Matrix metalloproteinases (MMPs) are a family of enzymes involved in a number of normal cellular processes, such as regulation of endometrial growth and menstruation, and abnormal processes, such as tumor growth, invasion, and metastasis [1]. There are now 18 distinct enzymes that fall into three functional classifications based on their substrate target: collagenases which degrade fibrillar collagen; gelatinases which degrade denatured and basement membrane collagens, and stromelysins which degrade proteoglycans and glycoproteins. The MMPs can also be separated into five categories based on structural and functional similarities [2, 3].

There are several observations that associate MMPs with tumor progression and metastasis:

- The number of different types of MMPs that can be detected in a tumor tends to increase with the progression of the cancer.
- The relative level of individual MMPs tends to increase with increasing tumor stage.
- Transfection of selected MMPs into cancer cells can increase the generation of distant metastases in vivo.
- Inactivation of these same enzymes reduces the production of tumor metastases.

MMPs are considered to be key enzymes involved in angiogenesis, since degradation of basement membrane and stromal tissue is an essential process for migration of endothelial cells needed to form new blood vessels. They are also critical for the entry and exit of tumor cells into existing blood vessels that are necessary for metastasis. For these reasons, the MMPs represent an attractive therapeutic target.

Matrix metalloproteinases may be produced by the tumor cells themselves or by surrounding stromal cells when stimulated by nearby tumor cells [4]. These enzymes are tightly regulated through gene expression and secretion in an inactive, pro-enzyme form that must be cleaved to become active. The function of MMPs is further controlled by local concentrations of specific inhibitors known as tissue inhibitors of metalloproteinases (TIMPs).

Over the past five years, matrix metalloproteinase inhibitors (MMPIs) have undergone rapid clinical development. Since MMPIs target enzymes that are more essential to tumor invasion and metastasis than to growth of the primary tumor, standard phase II clinical trial endpoints such as objective tumor response rate are not likely to accurately reflect the true clinical impact of the MMPIs. Indeed, in preclinical studies, tumor growth delay and reduction in metastasis formation, rather than tumor shrinkage, have been the main findings. This pattern has carried over into human trials, as well, where very few objective responses have been reported with single-agent trials of MMPIs. This presents a formidable challenge in their clinical development and evaluation. One approach has been to proceed from phase I directly to phase III trials, where overall survival and time to tumor progression for patients treated with MMPIs or MMPI-containing chemotherapy regimens could be compared directly to that achieved with patients treated with placebo or placebo-containing chemotherapy regimens. Another approach has been to look at surrogate endpoints, such as tumor marker levels or change in the rate of rise of tumor markers pre- and post-treatment with an MMPI. Another challenge has been to define criteria for identification.
of the doses that should be used for phase II and III trials of each MMPI. Dose escalation to the maximum tolerated dose may not be appropriate for agents which may induce optimal inhibition of target MMPs, such as MMP-2 and MMP-9, and relative sparing of MMP-1. There are now three MMPs undergoing phase III evaluation.

**Batimastat**

Batimastat (BB-94), a hydroxamic acid derivative that mimics the peptide structure of natural substrates, was the first matrix metalloproteinase inhibitor to enter clinical testing. Reports of the initial phase I trials began to appear in 1994 [5]. Because of poor solubility, batimastat could not be administered orally and was limited to direct injection into the pleural space or the abdomen to control fluid accumulation. Preliminary reports estimated that up to 47% of patients experienced a slowing in the reaccumulation of fluid and a reduction in the need for thoracentesis or paracentesis following treatment with batimastat [5]. Phase III trials were initiated, but closed soon afterward due to slow accrual, local tissue reaction (peritonitis) and the development of an orally bioavailable analog of batimastat.

**Marimastat**

Marimastat (BB-2516) is another hydroxamic acid analog that is structurally similar to batimastat. Unlike batimastat, however, marimastat is extensively absorbed following oral administration and has a long plasma half-life of 8-10 hours [6, 7]. The most common toxicity has been a syndrome of musculoskeletal pain and stiffness, often starting in the small joints of the hands and progressing to the arms and shoulders, mainly at tendon-insertion points. This toxicity occurs after three to five months in approximately 30% of patients treated with doses of 10 mg bid [8]. A one- to three-week drug “holiday” is sufficient for resolution of this toxicity, and most patients are able to continue treatment at a lower dosage.

