developed for arthritis (7). AG3340, BAY 12–9566, BMS-275291, and CGS 27023A are currently undergoing clinical evaluation in cancer patients.

BAY 12–9566. BAY 12–9566 is an orally bioavailable biphenyl compound that is a potent inhibitor of MMP-2, -3, and -9, with an IC$_{50}$ below 0.13 µg/mL (7). In preclinical studies, BAY 12–9566 inhibited the migration of HT1080 human fibrosarcoma cells through artificial Matrigel basement membrane at a concentration of 0.04 µg/mL (118). In vivo, the administration of BAY 12–9566 to mice reduced angiogenesis and inhibited the formation of lung metastases (119). Furthermore, BAY 12–9566 induced tumor growth inhibition against several human tumor xenografts, including breast and colon carcinoma cell lines (120,121). In animal studies (122), administration of BAY 12–9566 was adequately tolerated and resulted in only mild hepatotoxicity, anemia, and renal toxicity. The compound was rapidly and substantially absorbed after oral administration, with an oral bioavailability of 70%–98%, and reached peak plasma concentrations at 0.5–2 hours after dosing, with evidence of enterohepatic recirculation (123). The pharmacokinetics of BAY 12–9566 in normal volunteers were linear at doses of up to 100 mg/day. Repeated administration of the drug resulted in increased clearance and thus a reduction in drug exposure (124).

BAY 12–9566 has completed phase I evaluations in patients with malignant disease, both alone and in combination with chemotherapy. Table 5 summarizes the major conclusions observed in these studies (125–129). The drug has been very well tolerated, resulting in only mild thrombocytopenia (which was more pronounced when the drug was administered in combination with carboplatin) and minor elevations in liver function tests, of no clinical consequences. No musculoskeletal toxic effects have been associated with BAY 12–9566. The recommended dose for phase II clinical trials derived from these studies was 800 mg twice a day because of the occurrence of non-dose-proportional increases in steady-state plasma concentrations (C$_{ss}$) at higher doses of the drug, indicating saturation of absorption (130,131).

BAY 12–9566 has subsequently been evaluated in phase III clinical trials in patients with pancreatic, ovarian, and lung cancers (115). However, preliminary results from these trials have been disappointing. Administration of BAY 12–9566 to patients with advanced pancreatic cancer resulted in statistically significantly inferior survival time and time to treatment failure when compared with gemcitabine, necessitating early termination of the study (132). In addition, an interim analysis of a phase III study that compared continuous administration of BAY 12–9566 with placebo in patients with small-cell lung cancer following response to conventional therapy has also been prematurely closed because of the inferior survival of patients treated with BAY 12–9566. On the basis of these results, clinical development of BAY 12–9566 has been suspended.

AG3340. AG3340 is a nonpeptidic collagen-mimicking MMP inhibitor that was synthesized by use of a protein structure drug design program. The drug inhibits MMP-2, -9, -3, and -13, with IC$_{50}$s of below 0.13 ng/mL (133). AG3340 has antitumor activity against a broad array of rodent tumor models after intraperitoneal and oral administration (134). In addition, the agent inhibited tumor growth and angiogenesis and increased apoptosis in xenograft models of prostate, colon, breast, and non-small-cell lung carcinomas (133,135,136). In these studies, the activity of AG3340 was optimized by dose fractionation and was not related to the total daily dose, peak plasma concentration, or AUC but rather to the maintenance of minimum effective plasma concentrations (135). AG3340 also demonstrated synergistic effects on inhibition of tumor growth and angiogenesis when combined with carboplatin and paclitaxel against the chemotherapy-resistant MV522 human non-small-cell lung cancer model. Furthermore, the agent decreased the number of pulmonary metastases in the B16F10 melanoma metastasis model (133). AG3340 is a low-molecular-weight compound that is lipophilic and crosses the blood–brain barrier. On the basis of these physical properties, the drug was explored in a human glioblastoma cancer model and demonstrated significant tumor growth inhibition, concomitant with prolonged animal survival (137). Like other MMP inhibitors, AG3340 inhibited the growth of implanted tumor but did not affect in vitro cell proliferation (133,137). The mechanism underlying this effect is not understood, but it has been postulated to be due to the inhibition of neoangiogenesis and/or of mitogenic factors anchored in the extracellular matrix that stimulate tumor growth in a paracrine fashion (137).

