REVIEW: CANCER THERAPY

Matrix Metalloproteinase Inhibitors and Cancer: Trials and Tribulations

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For at least 30 years, matrix metalloproteinases (MMPs) have been heralded as promising targets for cancer therapy on the basis of their massive up-regulation in malignant tissues and their unique ability to degrade all components of the extracellular matrix. Preclinical studies testing the efficacy of MMP suppression in tumor models were so compelling that synthetic metalloproteinase inhibitors (MPIs) were rapidly developed and routed into human clinical trials. The results of these trials have been disappointing. Here we review the studies that brought MPIs into clinical testing and discuss the design and outcome of the trials in light of new information about the cellular source, substrates, and mode of action of MMPs at different stages of tumor progression. The important lessons learned from the MPI experience may be of great value for future studies of MPIs and for cancer drug development in general.

The MMP family currently consists of ~24 members characterized in humans, rodents, and amphibians [reviewed in (*1*)]. Initially classified as zinc-dependent proteinases capable of digesting the various structural components of the extracellular matrix (ECM), their specific proteolytic targets have since expanded to many other extracellular proteins. These substrates include an array of other proteinases, proteinase inhibitors, clotting factors, chemotactic molecules, latent growth factors, growth factor binding proteins, cell surface receptors, and cell-cell and cell-matrix adhesion molecules [reviewed in (*2*)]. Regulation of MMP function occurs at multiple levels. MMP mRNA expression is under tight, cell type–dependent control, with expression of individual MMPs associated with specific inflammatory, connective tissue, or epithelial cell types. MMP transcripts are generally expressed at low levels, but these levels rise rapidly when tissues undergo remodeling, such as in inflammation, wound healing, and cancer. MMPs are synthesized as latent enzymes that can be stored in inflammatory cell granules but are more often secreted and found anchored to the cell surface or tethered to other proteins on the cell surface or within the ECM. Latent MMPs are proteolytically activated in multiple steps resulting in the release of propeptide domains. Once active, MMPs are subject to inhibition by a family of endogenous tissue inhibitors (see below) as well as by α2-macroglobulin, a plasma inhibitor.

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MMPs: Factors That Promote Tumor Progression

Enzymes that degrade the ECM have long been viewed as essential for tumor progression. Tumor cells are envisioned to produce enzymes that destroy the matrix barriers surrounding the tumor, permitting invasion into surrounding connective tissues, entry and exit from blood vessels, and metastasis to distant organs (Fig. 1A). MMPs were prime candidates for these activities because MMP family members collectively degrade all structural components of the ECM. Moreover, MMPs are up-regulated in virtually all human and animal tumors as well as in most tumor cell lines [reviewed in (*3*)]. Indeed, several MMPs were first identified as a result of the cloning of their cDNAs from tumors or tumor cell lines (gelatinase A/MMP-2, stromelysin-1/MMP-3, matrilysin/MMP-7, gelatinase B/MMP-9, stromelysin-2/MMP-10, and MT1-MMP/MMP-14), or as metastasis-specific genes from advanced tumors (stromelysin-3/MMP-11, collagenase-3/MMP-13). In several cases, the stage of tumor progression is positively correlated with the expression of MMP family members (MMP-1/interstitial collagenase; MMPs 2, 3, 7, 9, 11, and 14) (*4*). Changes in MMP levels can markedly affect the invasive behavior of tumor cells and their ability to metastasize in experimental animal models (*3*).

Further evidence supporting the hypothesis that MMPs promote tumor progression came from studies of their endogenous tissue inhibitors (TIMPs). Several groups demonstrated that overexpression of TIMPs reduced experimental metastasis (*5*–*8*), as did intraperitoneal injection of recombinant TIMP-1 (*9, 10*). Other studies exploited transgenic technology to reveal TIMP/MMP function; for example, mouse 3T3 cells became tumorigenic after antisense depletion of TIMP-1 (*11*), and TIMP-1 overproduction slowed chemical carcinogenesis in skin (*12*) as well as SV40 large T antigen (T-Ag)–induced liver carcinogenesis in transgenic mice (*13*). Taken together, these results suggested that MMPs were important contributors to tumor progression and provided the rationale for developing new cancer drugs that targeted MMP activity.

Although the endogenous MMP inhibitors TIMP-1 and TIMP-2 were initially considered as potential therapeutics for cancer and other diseases, technical difficulties prevented their development into useful drugs. MMPs made an attractive target for small-molecule inhibitors, and a great deal of effort went into determining the structure and substrate specificities of these enzymes [reviewed in (*14*)]. Small molecules containing both hydroxamate and non-hydroxamate zinc binding sites, as well as natural products such as tetracyclines and their derivatives, were developed as MMP inhibitors (MPIs). As early as 1988, the broad-spectrum MPI SC-44463 was shown to block experimental metastasis in mouse models (*15*). Subsequent studies confirmed these results with other MPIs and extended the testing to more complex and clinically relevant models [reviewed in (*16, 17*)]. For example, treatment of nude mice with batimastat (a broad-spectrum hydroxamate inhibitor) after resection of human breast cancer xenografts was found to reduce metastasis and inhibit local regrowth of the cancer (*18*).