The data from early clinical trials of marimastat are difficult to interpret for several reasons: A) most trials have been reported in abstract form only; B) most trials were conducted as combination phase I/II studies and the results reported primarily in terms of activity across all or a selected range of doses; C) clinical activity has been reported primarily in terms of change in the rate of rise of tumor markers pre- and post-treatment, an endpoint with unclear clinical implications, and D) mechanisms underlying the principle toxicity of marimastat—joint pain and stiffness—and measures to prevent or to minimize this toxicity are poorly understood.

Phase I/II trials performed with marimastat have demonstrated a significant impact in reducing the rate of rise in the tumor markers of a variety of malignancies, such as CEA (colorectal cancer), CA 19-9 (pancreatic cancer), CA-125 (ovarian cancer) and PSA (prostate cancer) [8-12]. Marimastat appears to reduce the rate of rise of these markers in a dose-dependent fashion. Patients experiencing such a reduction tended to survive for longer periods of time than those who did not [8, 13]. Accrual to a phase III trial comparing marimastat to gemcitabine in patients with advanced pancreatic cancer has been completed, as has a second phase III trial in which the combination of marimastat plus gemcitabine was compared to gemcitabine in the same group of patients. The results of these trials should become available within the next one to two years. Phase III trials have also been completed in patients with extensive-stage small cell lung cancer and glioblastoma multiforme. Phase III trials are ongoing in patients with non-small cell lung cancer and gastric cancer. A phase I study of marimastat plus carboplatin has been performed in patients with ovarian cancer who had previously responded to a platinum-based regimen [14]. Marimastat, in doses of 5-20 mg bid, was administered concurrently with six cycles of carboplatin (AUC=6). The spectrum and severity of toxicities were no different than what would be expected from each drug administered as a single agent. Marimastat had to be interrupted in 9 of 20 patients due to musculoskeletal toxicities. Seven were able to restart marimastat after the break. Eight of 25 assessable patients attained objective responses, including three patients with complete responses. Based on these data, a randomized, double-blind, placebo-controlled study has been initiated in women with platinum-sensitive, relapsed ovarian cancer.

**AG3340**

AG3340 is a hydroxamic acid derivative that was synthesized based on knowledge obtained from the x-ray crystallographic structure of selected MMPs. As a result, it is more selective for certain MMPs (e.g., MMP-2, 3, 9 and 13) that are felt to be involved in tumor invasion and metastasis and less potent against MMP-1, which is believed to be associated with the primary toxicity of this class of agents, arthralgia. A phase I trial of AG3340 given twice daily on a continuous basis until tumor progression has recently been completed [15]. Although no dose-limiting toxicities were noted in the first four weeks of treatment at doses from 10-100 mg bid, delayed-onset joint-related complaints involving the shoulders, knees and hands occurred in a dose- and time-dependent manner, necessitating treatment interruption for two to four weeks. Plasma exposure of AG3340 at the lower dose levels is similar to the optimal plasma profile obtained in mice which resulted in nearly continuous inhibition of targeted MMPs and only transient inhibition of MMP-1.

In an attempt to develop a strategy for integrating MMPIs into the treatment of hormone-refractory prostate cancer, Wilding and colleagues have performed a phase I trial in which AG3340 was combined with mitoxantrone and prednisone [16]. AG3340 was administered twice a day at doses of 5 and 25 mg. No significant pharmacological interactions were noted.
with this combination and no additional or unexpected toxicities were observed with this combination. This strategy has now been taken directly into phase III in which men with hormone-refractory prostate cancer are treated with mitoxantrone plus prednisone plus placebo or AG3340. A similar design is being used in patients with Stage IV non-small cell lung cancer, in which patients are randomized to carboplatin plus paclitaxel plus placebo or AG3340. Primary endpoints in both trials include overall survival and time to tumor progression.

