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Experimental and Clinical Studies on the Use of Matrix Metalloproteinase Inhibitors for the Treatment of Cancer

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MATRIX METALLOPROTEINASES: BACKGROUND AND RECENT DEVELOPMENTS

MATRIX METALLOPROTEINASES (MMPs) are a family of structurally related zinc-containing enzymes which function in the degradation of extracellular matrix proteins that constitute connective tissue. The first MMP to be described was interstitial collagenase which was purified in 1962 as the factor responsible for the resorption of tadpole tails [1]. Collagenase-mediated degradation of fibrillar type I and type III collagens remained the focus of research for much of the next two decades. There then followed a period of research and rapid discovery between 1985 and 1990. During this time, seven new members of the family were identified and cloned [2–9], as were the first two native tissue inhibitors of metalloproteinases, TIMP- 1 and TIMP-2 [10, 11]. The current members of the MMP family and their substrate preferences are shown in Table 1.

In the past 5 years, attention has focused on the regulation of MMP gene expression and enzymic activity in normal and diseased tissues. As a result of such studies, MMPs have been implicated in the pathogenesis of several diseases including arthritis [12], cancer [13] and, more recently, neurodegenerative diseases such as multiple sclerosis [14]. There have been several correlative studies in human cancer showing high levels of expression of various MMP mRNA species in tumour tissue or in adjacent stroma, with little or no expression in surrounding normal tissue [8, 15]. Other studies have shown the expression of activated forms of MMPs, particularly gelatinase A, to be markedly increased in tumour relative to normal tissue [16, 17].

The recent discovery of a new member of the MMP family, membrane-type or MT-MMP, has shed new light on this last observation [18]. MT-MMP, a membrane-bound enzyme with a recognised transmembrane domain, appears to be a specific activator of progelatinase A [19]. The expression of MT-MMP by cancer cells would confer the ability to activate gelatinase A produced locally by stromal cells at the invasive tumour margins. In a study of gastric cancer, MT-MMP was expressed exclusively in the tumour tissue and co-localised with activated gelatinase A in invasive carcinoma cell nests [20]. Since the discovery of MT-MMP, genes for three other membrane-type MMPs have been identified, and these enzymes now constitute a new subfamily of MMPs [21]. The substrate preference for these new membrane-bound enzymes has not been established, but may involve the processing of a variety of cell-bound cytokines [22]. These correlative studies suggest a role for MMPs in the processes of angiogenesis, tumour invasion and metastasis. The use of specific MMP inhibitors has helped in elucidating the function of these enzymes and in defining how critical their roles are.

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MATRIX METALLOPROTEINASE INHIBITORS

Matrix metalloproteinase inhibitors (MMPIs) range from simple chelating agents, such as EDTA, to synthetic peptide-based inhibitors, and to the naturally occurring inhibitory proteins, TIMPs. At least three distinct TIMPs have now been identified [10, 11, 23]. These proteins, 21–28 kDa in size, have broad activity inhibiting all known classes of MMP. Studies with TIMPs have demonstrated their ability to inhibit tumour cell invasion *in vitro* and lung colonisation by murine B16-F10 melanoma cells *in vivo* [24, 25]. TIMPs have also been shown to block the formation of vascular-like structures within a collagen gel by human umbilical vein endothelial cells *in vitro* [26] and through inhibition of endothelial cell migration during angiogenesis *in vivo* [27].