Although these exciting results validated the concept of MMPs as therapeutic targets for cancer and confirmed the efficacy of specific MPIs, they quickly led to more questions that needed to be urgently addressed: Which MMPs were important in which cancers and at what stages, and precisely what were these enzymes doing during tumor progression?

New Concepts of MMP Action

More recent studies have led to a rethinking of the potential roles of MMPs in cancer

progression (Fig. 1B). Examination of MMP expression in human tumor tissue sections revealed that MMPs are largely produced by reactive stromal cells recruited to the neoplastic environment (*19*). Although there are exceptions [e.g., MMP-7 is primarily expressed by glandular epithelia, and in a few situations, MMPs 2, 9, and 14 originate in epithelia], the picture that has emerged indicates that the increased presence of MMPs is largely the result of a host response induced by tumors. These observations call into question the biological relevance of some of the earlier studies in which tumor cell lines were engineered to overexpress MMPs and were tested in invasion and metastasis assays. Furthermore, MMPs were shown to be up-regulated and present in an active form in neoplastic tissue before the destruction of basement membrane and emergence of malignant tumors (*19*), which suggests that MMPs do more than merely create gaps in basement membranes through which tumor cells escape.

Overexpression of MMPs 3, 7, and 13 at various epithelial sites in transgenic mice invariably induces cellular hyperproliferation; when challenged by chemical carcinogens or oncogene activation, these mice exhibit increased tumor frequency (*20–22*). The corollary is also true: Tumor-prone mice in specific MMP-null backgrounds develop fewer de novo tumors (*23–26*). These later experiments have been instrumental in revealing which stage of tumor progression and which biologic events are regulated by individual MMPs. The range of biological activities is impressive: MMPs can mediate cell death, cell proliferation, cell differentiation, tumor-associated angiogenesis, and malignant conversion. Although the matrix-degrading abilities of the MMPs are likely to be important, these activities alone do not account for the diversity of biologic responses modulated by the enzymes. More likely, it is the activity of MMPs on nonmatrix substrates (e.g., chemokines, growth factors, growth factor receptors, adhesion molecules, and apoptotic mediators) that yields the rapid and critical cellular responses required for tumor growth and progression.

De novo tumor models have also provided valuable insights regarding the stage-specific and tumor-specific efficacy of MPIs. For example, batimastat treatment decreases the number of intestinal adenomas and pancreatic islet cell tumors in the *min* and RIP-TAg mouse models, respectively (*27, 28*); however, tumor burden is diminished in RIP-TAg mice only if the drug is administered before the emergence of large invasive carcinomas (*28*). When batimastat is given at advanced tumor stages, no efficacy is observed (*28*). In the RIP-TAg islet cell carcinoma model, MMP-9 (but not MMP-2) is critical for tumor angiogenesis; either MMP-9 deficiency or an MPI (batimastat) inhibits tumor development (*25*). Together, these studies assign unique functions to individual MMPs and establish their spatial and temporal significance during tumorigenesis. Most important, this information has served to alter the focus of attention on MMPs as targets during metastasis to proteins that contribute to tumor progression at multiple stages. In particular, MPIs are now viewed as potential antiangiogenic agents for primary tumors (*29*) and as a therapy that can help to maintain small clusters of metastatic cells in a dormant state.