AG3340 was evaluated in phase I clinical trials as well as in phase I–II feasibility studies in combination with chemotherapy, and it is currently being evaluated in phase III randomized clinical trials in patients with non-small-cell lung and prostate cancers (Table 6) (138–140). The agent has been administered on a continuous oral dosing schedule at doses that ranged from 2 to 100 mg/day given in two doses per day. Although treatment with AG3340 did not result in severe dose-limiting toxicity, doses above 25 mg/day induced musculoskeletal effects that required dose discontinuation in more than half of the subjects. At this dose, AG3340 can be safely combined with mitoxantrone/ prednisone and carboplatin/paclitaxel, and these combinations are currently being tested in randomized placebo-controlled
concentration; PK/H11505

Erlichman D Wilding Goel Grochow inhibited the growth of B16F10 murine melanoma and reduced antiangiogenic effects in several assays against MMP-2 and MMP-9. Studies, BMS-275291 demonstrated potent inhibitory activity (141).

Erlichman et al. (127) Continuous oral dosing 400 mg od 400 mg bid and tid 800 mg bid All 11 NA NA NA No changes in plasma levels of VEGF, pyridinoline, and deoxypyridinoline

Goel et al. (126) Continuous oral dosing 100–400 od 400 mg bid, tid, and qid 800 mg bid All 29 400 bid Liver dysfunction and thrombocytopenia No objective responses; 18 of 29 patients had SD Recommended phase II dose 800 mg bid based on saturation of absorption at higher doses; Csa >100 μg/mL was achieved at doses of 800 mg bid

Grochow et al. (128) Continuous oral dosing 100–400 od 1600 mg divided doses All 26 NA NA 11 of 26 pts had SD for longer than 4 mo Csa ranged from 41 to 150 μg/mL, and did not increase proportionally with dose

Tolcher et al. (129) Combined with paclitaxel† with paclitaxel/carboplatin‡ or with carboplatin¶ 800 mg bid All 19 NA NA Thrombocytopenia in the BAY 12-9566/ carboplatin combination NA No pharmacokinetic interaction between Bay 12-9566 and any of the chemotherapy regimens

Table 5. Phase I and II trials of BAY 12-9566*

Investigator(s) (reference No.) Regimen Dose Tumor type No. of pts MTD DLT Activity Comments
Rowinsky et al. (125) Continuous oral dosing 100 and 400 mg od 400 mg bid, tid, and qid 800 mg bid All 28 NA NA NA No consistent effect on plasma concentrations of MMPs; recommended dose 800 mg bid based on saturation of absorption at higher doses; at the recommended dose, Csa averaged 141.86 ± 53.57 μg/mL.

Erlichman et al. (127) Continuous oral dosing 400 mg od 400 mg bid and tid 800 mg bid All 11 NA NA NA No changes in plasma levels of VEGF, pyridinoline, and deoxypyridinoline

Goel et al. (126) Continuous oral dosing 100–400 od 400 mg bid, tid, and qid 800 mg bid All 29 400 bid Liver dysfunction and thrombocytopenia No objective responses; 18 of 29 patients had SD Recommended phase II dose 800 mg bid based on saturation of absorption at higher doses; Csa >100 μg/mL was achieved at doses of 800 mg bid

Grochow et al. (128) Continuous oral dosing 100–400 od 1600 mg divided doses All 26 NA NA 11 of 26 pts had SD for longer than 4 mo Csa ranged from 41 to 150 μg/mL, and did not increase proportionally with dose

Tolcher et al. (129) Combined with paclitaxel† with paclitaxel/carboplatin‡ or with carboplatin¶ 800 mg bid All 19 NA NA Thrombocytopenia in the BAY 12-9566/ carboplatin combination NA No pharmacokinetic interaction between Bay 12-9566 and any of the chemotherapy regimens

*pts = patients; MTD = maximum tolerated dose; DLT = dose-limiting toxic effects; od = once a day; NA = not available; bid = twice a day; tid = three times a day; qid = four times a day; SD = stable disease; VEGF = vascular endothelial growth factor; Csa = plasma steady-state concentration.
†IC50 (concentration that causes 50% inhibition of enzyme activity) for MMP-2, -3, and -9 inhibition were less than 0.13 μg/mL, respectively.
‡Paclitaxel was given at 175 mg/m² for 3 hours and carboplatin was given to an area under the concentration-versus-time curve (AUC) of 6 mg.min/mL.
¶Paclitaxel was given at 175 mg/m² for 3 hours and carboplatin was given to an area under the concentration-versus-time curve (AUC) of 6 mg.min/mL.
§Paclitaxel was given at 175 mg/m² for 3 hours and carboplatin was given to an area under the concentration-versus-time curve (AUC) of 6 mg.min/mL.