The development of synthetic inhibitors started in the early 1980s with research programmes investigating their potential use in preventing bone and cartilage destruction in rheumatoid arthritis [28]. These early inhibitors were essentially peptide mimetics of the P′ side of the cleavage site in the collagen molecule (Figure 1). Inhibition is achieved through a zinc-binding group adjacent to the P1′ position. Several zinc-binding chemical groups have now been studied including hydroxamates, carboxylates, aminocarboxylates, sulphydryls, and phosphorus acid derivatives [29]. Partly as a result of the model of metastasis and the role of type IV collagenase activity proposed by Liotta and others [30], the focus of MMPI research began to switch from arthritis to cancer. MMPIs were initially considered simply as agents which might prevent metastasis by blocking the passage of malignant cells across basal laminae. However, studies with synthetic MMPIs showed that these inhibitors

Table 1. Matrix metalloproteinase family

Matrix metalloproteinase	MMP number	Preferred substrate
Interstitial collagenase	MMP-1	Fibrillar collagens, types I, II, III
PMN collagenase	MMP-8	Fibrillar collagens, types I, II, III
Collagenase-3	MMP-13	Fibrillar collagens, types I, II, III
Stromelysin-1	MMP-3	Laminin, fibronectin, proteoglycans
Stromelysin-2	MMP-10	Laminin, fibronectin, proteoglycans
Stromelysin-3	MMP-11	1-Antitrypsin?
Matrilysin (pump)	MMP-7	Laminin, fibronectin, proteoglycans
Gelatinase A (72 kDa)	MMP-2	Collagen types IV, V, gelatin
Gelatinase B (92 kDa)	MMP-9	Collagen types IV, V, gelatin
Metalloelastase	MMP-12	Elastin
Membrane-type MMP*	MMP-14	Progelatinase A

*At least four separate MT-MMPs have now been identified at the mRNA level.

could also inhibit angiogenesis, an essential process in tumour growth and invasion, and thereby block invasive tumour growth [31] (Figure 2). These studies are the subject of a recent review [32].

Two recent studies illustrate the way in which MMPIs might be applied clinically. The MMPI batimastat (BB-94, British Biotech, Oxford, U.K.) was used to treat rats bearing the HOSP1 syngeneic mammary tumour. The primary tumour in the mammary fat pad was resected early and the animals left to develop metastatic disease. Animals receiving a 7-day course of batimastat had a reduced incidence of lung metastases from the primary tumour, although they developed a similar incidence of lymph node metastases when compared with untreated animals. However, no visible metastases were observed in 13/14 rats receiving a 58 day maintenance course of batimastat starting 2 days before resection of the primary tumour [33]. This result suggests that MMPI treatment, if maintained, may suppress the growth and development of established lymphatic metastases. It is thought that the anti-angiogenic properties of batimastat may have contributed to the inhibition of secondary tumour growth in this model [31]. The second study examined the efficacy of combining a gelatinase selective synthetic MMPI (CT1746, Celltech, Slough, U.K.) with the cytotoxic agent cyclophosphamide in the treatment of murine Lewis lung carcinoma. The combination was shown to be significantly more effective than either agent used alone, both with respect to the growth delay of the primary lesion and the number and size of resultant pulmonary metastases [34]. The use of intraperitoneal batimastat in the treatment of malignant ascites was evaluated in a human ovarian carcinoma xenograft in a nude mouse model [35]. Batimastat resulted in resolution of ascites, reduced tumour burden and a dose-dependent increase in survival of at least 5-fold. Histological examination revealed treated tumours had become avascular and necrotic. The inactive diastereoisomer of the drug had no effect on tumour behaviour. Collectively, these preclinical investigations indicate that MMPI therapy could have potential clinical utility in the prevention of tumour growth possibly through inhibition of angiogenesis.

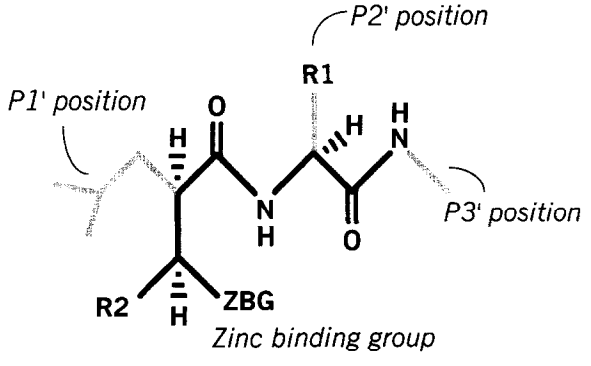

Figure 1. Generic structure of a typical 'right-hand side' or P' based MMP inhibitor. The peptide backbone is shown in black with substituents in grey. The molecule mimics the right-hand side of the cleavage site made by interstitial collagenase in the α-chain of type I collagen. Substitutions at the R1 and R2 positions affect the selectivity and pharmacological properties of MMP inhibitors.