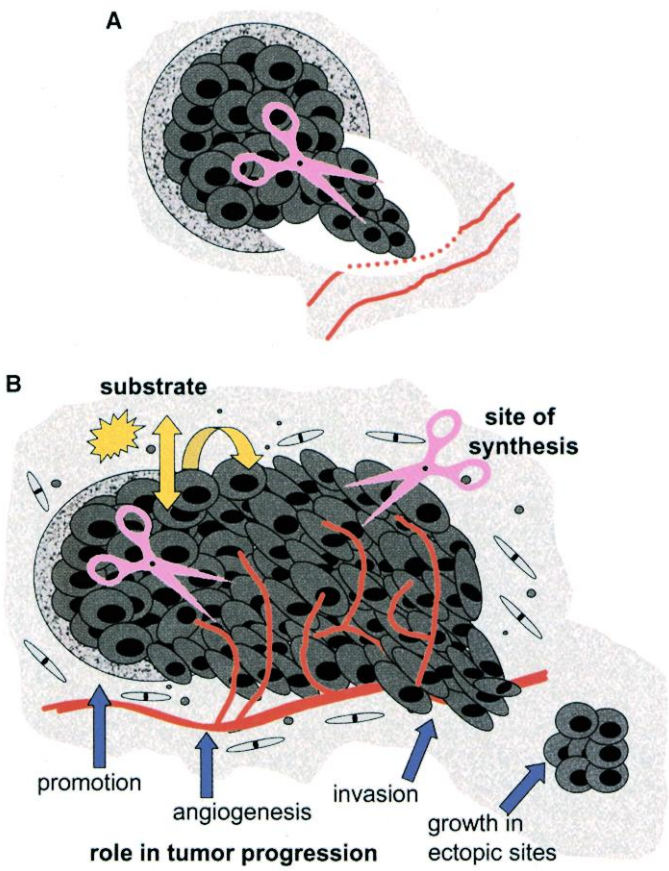

Fig. 1. (**A**) Early view of MMP action in cancer. MMPs (represented by scissors) were classically viewed as being produced and secreted by tumor cells, degrading basement membrane and extracellular matrix components, thereby facilitating tumor cell invasion and metastasis. (**B**) Current view of MMP action in cancer. MMPs are now known to contribute to multiple steps of tumor progression in addition to invasion, including tumor promotion, angiogenesis, and the establishment and growth of metastatic lesions in distant organ sites. In addition, it is recognized that MMPs not only can be synthesized by tumor cells but are frequently produced by surrounding stromal cells, including fibroblasts and infiltrating inflammatory cells. Finally, although creating gaps in matrix barriers remains a role for MMP activity, MMPs are also known to solubilize cell surface and matrix-bound factors that can then act in an autocrine or paracrine manner to influence cellular properties such as growth, death, and migration.

MPI Clinical Trials

The clinical development of MPIs had to overcome multiple unforeseen problems. First-generation MPIs were hampered by poor bioavailability and were rapidly replaced by second-generation orally active drugs [reviewed in (*14*)]. Early phase I clinical trials (dose escalation studies designed to evaluate safety) revealed that prolonged treatment with MPIs caused musculoskeletal pain and inflammation, complications not seen in preclinical models. Although the conditions were reversible and patients were able to continue treatment after a brief drug holiday, the unexpected side effects limited MPI dosages administered in subsequent trials. The critical question of which MMPs were responsible for the musculoskeletal side effects and which ones were valid targets for anticancer therapy received considerable attention but initially remained largely unanswered. In an attempt to minimize or eliminate these side effects, Agouron and Bayer developed "deep-pocket" MPIs (prinomastat and tanomastat, respectively), which are potent inhibitors of MMPs 2 and 9 but are much less effective against MMPs 1, 7, and 11; however, prinomastat still produced similar side effects (*14*). Recently, inhibition of "sheddase" activity attributed to non-MMP metalloproteinases [such as those of the "Adamalysin" (ADAM and ADAM-TS) family] has been implicated in the musculoskeletal side effects (*30*). Indeed, in studies to date, patients treated with the broad-spectrum MPI BMS-275291, which has reduced activity against sheddases, have not experienced these side effects (*17*).

Phase II trials (designed to examine efficacy) turned out to be problematic as well. Because MPIs are cytostatic (cells are growth-arrested but viable) rather than cytotoxic (cells are killed), conventional measures of efficacy such as reduction in tumor size could not be used to monitor drug

activity. Instead, reduction in the rate of increase of tumor markers in serum was used to define a biologically active dose of marimastat (*31–33*). This was criticized as an endpoint because changes in biomarker levels in serum do not necessarily reflect tumor regression (*34*). As a consequence of these and other issues, phase I trials were often followed immediately by phase II/III combination trials without the benefit of efficacy information from smaller studies. Phase III trials (large-scale studies that evaluate efficacy in comparison to standard treatments) were initiated in the mid-1990s. The design of these trials evolved as the clinical and ongoing laboratory studies provided more detailed information about the role of MMPs in cancer (Table 1). The first trials examined the efficacy of the MPI alone versus that of cytotoxic drugs, whereas later trials examined the effect of an MPI, either in combination with or after treatment with cytotoxic drugs, compared with the effect of the cytotoxic drugs alone (*19*).