Table 6. Phase I and II trials with AG3340*

Investigator(s) (reference No.) Dose Tumor type No. of pts MTD DLT Activity Comments
Hande et al. (138) 10–100 mg bid All 45 10–25 mg Musculoskeletal 1 PR in melanoma and non-small-cell lung cancer tmax 2–3 h, linear PK; t1/2 2–3 h
Wilding et al. (139)† 25 mg bid starting on day 15 of the first course Prostate 15 NA NA 4 pts had PD on the first evaluation
D’Olimpo et al. (140)‡ 25 mg bid starting on day 15 of the first course All 15 NA NA NA

*pts = patients; MTD = maximum tolerated dose; DLT = dose-limiting toxicity; bid = twice a day; NA = not available; tmax = time to peak plasma concentration; PK = pharmacokinetics; t1/2 = half-life; NA = not available; PD = progressive disease.
†Mitoxantrone was also given intravenously at 12 mg every 3 weeks, and prednisone was given at 5 mg twice a day.
‡Carboplatin was given to an area under the time-versus-concentration curve of 6 mg.min/mL, and paclitaxel was given at 200 mg/m².

phase III clinical trials as first-line therapy for patients with hormone-refractory prostate cancer and non-small-cell lung carcinoma, respectively (139,140).

BMS-275291. BMS-275291 is an orally bioavailable MMP inhibitor in phase I clinical development (141). In preclinical studies, BMS-275291 demonstrated potent inhibitory activity against MMP-2 and MMP-9. In vitro BMS-275291 exhibited antiangiogenic effects in several assays (141), and in vivo it inhibited the growth of B16F10 murine melanoma and reduced the size and metastases of the rat HOSP-1 mammary carcinoma (141). More important, this compound does not cleave the extracellular domain of the TNF receptor, which is thought to be responsible for some of the musculoskeletal effects of nonpeptide MMP inhibitors (141). Indeed, in the marmoset monkey, BMS-275291 did not induce joint inflammation toxicity. The drug has completed a phase I dose escalation study in normal volunteers and is currently in phase I clinical trials in patients with advanced cancer (141).
CGS-27023A. CGS-27023A is a broad-spectrum inhibitor of MMPs. The agent does not inhibit the proliferation of cancer cell lines in vitro, but it does inhibit the invasion of tumor cells through matrigel as well as angiogenesis in various preclinical models (142). For example, it inhibited the matrigel invasion of HT 1080 tumor cells and human umbilical vein endothelial cells and substantially reduced the formation of blood vessels induced by platelet-derived growth factor and bFGF in a murine subcutaneous implant model (142). In vivo, CGS-27023A administered orally inhibited the growth of a broad range of subcutaneous human tumor xenografts and reduced the formation of lung metastasis in the B16F10 melanoma experimental metastasis model (143). The combination of CGS-27023A with conventional cytotoxic agents resulted in additive or synergistic effects (142). CGS-27023A has been evaluated in a phase I clinical trial administered orally on a continuous dosing schedule at doses ranging from 150 to 600 mg in divided doses (144). The major toxic effects, which were encountered at doses exceeding 300 mg twice a day, consisted of cutaneous and musculoskeletal toxicity. Pharmacokinetic analysis revealed that administration of CGS-27023A at clinically tolerable doses yielded plasma concentrations that were several-fold greater than the in vitro IC_{50}s for MMP-2, -3, and -9 and were sustained for longer than 10 hours after dosing.