THE FIRST CLINICAL TRIALS OF MMPIs IN CANCER PATIENTS

Initially, it was considered that TIMPs could be employed as therapeutic agents in their own right. The supposition that MMPIs may require prolonged periods of maintenance therapy and the likelihood that they would have inadequate activity after oral administration limited the attractiveness of these proteins as drugs. The first generation of synthetic MMPIs indeed proved to have poor oral bioavailability and consequently the first synthetic MMPIs to enter clinical trials were administered by other routes. These were galardin (Glycomed), currently being developed as a topical treatment for corneal ulceration [36] and batimastat, the first MMPI to be used in clinical trials in cancer. The phase I study of oral batimastat, initiated in 1992, indicated poor bioavailability by the oral route of administration and consequently the development of this formulation of the drug was discontinued. As the results of the preclinical study of intraperitoneal batimastat in the ovarian carcinoma xenograft mouse model became available [35], an intraperitoneal formulation was developed for clinical evaluation.

Intraperitoneal batimastat

The phase I study enrolled 23 patients with malignant ascites requiring paracentesis for symptomatic relief (Beattie and Smyth, unpublished data). Following drainage of ascites by peritoneal dialysis catheter, a single dose of batimastat was instilled into the peritoneal cavity as a suspension in 500 ml 5% dextrose. The doses given were 150, 300 and 600 mg/m^2 (3 patients each) 1050 and 1350 mg/m^2 (7