Results from phase III trials have been disappointing (Table 1) and have led many investigators to conclude that MPIs have no therapeutic benefit in human cancer. In light of the extensive preclinical data that support a role for MMP inhibition as a therapeutic approach, the clinical data need to be evaluated from a scientific perspective. It becomes immediately clear that the difference between the preclinical and clinical experiments extends beyond the differences between mouse and human in several respects. In the clinical trials, MPIs at their maximum tolerated dose were administered to patients with advanced cancer, with survival or time to progression (versus standard chemotherapy) as the endpoint. In contrast, in the mouse models, MMP inhibition (MPIs or genetic ablation) was generally initiated at early stages of disease and maintained throughout tumor progression, with size or number of tumors as the endpoint. Given our current understanding of MMPs as contributors to multiple stages of tumor progression (Fig. 1B), one can predict that MMP inhibition would decrease the rate of tumor progression and that the therapeutic benefit of this decrease would be minimized at late stages of disease (Fig. 2). As mentioned above, this premise is supported by the observation that batimastat treatment of RIP-TAg mice reduced tumor burden when administered at both early and intermediate stages of the disease but had no effect on mice with advanced tumors (*28*).

Evaluation of the clinical trial data also suggests that MPIs benefit patients with earlier stage disease, although it must be recognized that these conclusions are drawn from the analysis of subgroups of patients and should be considered hypothesis-generating observations that require follow-up with prospectively designed, adequately powered clinical trials. Patients with nonmetastatic pancreatic cancer who received high doses of marimastat had 1-year survival rates that were comparable to those seen after treatment with gemcitabine, the most effective chemotherapeutic agent against this highly aggressive cancer (*35*). In the most encouraging clinical trial, patients with unresectable gastric cancer who were treated with marimastat were reported to show a modest increase in survival, although this interpretation has been disputed on the basis of a P value of 0.07 (*36, 37*). However, a significant survival benefit was identified in those patients who had received prior chemotherapy, and 2-year survival in these patients increased from 5% to 18% ($P = 0.006$). This subgroup excludes those patients with more advanced or rapidly progressing disease, and perhaps represents a shift to the left on the tumor progression pathway (Fig. 2).

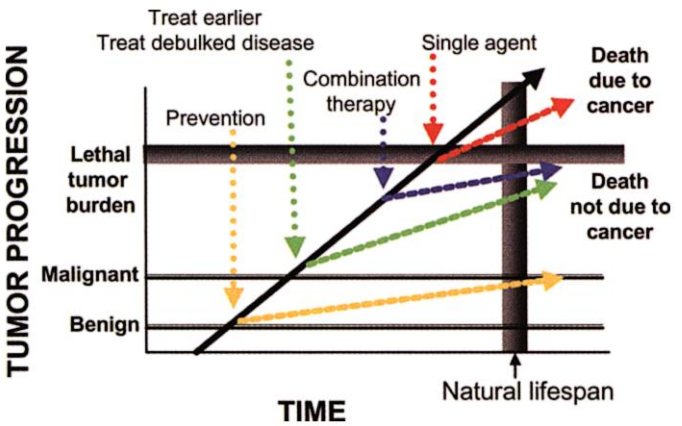

Fig. 2. MMP inhibition to control tumor progression. Tumor progression is presumed to be a linear function (black arrow) progressing through stages of benign disease, malignant conversion, and a lethal tumor burden. Effective therapies would reduce the rate of tumor progression so that the arrow crosses the "natural life-span" line before the "death due to cancer" line. Effective cancer prevention would reduce the rate of tumor progression so that the "natural life-span" line is crossed before the "malignant disease" threshold is reached. Treatment of patients with an MPI as a single agent at advanced stages of disease is likely to have minimal impact on survival, resulting in death due to cancer (red arrow). Treatment with an MPI, in combination with other cytotoxic or cytostatic agents, is likely to cause a steeper reduction in the rate of tumor progression (blue arrow). Treatment at earlier disease stages, or treatment of debulked disease (i.e., treated by surgery, radiation, or cytotoxic therapy), should significantly affect tumor progression and prevent death due to cancer (green arrow). Treatment of premalignant disease is likely to prevent malignant conversion (gold arrow) and may be an effective strategy for cancer prevention.

Although several trials have been terminated for lack of efficacy, the early termination of studies of tanomastat in patients with pancreatic and small-cell lung cancer is of even greater concern because patients receiving the MPI showed significantly poorer survival than patients receiving placebo (*38, 39*). No adverse effect on survival was observed in similar patients treated with another MPI (marimastat), which suggests that the negative effects were not mechanism-based. Nevertheless, there are preclinical data suggesting that in some instances MMP inhibition stimulates disease progression. In a mouse model of skin carcinogenesis driven by human papilloma virus (HPV) 16, the absence of MMP-9 results in fewer squamous cell carcinomas, but the tumors that arise have a less differentiated morphology and are representative of more aggressive tumors (*26*). MPIs have also been shown to increase the number of liver micrometastases from breast and lymphoma cells (*40*). Other studies indicate that an elevation in MMP levels results in increased conversion of plasminogen to angiostatin and decreased vascularization of transplanted tumors, and in this context MPIs can enhance tumor vascularization (*41, 42*). These findings emphasize that the substrates of MMPs are complex and diverse, with biological activities that range from stimulation of proliferation to induction of apoptosis (*2*). It is likely that in specific situations MMP inhibition can result in a decrease in a beneficial natural product (e.g., angiostatin) that is not balanced by decreases in tumor-promoting activities. Preclinical experiments and most of the clinical experiments, however, indicate that this is a rare event and the predominant effect is more likely to be beneficial given appropriate conditions of disease stage and drug efficacy.