Tetracycline Derivatives

The tetracycline derivatives inhibit not only the activity but also the production of MMPs and are thus being investigated for the treatment of disorders in which the MMP system becomes amplified, such as degenerative osteoarthritis, periodontitis, and cancer (145–149). This family of agents comprises both the classic tetracycline antibiotics, such as tetracycline, doxycycline, and minocycline, and as the newer tetracycline analogues that have been chemically modified to eliminate their antimicrobial activity (e.g., removal of the dimethylamino group from carbox-4 of the “A” ring). These agents inhibit the collagenases, MMP-1, -3, and -13, and the gelatinases, MMP-2 and -9, via multiple mechanisms (145,150), including 1) blocking the activity of mature MMPs by chelation of the zinc atom at the enzyme binding site, 2) interfering with the proteolytic activation of pro-MMP into their active form, 3) reducing the expression of MMPs, and 4) protecting MMPs from proteolytic and oxidative degradation (145,151). Some tetracycline derivatives have been evaluated in preclinical cancer models and have entered early clinical trials in patients with malignant diseases, including doxycycline and Col-3.

Doxycycline. Doxycycline is one of the classic antimicrobial tetracyclines that has been studied extensively in patients as an anticancer agent (147,152). Like related compounds, doxycycline exerts diverse inhibitory effects on MMP production and activity (153). In cultured MDA-MB-435 cancer cell lines, doxycycline inhibited the secretion of MMP-2 and -9 and noncompetitively inhibited their activity (154). Furthermore, in vitro, doxycycline inhibited the proliferation of the U2OS osteosarcoma, PC-3 prostate, and MDA-MB-435 breast cancer cell lines; it also induced apoptosis and decreased the invasion and metastatic potential of the MDA-MB-435 breast cancer and B16F10 melanoma cell lines at concentrations of 5–10 μg/mL (18,146,147,154–156). In vivo, the inhibitory effects of doxycycline on breast cancer tumor metastasis formation was potentiated by the addition of batimastat, suggesting that targeting MMPs through multiple distinct pathways may improve treatment efficacy (157). In phase I evaluation studies of cancer patients, oral doses of 400 mg administered twice a day resulted in dose-limiting toxicity that consisted of fatigue, confusion, nausea, and vomiting. At the maximum tolerated dose of 300 mg twice a day, mean through plasma concentrations were comparable to those associated with antiangiogenic effects in vivo (158).

Col-3 (metastat). The chemically modified tetracyclines (CMTs) comprise a group of at least 10 analogues (CMT-1 to -10) that differ in their MMP specificity and potency. CMTs have several potential advantages over conventional tetracyclines: Their long-term systemic administration does not result in gastrointestinal toxicity, they reach greater plasma concentrations, and they have a longer elimination half-life, requiring less frequent drug administration. CMT-3, also known as Col-3 (metastat), is one of the most potent CMTs studied to date and has demonstrated antitumor activity both in vitro and in vivo. In vitro, Col-3 inhibited the expression and activity of MMP-2 and MMP-9 in cancer cell lines. Moreover, Col-3 inhibited proliferation of human prostate PC-3 and DU-145 cancer cell lines with an IC_{50} of 12 μg/mL, and it abrogated their invasiveness into Matrigel at concentrations of 3–5 μg/mL (146). In experiments using the rat subcutaneous Dunning MAT LyLu prostate cancer model, Col-3 inhibited tumor growth and reduced lung and bone metastases (146,159). It also induced apoptosis in the Dunning MAT LyLu prostate cancer cell line at concentrations of 10 μg/mL (146).

On the basis of these preclinical studies, Col-3 is currently being evaluated in phase I clinical trials in cancer patients treated by oral administration on a continuous dosing schedule (160,161). The agent seems to be well tolerated at doses of up to 70 mg/m^2 per day, with photosensitivity and fatigue being the most frequently encountered side effect. Preliminary pharmacologic analysis indicates that Col-3 has a prolonged elimination half-life and that, at clinically tolerable doses, steady-state plasma concentrations exceed the IC_{50} required for in vitro activity (160,161). In addition, evaluation of several biologic correlative studies (160,161) suggests that, at the doses and plasma concentrations achieved in clinical trials, Col-3 reduces the plasma concentrations of MMP-2 and MMP-9 as well as the production of MMP-9 by peripheral blood mononuclear cells cultured ex vivo.