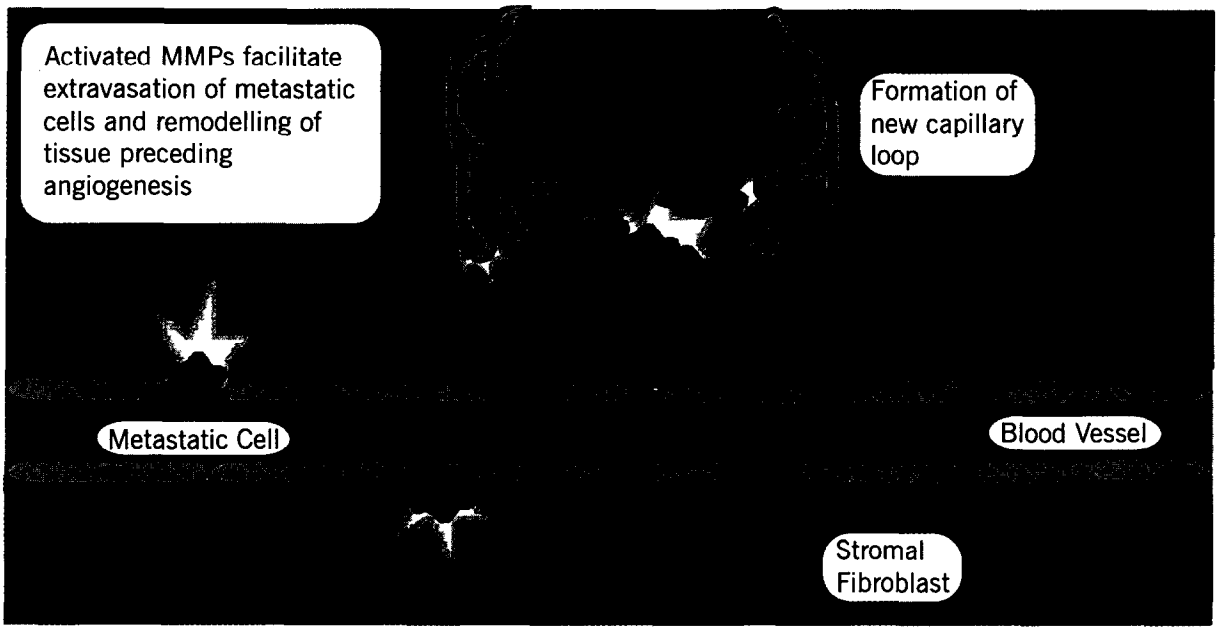

Figure 2. Tumour stimulated angiogenesis, the formation of new capillary loops, is an essential process in the growth of metastatic lesions. Capillary endothelial cells are believed to induce proteases, including MMPs, to degrade the stromal tissue allowing the establishment of a new capillary bed. MMP inhibitors have been shown to inhibit angiogenesis in experimental models.

patients each). Of the patients, 16 had ovarian carcinoma, 2 patients had sarcomas and 1 patient each having breast cancer, colon cancer, endometrial cancer, renal cancer and pancreatic cancer. Mild abdominal pain associated with nausea and vomiting was recorded in approximately half the patients, but this was usually short-lived. Bowel obstruction occurred in 3/23 (13%) patients but this did not appear to be dose-related and resolved following conservative management in each case. Fatigue was reported by 9/23 (39%) patients. Apart from 2/23 (9%) cases of anaemia, there were no significant changes in any of the standard biochemical or haematological parameters assessed and the drug was otherwise well tolerated.

A high and sustained plasma concentration of batimastat occurred following the single intraperitoneal dose. The highest mean plasma concentrations were recorded within the first 24 h and concentrations 28 days after administration ranged from 19.8 ng/ml following the 150 mg/m^2 batimastat dose level to 226.1 ng/ml after the 1350 mg/m^2 dose. The plasma half-life of batimastat was 19 days and the area under the plasma concentration × time curve (AUC) appeared to increase linearly with dose. The pharmacokinetic profile of batimastat following intraperitoneal injection is thought to be due to the slow dissolution of the suspension followed by rapid absorption across the peritoneal membrane. Studies in animals suggest that batimastat is metabolised by the liver and removed rapidly from the circulation (mean elimination half-life 9–10 h, depending on species). Although it was not possible to assess efficacy in this study, 16/23 (70%) patients did not have further paracenteses during this initial 28-day study period. 7/23 (30%) patients died without reaccumulation of ascites and 5/23 (22%) patients were alive and had not had further paracenteses at the end of the 112-day follow-up period.

A phase II study of batimastat in malignant ascites was performed. The preliminary findings were broadly similar to those of the phase I study, although benefits from treatment appeared to be confined to patients with ovarian cancer. Further development of batimastat for this indication has been halted by the finding that the drug induced acute bowel toxicity in a significant proportion of patients in a larger study and in trials evaluating intraperitoneal administration in patients with advanced cancer without ascites (Peter Brown, British Biotech, Oxford, U.K.).

Intrapleural batimastat

Malignant pleural effusions are a common manifestation of malignancy occurring in nearly half of all patients with breast or lung cancer. The presence of a pleural effusion causes significant morbidity and is an adverse prognostic factor. Current management of malignant pleural effusions usually involves pleural drainage followed by talc pleurodesis or injection of an intracavatory sclerosing agent or cytotoxic drug. Unfortunately, this approach is able to control pleural fluid accumulation in only a third of patients. Substantial quantities of MMP-2 and MMP-9 are present in human pleural effusions, with concentrations of up to five times that in corresponding serum samples [37]. This, together with the effects of intraperitoneal batimastat on malignant ascites in animal model systems [35] prompted the clinical evaluation of the agent in malignant pleural effusions.