There are currently five MPIs in advanced stages of clinical development. Marimastat testing has continued with a modified trial design to examine effects on patients with curatively resected pancreatic cancer (*36*). A phase III trial testing the efficacy of BMS-275291 in advanced non–small-cell lung cancer is now open; it differs from many of the previous studies in that dosing does not appear to be limited by musculoskeletal side effects (*43*). Multiple phase II trials are testing prinomastat in diverse tumor settings and

earlier stages of disease (*44*). In addition, phase II trials in Kaposi's sarcoma are assessing metastat, a tetracycline analog designed as a MMP inhibitor (*45*). Finally, neovastat, an extract of shark cartilage with MMP-inhibitory activity, is in phase III trials for treatment of unresectable renal cell carcinoma (*46*). There is optimism that the changes in trial design and/or compound characteristics may finally lead to the registration of an MPI for a cancer indication. However, to date, Periostat (doxycycline hydrate, a tetracycline analog) is the only MPI licensed in the United States, and the application is for periodontal disease (*47*).

Lessons for the Future

So what have we learned from the MPI experience? One important lesson is the need for attention to the stage and type of cancer that is likely to be evaluated in clinical versus preclinical studies. For example, the selection of advanced pancreatic and lung cancers for clinical trials was based on considerations such as expected survival time and patient availability, both of which affect the time and financial resources required to achieve statistically significant results. Patent issues, competition, and impatience contributed to the decision to proceed at an unprecedented pace in an inappropriate setting, and these factors will undoubtedly continue to influence drug development decisions in the future. The rapidity with which the MPIs moved into clinical trials raised questions that were unanswerable at the time: Do any MMPs play an important role in advanced lung and pancreatic cancer, and if so, which ones? An analysis of the expression patterns of the entire family of MMPs in the cancer type and stage used for clinical trials, and correlation with clinical outcome to determine prognostic significance, may have allowed a more rational decision on the appropriate enzymes to target. For example, it is now known that the expression of MMP-11 and/or MMP-14 is a negative prognostic indicator for small-cell lung cancer, and that this tumor type has undetectable expression of MMP-2 (*48*). This knowledge could have guided the selection of a more appropriate tumor type for the clinical testing of tanomastat, which targets MMP-2 and has very little activity toward MMP-11. The abundant "proof-of-principle" evidence for the efficacy of MMP inhibition in mouse models has turned out to have little relationship with the types of human cancers examined in clinical trials. Mouse models of pancreatic adenocarcinoma and small-cell lung cancer in particular are woefully lacking. Classical animal models of subcutaneous or intravenous injection of human tumor cells into immunodeficient mice are inadequate to evaluate the activity of molecules such as MMPs in that they do not recapitulate host-tumor interactions. Special consideration must be given to understanding the stage of tumor progression at which cytostatic agents are likely to work alone, and to understanding where an experimentally assessable advantage is provided when MPIs are combined with standard debulking or cytotoxic thera-

Table 1. Phase III clinical trials with MPIs.

MPI	Cancer	Stage	Treatment	Result
Marimastat (BB-2516)	Pancreatic (*35*)	II, III, IV; unresectable	5, 10, 25 mg vs. gemcitabine	No significant difference in overall survival; in subset analysis, 25 mg had 1-year survival rate similar to gemcitabine
	Pancreatic (*17*)	II, III, IV; unresectable	gemcitabine with 10 mg or placebo	No survival benefit
	Pancreatic (*36*)	I, II, III; resectable	20 mg versus placebo	Result expected December 2002
	Gastric (*36, 37*)	Advanced unresectable	10 mg versus placebo	No or very modest survival benefit (P = 0.07); in subset analysis, significant survival benefit in patients who received prior Rx (P = 0.045)
	Glioblastoma (*17, 56*)	Unresectable	10 mg versus placebo	No survival benefit
	Small-cell lung (*57*)	Any; PR or CR after first Rx	10 mg versus placebo	No survival benefit
	Non–small-cell lung (*36*)	IIIA or IIIB	10 mg versus placebo carboplatin with 10 mg	No survival benefit
	Ovarian (*58*)*	Advanced second Rx	or placebo	No difference in response
Prinomastat (AG3340)	Non–small-cell lung (*44*)	IIIBT4 or IV	Carboplatin + paclitaxel with prinomastat or placebo	No survival benefit
	Non–small-cell lung (*44*)	IIIBT4 or IV	Cisplatin + gemcitabine with prinomastat or placebo	Terminated early because of lack of efficacy
	Prostate (*44*)†	Metastatic, hormone refractory	Mitoxantrone + prednisone with prinomastat or placebo	No difference in symptomatic progression
Tanomastat (BAY 12-9566)	Small-cell lung (*38*)	Extensive; PR or CR after first Rx	Tanomastat versus placebo	Terminated prematurely because tanomastat-treated patients showed poorer survival than placebo-treated patients
	Pancreatic (*39*)	Metastatic; unresectable; no prior Rx	Gemcitabine versus tanomastat	Terminated prematurely because tanomastat-treated patients showed poorer survival than gemcitabine-treated patients
BMS-275291	Non–small-cell lung (*43*)	IIIB or IV	Carboplatin + paclitaxel with BMS-275291 or placebo	Currently recruiting patients
Neovastat	Renal cell carcinoma (*46*)	IV	Neovastat vs. placebo	Currently recruiting patients