**BAY 12-9566**

BAY 12-9566 is a butanoic acid analog and is, therefore, structurally distinct from other MMPIs. BAY 12-9566 has a very long terminal plasma half-life (90-100 hours), but also has an extremely high plasma protein binding fraction (>99.99%). Four phase I trials have been conducted, all using a daily dosing schedule [17]. Initial schedules called for treatment on a daily basis for four out of every five weeks. When this appeared to be quite tolerable, the schedule was changed to continuous administration until disease progression. Doses from 100 mg qd to 800 mg bid have been evaluated. The primary toxicities observed have been mild to moderate thrombocytopenia, liver enzyme and bilirubin elevations (especially in patients with compromised hematopoietic or liver reserve) and nausea [18-20]. Interestingly, no drug-related arthralgias have been reported, suggesting that the unique structure of BAY 12-9566 may confer a certain degree of enzyme specificity that the hydroxamic acid derivatives do not. Although no objective responses have been observed in phase I trials of BAY 12-9566, the median time to tumor progression has been four months, which is relatively long for a phase I trial. An attempt to measure the effect of BAY 12-9566 on surrogate endpoints in one phase I trial failed to discern a change in plasma vascular endothelial growth factor, basic fibroblast growth factor, or urinary pyridinoline or deoxypridinoline crosslink levels [21].

Phase I trials evaluating the tolerability and pharmacokinetic interaction between BAY 12-9566 and doxorubicin and 5-FU plus leucovorin are under way. Phase III trials have also been initiated in four diseases. In patients with small cell lung cancer, Stage IIIA and IIIB non-small cell lung cancer, and ovarian cancer, patients are randomized to placebo or BAY 12-9566 after attaining best response to standard front-line therapy. In patients with advanced pancreatic cancer, patients are being randomized to front-line therapy consisting of either BAY 12-9566 or gemcitabine.

**CGS27023A**

CGS27023A is the latest entry into the matrix metalloproteinase inhibitor field. A single phase I trial has been reported with this compound [22]. Doses from 150 mg bid to 600 mg tid were explored. Two main toxicities were observed: a widespread, self-limiting maculopapular rash at doses of 300 mg bid or higher, and mild to moderate arthralgias and myalgias which did not appear to be dose-related.

**SUMMARY**

The implication of matrix metalloproteinases in processes critical to tumor invasion, angiogenesis, and metastasis and the great therapeutic potential of agents capable of inhibiting tumor progression through this novel mechanism have prompted the aggressive and rapid clinical development of a new class of agents known as matrix metalloproteinase inhibitors. There are currently four MMPIs in clinical development (three of which are in phase III) and several others poised to enter clinical development within the year. The agents are all orally administered, making them suitable for chronic administration which appears to be necessary for optimal effect. Some are relatively non-specific inhibitors of MMPs (such as marimastat), while others are more selective (AG3340 and BAY 12-9566). It is not yet known which strategy will yield superior clinical results, but it is becoming apparent that this selectivity (or lack of it) may be responsible for the pattern of clinical toxicity that has been observed in early clinical trials.

Three main strategies have been pursued in phase III trials: A) comparing the activity of single-agent MMPI to the best known standard therapy (e.g., marimastat versus gemcitabine in pancreatic cancer); B) combining the MMPI

### Table 1. Inhibitory concentrations of MMPIs

<table>
<thead>
<tr>
<th>MMP</th>
<th>Batimastat</th>
<th>Marimastat</th>
<th>AG3340</th>
<th>BAY 12-9566</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1 (interstitial collagenase)</td>
<td>3</td>
<td>5</td>
<td>8.3</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>MMP-2 (gelatinase A)</td>
<td>4</td>
<td>6</td>
<td>0.05</td>
<td>11</td>
</tr>
<tr>
<td>MMP-3 (stromelysin-1)</td>
<td>20</td>
<td>200</td>
<td>0.03</td>
<td>134</td>
</tr>
<tr>
<td>MMP-7 (matrilysin)</td>
<td>6</td>
<td>—</td>
<td>54.0</td>
<td>—</td>
</tr>
<tr>
<td>MMP-9 (gelatinase B)</td>
<td>4</td>
<td>—</td>
<td>0.26</td>
<td>301</td>
</tr>
</tbody>
</table>
with cytotoxic chemotherapy and comparing it to cytotoxic chemotherapy alone (e.g., paclitaxel plus carboplatin plus AG3340 or placebo in non-small cell lung cancer), or C) comparing MMPI to placebo in patients who have completed cytotoxic chemotherapy and have achieved their best clinical response (e.g., BAY 12-9566 versus placebo in patients with small cell lung cancer who have completed planned induction therapy). Appropriately, the primary endpoint for all of these phase III trials is survival, with disease- or progression-free survival as an important secondary endpoint. The results from these trials are eagerly awaited and will determine whether inhibition of matrix metalloproteinases can translate into clinically meaningful effects.

REFERENCES

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