Bisphosphonates

The bisphosphonates are a class of drugs developed during the last three decades for use in disturbances of calcium homeostasis and, more recently, for the palliation and prevention of bone metastases in patients with breast cancer and multiple myeloma (162,163). These agents are synthetic compounds with a high affinity for the hydroxyapatite crystal of bone. Their mechanism of action has not been completely elucidated, but it probably involves direct inhibition of osteoclast function, incorporation into the skeletal matrix interfering with bone resorption, as well as direct inhibition of osteoclast cytokine production (164). The agent exerts varied inhibitory effects on MMPs, including inhibition of their enzymatic activity. In addition, these agents inhibited TGF-β1-induced MMP-2 secretion in PC-3 prostate cancer cell lines, resulting in inhibition of collagen degradation (165,166). Clodronate, one of the most frequently used bisphosphonates, also inhibited the expression of the MT1-MMP.
protein and messenger RNA in the HT1080 fibrosarcoma cell line and decreased the invasion of C8161 melanoma and HT1080 fibrosarcoma cell lines through artificial basement membranes at IC_{50}s ranging from 10 to 35 μg/mL (167). It is, therefore, possible that some of the beneficial clinical effects of the bisphosphonates may be a consequence of their impact on the MMP system. The fact that these agents have been used clinically for a number of years and are well known both pharmacologically and toxicologically suggests that they should be investigated in properly designed clinical trials in patients with cancer or other diseases for which inhibition of MMPs may be beneficial.

**Clinical Development of MMP Inhibitors**

The clinical development strategies of MMP inhibitors, similar to those of other novel targeted agents that are thought to induce predominantly antiproliferative, rather than directly cytotoxic, effects, pose considerable difficulties. The paradigms used for the development of classic cytotoxic compounds may not be appropriate for this new class of agents, and alternative clinical strategies may be required in all aspects of clinical development, from dose escalation to phase III clinical trials (168).

One difficulty pertains to the design of dose escalation studies and the selection of doses for subsequent efficacy evaluations. The classical endpoint of phase I clinical trials has been the definition of the maximum tolerated dose of an agent. However, the objective of phase I evaluations of MMP inhibitors should instead be the definition of a dose that is suitable for continuous oral administration and results in sustained plasma concentrations that exceed the inhibitory concentrations for MMPs in vitro with tolerable toxicity. Currently, biologic correlative studies in phase I clinical trials are being incorporated to determine whether the desired pharmacologic effect (i.e., MMP inhibition) is occurring in vivo and whether this effect relates to dose, plasma concentration, and clinical parameters. Unfortunately, none of the studies reported to date have been able to demonstrate consistent, reproducible, in vivo biologic effects of MMP inhibition, possibly because of the paucity of assays of MMP inhibition that are technically feasible using patient specimens. Moreover, in addition to using plasma as a surrogate fluid to monitor MMP inhibition in clinical trials, MMPs could be analyzed in other tissues, such as circulating blood cells or periodontal fluids (169–173).

The principal question remaining at the completion of phase I clinical trials is whether the agent should be moved immediately to randomized phase III clinical trials, either alone or in combination with standard therapy, or whether phase II trials are necessary. Although randomized phase III clinical trials represent the ultimate proof of efficacy, they require large numbers of patients and expensive resources, which may be difficult to justify in the absence of established efficacy or biologic effect. However, typical single-arm phase II trials of MMP inhibitors may not be useful because the absence of direct cytotoxicity may not result in objective tumor responses. To circumvent this difficulty, novel endpoints for assessing the efficacy of these agents in phase II trials must be identified.

Two areas are of particular interest in this context. First, biologic imaging modalities, such as magnetic resonance imaging and positron emission tomography, may be used to measure tumor blood flow and angiogenesis, glucose uptake, and thymidine metabolism and thus could noninvasively detect changes in tumor biology rather than simply a geometric reduction in tumor size (168). Second, novel clinical trial designs are needed to properly capture the potential beneficial effects of these compounds. Proposed designs include the use of parameters of tumor progression (e.g., time to treatment failure on the experimental agent versus time to treatment failure on the previous standard agent; proportion of patients with early progressive disease) (174,175) instead of response rate as the principal endpoint in phase II trials, randomized instead of single-arm phase II trials, and randomized discontinuation trials. In the latter trial design, all patients are treated with an investigational drug; after 2–4 months of treatment, those patients who are not removed from the study because of disease progression, toxicity, or lack of compliance are randomly assigned to receive either continued drug treatment or a placebo. The principal advantage of this design is that it enriches the randomized population and substantially reduces the number of patients who need to be enrolled (176).