Endpoint for all studies was survival except: *Endpoint was response. †Endpoint was time to symptomatic progressive disease. Abbreviations: PR, partial response; CR, complete response; Rx, treatment.

pies. Mouse models that more closely mimic human cancers are rapidly becoming available and must be applied in a way that also recapitulates the clinical presentation of and current therapeutic approach to the corresponding human disease.

A second important lesson from the MPI experience is that standard clinical trial endpoints are insufficient for the evaluation of molecularly targeted cytostatic agents. The development of MPIs lacked endpoint assessments for (i) the efficacy of the compound in modulating the activity of its target, and (ii) the relationship between target modulation and clinical response. Despite the large number of patients accrued in MPI clinical trials, there remains no clear demonstration that any dosing schedule or compound tested has reached levels sufficient to inhibit target MMP activity within the tumor tissue. A reliable surrogate marker for the assessment of MMP-inhibitory activity in phase I/II correlative studies would have helped enormously in determining the optimal biologic dose and optimal dose scheduling to sustain activity during trough periods while maintaining selectivity during peak periods and limiting potential side effects. In the absence of this information, phase III trials proceeded with several doses (diluting the power of each arm of the study), or with maximum tolerated doses without the benefit of knowing whether tumor-associated MMP activity was inhibited. Unfortunately, surrogate markers for MPI activity have proved elusive. Analysis of serum or plasma levels of the gelatinases by zymography has been uninformative (*49*), although the activity of the tetracycline analog col-3, an unusual MPI that affects MMP synthesis as well as activity, can be monitored by analysis of changes in plasma levels of MMP-9, a measurement that does appear to correlate with efficacy (*50*). Measurement of specific degradation products of MMPs has also been suggested as a tool for assessing inhibitor activity, but again, this strategy has not yet proved to be informative (*49*). One recent study used whole-animal fluorescent imaging techniques to show that tumor-associated proteolytic activity was inhibited in vivo by the MPI prinomastat (*51*). The MPI experience indicates that the development of such minimally invasive techniques for the analysis of drug efficacy should be considered essential for the clinical evaluation of any molecularly targeted therapy.

Once effective drug concentrations to disrupt target function can be demonstrated, therapeutic endpoints to assess the effectiveness of specific target elimination should be addressed before large-scale phase III trials are initiated. Because tumor shrinkage is not a likely event with cytostatic agents, a new means of defining an objective response in phase II trials is required. There is a pressing need to develop and validate markers of tumor progression, a task that might best be approached by radiologists and imaging scientists. Positron emission tomography has been used to measure tumor blood flow, glucose metabolism, and cell growth by thymidine incorporation, and thus could be useful in correlative studies to differentiate between benign and malignant tumor masses or to identify large tumors with impaired blood flow or extensive necrotic areas (*52*). Magnetic resonance imaging offers the advantage of supplying both anatomical and functional measurements at high resolution. Recent developments in the field include the generation of contrast agents that can be activated only in the presence of particular enzymatic activities (*53*). Clinical development and validation of techniques such as these will be of great importance for future monitoring of molecularly targeted antitumor agents before the ultimate tests of efficacy—large-scale trials with survival endpoints—are initiated.

In contrast to the difficulties encountered in MPI development, at least one molecularly targeted agent, STI-571 (produced by Novartis under the name Gleevec), gives us an example of how well things can work with appropriate attention to some of these issues. STI-571 was developed as a tyrosine kinase inhibitor with specificity for the Abl tyrosine kinase, although c-Kit and the platelet-derived growth factor receptor are also targets. In chronic myelogenous leukemia, 95% of patients have the Philadelphia chromosome that results in generation of the oncogenic Bcr-Abl fusion protein. Studies have shown that the activity of this kinase is sufficient to cause the disease, thus allowing therapeutic efforts to be focused on a highly appropriate patient population (*54*). Inhibition of tyrosine kinase activity was readily assayed by the analysis of levels of phospho-CrkL, the predominant Bcr-Abl substrate in neutrophils. This endpoint assay assisted in the identification of a biologically active dose. Because tumor tissue could be easily obtained from leukemia patients, analysis of the presence of the Philadelphia chromosome in patients' blood samples provided a surrogate marker for disease activity (*55*). The rapid and successful clinical development of Gleevec largely rests on the fact that the disease in the patient population tested was dependent on ABL activity, and there were methods available for the determination of ABL inhibition and disease remission in patients. From this viewpoint, one could conclude that the chances of developing effective anti-MMP therapies would greatly increase with improved knowledge of the contribution of MMPs to the progression of specific cancer types and stages, and with appropriate tools for evaluating MPI activity at both the molecular and clinical levels.

So what is the future of MMP inhibitors in the treatment of cancer? At the moment, their fate appears to lie primarily in the hands of the pharmaceutical companies, where decisions to invest in enormously expensive clinical trials must include a consideration of past performance records. What remains clear is that there is still much groundwork to be done. Basic and translational researchers must make greater efforts to develop and validate assays that identify tumors expressing target enzymes and to assess the efficacy of specific compounds and optimal doses that reduce tumor-associated proteolytic activity. They need to continue with preclinical studies to determine the role of specific MMPs in specific stages of tumor progression, as well as to develop animal models that recapitulate clinical trial design. They are presented with an almost unprecedented opportunity to use the available clinical data to assess the validity of new animal models in understudied diseases (such as pancreatic adenocarcinoma and small-cell lung cancer) in predicting clinical outcome. Clinical researchers need to focus on improving the design of trials that better assess agents with little or no direct tumor cell–killing activity. Finally, government agencies such as the NIH and National Cancer Institute that fund and conduct many clinical trials, or the FDA that regulates the process of clinical drug development, must recognize the need for molecular target assays and relevant therapeutic endpoints during clinical trials. These agencies must continue their efforts to support the development and approval of targeted cancer therapies, as well as work toward addressing the urgent need to increase the number of patients who enter clinical trials. The current gaps between basic cancer researchers, clinicians, the pharmaceutical industry, and the regulatory agencies can be closed by improved communication and the realization that they aspire to a common goal. Whether MPIs become a standard in the cancer armament is unclear. However, the lessons learned from the MPI experience are likely to be invaluable in advancing the application of whole new generations of cytostatic therapies in the treatment of cancer.

References and Notes

1. J. F. Woessner, H. Nagase, *Matrix Metalloproteinases and TIMPs* (Oxford Univ. Press, New York, ed. 1, 2000).
2. L. J. McCawley, L. M. Matrisian, *Curr. Opin. Cell Biol.* **13**, 534 (2001).
3. _____, *Mol. Med. Today* **6**, 149 (2000).
4. W. G. Stetler-Stevenson, *Am. J. Pathol.* **148**, 1345 (1996).
5. Y. A. DeClerck, S. Imren, *Eur. J. Cancer* **30A**, 2170 (1994).
6. A. M. P. Montgomery, B. M. Mueller, R. A. Reisfeld, S. M. Taylor, Y. A. DeClerck, *Cancer Res.* **54**, 5467 (1994).
7. R. Khokha, *J. Natl. Cancer Inst.* **86**, 299 (1994).
8. S. Koop *et al.*, *Cancer Res.* **54**, 4791 (1994).