A final issue is the optimum design of phase III trials. Phase III trial designs used thus far with MMP inhibitors include direct comparison of an MMP inhibitor to standard therapy in patients with advanced disease, the combination of an MMP inhibitor with standard therapy versus standard therapy alone in patients with metastatic or locally advanced disease, and comparison of an MMP inhibitor to placebo in patients with minimal residual disease after standard therapy or as adjuvant treatment in patients with completely resected disease. All of these designs have potential advantages and disadvantages. However, the available preclinical data suggest that the antitumor activity of this class of compounds may be optimized when used in situations of minimal disease and that combinations of MMP inhibitors with conventional chemotherapy and/or radiation therapy may result in additive or synergistic antitumor effects. Incorporating these data, along with recent preliminary results from phase III trials, in subsequent phase III strategies will allow MMP inhibitors to be tested in settings that are consistent with their hypothesized biologic effects.

**Future Directions**

As we have discussed in this review, the MMP inhibitors represent a new class of potential anticancer agents that are currently undergoing intensive clinical evaluation in a variety of malignant diseases. Many of these compounds have successfully completed early clinical trials and are currently being assessed in definitive randomized clinical trials. The results of these studies, which should be available in the near future, will determine whether currently available compounds result in clinically meaningful antitumor effects. Additional areas being investigated include the development of more specific inhibitors, the refinement of clinical trial design, and the development of treatment strategies that combine MMP inhibitors with standard cytotoxic agents or other biologic agents.

Many new MMP inhibitors have been rationally synthesized on the basis of a greater understanding of the MMP spatial structure and catalytic center configuration, and several pharmaceutical and academic laboratories are actively engaged in the development of these compounds (177–183). The new inhibitors are characterized by their specificity toward subtypes of MMPs that result in distinct toxicity and efficacy profiles (184). For example, the inhibition of MMP-1 has been associated with the development of musculoskeletal toxic effects (185). Thus,
agents such as BAY 12–9566 were developed that lack MMP-1 inhibitory activity. Furthermore, the development of musculoskeletal toxic effects has been associated with inhibition of shedding of the extracellular domain of the TNF receptor from synovial membranes by the MMP inhibitors. Newer inhibitors that lack this so-called antisheddase activity are anticipated to be less toxic. Another example of specific targeting of a particular MMP is the development of MMP-7 inhibitors as chemopreventive agents. MMP-7 has been implicated in the early steps of tumor development; thus, specific inhibitors of this protein could reduce tumor development (43). However, although the development of specific MMP inhibitors may result in more effective compounds, the MMP system is extremely complex, and it is not yet clear which subtypes should be targeted therapeutically to achieve antitumor effects.

A new and interesting area of MMP inhibitor research relates to the ability of these compounds to inhibit the shedding of membrane-bound proteins (186). Many membrane receptors and proteins, including TGF-α (the EGF receptor), Her2/Neu (the folic acid receptor), CD40, and CD95, are cleaved and shed into the extracellular space by MMPs. MMP inhibitors have been shown to inhibit the shedding of these molecules. As mentioned above, this property may be associated with the development of side effects. However, depending on which plasma membrane protein or receptor shed is inhibited, this effect can also have therapeutic implications (187,188). For example, in vitro experiments in cancer cell lines have demonstrated that inhibition of the MMP that regulates the shedding of TGF-α decreased the release of TGF-α from the plasma membrane and, consequently, the paracrine activation of the EGF receptor resulting in cancer cell growth inhibition. This effect was potentiated by the addition of monoclonal antibodies to the EGF receptor, a result that may provide a rationale for combining both agents in clinical trials (189).

In conclusion, the MMPs represent an attractive target for cancer treatment, and a number of MMP inhibitor are undergoing clinical trials to establish whether any of these compounds are therapeutically useful. Independent of the conclusions from the first generation of studies, the field of MMP inhibitors remains attractive for creative and innovative research. The development of novel, less toxic, and more effective MMP inhibitors as well as the combination of conventional agents with these novel anticancer agents will constitute the main focus of future research efforts.

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NOTES

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