9. R. M. Schultz, S. Silberman, B. Persky, A. S. Bajkowski, D. F. Carmichael, *Cancer Res.* **48**, 5539 (1988).
10. O. A. Alvarez, D. F. Carmichael, Y. A. DeClerck, *J. Natl. Cancer Inst.* **82**, 589 (1990).
11. R. Khokha *et al.*, *Science* **244**, 947 (1989).
12. T. B. Buck, H. Yoshiji, S. R. Harris, O. R. Bunce, U. P. Thorgeirsson, *Ann. N.Y. Acad. Sci.* **878**, 732 (1999).
13. D. C. Martin, U. Ruther, O. H. Sanchez-Sweatman, F. W. Orr, R. Khokha, *Oncogene* **13**, 569 (1996).
14. M. Whittaker, C. D. Floyd, P. Brown, A. J. H. Gearing, *Chem. Rev.* **99**, 2735 (1999).
15. R. Reich *et al.*, *Cancer Res.* **48**, 3307 (1988).
16. M. D. Sternlicht, G. Bergers, *Emerg. Ther. Targets* **4**, 609 (2000).
17. P. D. Brown, *Expert. Opin. Investig. Drugs* **9**, 2167 (2000).
18. G. W. Sledge Jr., M. Qulali, R. Goulet, E. A. Bone, R. Fife, *J. Natl. Cancer Inst.* **87**, 1546 (1995).
19. A. R. Nelson, B. Fingleton, M. L. Rothenberg, L. M. Matrisian, *J. Clin. Oncol.* **18**, 1135 (2000).
20. J. D'Armiento *et al.*, *Mol. Cell. Biol.* **15**, 5732 (1995).
21. L. A. Rudolph-Owen, R. Chan, W. J. Muller, L. M. Matrisian, *Cancer Res.* **58**, 5500 (1998).
22. M. D. Sternlicht *et al.*, *Cell* **98**, 137 (1999).
23. C. L. Wilson, K. J. Heppner, P. A. Labosky, B. L. M. Hogan, L. M. Matrisian, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1402 (1997).
24. R. Masson *et al.*, *J. Cell Biol.* **140**, 1535 (1998).
25. G. Bergers *et al.*, *Nature Cell Biol.* **2**, 737 (2000).
26. L. M. Coussens, C. L. Tinkle, D. Hanahan, Z. Werb, *Cell* **103**, 481 (2000).
27. K. J. Heppner Goss, P. D. Brown, L. M. Matrisian, *Int. J. Cancer* **78**, 629 (1998).
28. G. Bergers, K. Javaherian, K. M. Lo, J. Folkman, D. Hanahan, *Science* **284**, 808 (1999).
29. W. G. Stetler-Stevenson, *J. Clin. Invest.* **103**, 1237 (1999).
30. J. Bird, J. G. Montana, R. E. Wills, A. D. Baxter, D. A. Owens, *Chem. Abstr.* **129**, 22571 (1998).
31. J. Nemunaitis *et al.*, *Clin. Cancer Res.* **4**, 1101 (1998).
32. J. N. Primrose *et al.*, *Br. J. Cancer* **79**, 509 (1999).
33. A. Rosemurgy *et al.*, *Am. J. Clin. Oncol.* **22**, 247 (1999).
34. M. Gore, R. A'Hern, M. Stankiewicz, M. Slevin, *Lancet* **348**, 263 (1996).
35. S. R. Bramhall, A. Rosemurgy, P. D. Brown, C. Bowry, J. A. Buckels, *J. Clin. Oncol.* **19**, 3447 (2001).
36. British Biotech PLC, www.britbio.com/news/173,183analysis.pdf (2001).
37. J. Fielding *et al.*, *Proc. Am. Soc. Clin. Oncol.* **19**, 240A (2000).
38. Bayer Corporation, www.bayerusa.com/news/co0221.asp (1999).
39. M. Moore *et al.*, *Proc. Am. Soc. Clin. Oncol.* **19**, 240a (2001).
40. A. Kruger *et al.*, *Cancer Res.* **61**, 1272 (2001).
41. A. Pozzi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 2202 (2000).
42. A. Pozzi, W. F. LeVine, H. A. Gardner, *Oncogene* **21**, 272 (2002).
43. http://clinicaltrials.gov/ct/gui/c/a2b/show/NCT00006229 (2001).
44. Agouron Pharmaceuticals Inc., www.agouron.com/Pages/press_releases/pr080400.html (2000).
45. http://clinicaltrials.gov/ct/gui/c/w2b/show/NCT00020683 (2001).
46. http://clinicaltrials.gov/ct/gui/c/w2b/show/NCT00005995 (2001).
47. R. L. Wynn, *Gen. Dent.* **47**, 19 (1999).
48. M. Michael *et al.*, *J. Clin. Oncol.* **17**, 1802 (1999).
49. M. Hidalgo, S. G. Eckhardt, *J. Natl. Cancer Inst.* **93**, 178 (2001).
50. M. Cianfrocca *et al.*, *J. Clin. Oncol.* **20**, 153 (2002).
51. C. Bremer, C. H. Tung, R. Weissleder, *Nature Med.* **7**, 743 (2001).
52. R. M. Glasspool, T. R. Evans, *Eur. J. Cancer* **36**, 1661 (2000).
53. A. Y. Louie *et al.*, *Nature Biotechnol.* **18**, 321 (2000).
54. B. J. Druker, N. B. Lydon, *J. Clin. Invest.* **105**, 3 (2000).
55. B. J. Druker *et al.*, *N. Engl. J. Med.* **344**, 1031 (2001).
56. British Biotech PLC, www.britbio.com/news/131.pdf (2000).
57. British Biotech PLC, www.britbio.com/news/140_result.pdf (2001).
58. British Biotech PLC, www.britbio.com/news/186.pdf (2000).

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