The phase I trial of batimastat enrolled patients with cytologically proven malignant pleural effusions who had had no prior intrapleural treatment and for whom pleural drainage was indicated. Patients with a history of cardiac disease, obstructive jaundice or surgery within the previous month were ineligible. Patients were admitted to the ward for insertion of a chest drain and the fluid drained under gravity followed by suction to ensure the effusion had been drained to dryness. A single dose of batimastat, dissolved in 50 ml 5% dextrose, was instilled into the pleural cavity and the chest drain removed. Of the patients, 3 were treated at each of the following doses: 15, 30, 60, 105, 135 and 300

mg/m^2. Patients were assessed weekly for 12 weeks for the presence and amount (measured by chest ultrasound) of any pleural fluid. At these times, blood for haematological and biochemical profiles and batimastat pharmacokinetics was drawn and dyspnoea scores, measured by linear analogue scale, were documented. Zymography of pleural fluid was performed to assess the activity of MMP-2 and MMP-9.

18 patients (12 female, 6 male; age range 39–72 years) were enrolled. Primary tumours were: breast cancer, 8 patients; adenocarcinoma of unknown primary site, 4 patients; non-small cell lung cancer, 3 patients; melanoma, renal cell cancer and mesothelioma, 1 patient each. The treatment was generally well tolerated. Acute effects attributable to batimastat were mild local discomfort (5 patients), low grade fever for up to 48 h (7 patients) and reversible elevation of serum aspartate transaminase, gamma glutamyl transferase and alkaline phosphatase (7 patients). No other changes in standard biochemical or haematological indices were observed. Batimastat was detectable in plasma 1 h after administration. Peak plasma levels achieved ranged between 12 and 170 times the IC_{50} for interstitial collagenase and 72 and 92 kDa gelatenases, 6 and 115 times that of matrilysin and 2 and 20 times that of stromelysin-1. Plans for the further evaluation of the use of intrapleural batimastat in the management of malignant pleural effusions include a randomised trial comparing the agent with standard intracavity sclerosing agents.

SECOND GENERATION INHIBITORS: ORAL BIOAVAILABILITY

Knowledge of the crystallographic structure of MMP-inhibitor complexes has improved the design of molecules with the potential of enhanced selectivity for specific MMPs, and has allowed alteration of the physicochemical properties of the inhibitor to improve its oral bioavailability [38] (Figure 3). Given the possible indication for MMPIs to be administered as long-term treatments, the quest for compounds having good oral bioavailability has been the primary goal in the development of second generation agents. The first compounds to be developed with improved oral bioavailability were Ro 31-9790 (Roche), marimastat (BB-2516, British Biotech) and CGS 27023A (Ciba-Geigy). All have hydroxamic acid as the zinc-binding group, as do galardin and batimastat. MMPIs with alternative zinc-binding groups have recently entered development.

Ro 31-9790 has not yet been extensively tested in the clinic and may shortly be replaced by another second generation MMPI developed jointly by Roche and Agouron. CGS 27023A is currently undergoing phase I clinical trials. Marimastat has been tested extensively in healthy volunteers and cancer patients. Single and multiple doses (of up to 200 mg twice daily for 6.5 days) were well tolerated in healthy volunteers and proved to have good oral bioavailability. Marimastat has also been studied in a series of phase I studies in cancer patients examining the effect of the MMPI on the serum concentration of cancer-associated antigens. Patients with inoperable cancer in whom the relevant antigen had increased by more than 25% over 28 days were enrolled into a single arm study of marimastat. The tumour markers studied were CA125 in ovarian cancer, CEA in colorectal cancer, CA19-9 in pancreatic cancer and PSA in prostatic cancer. Patients were treated for a period of 28 days at doses ranging from 5 mg once daily to 75 mg twice daily. Variability was observed in antigen values, both before and during treatment. However, there were indications of a treatment-related reduction in tumour marker concentrations [39, 40], and the relevance of these findings has been discussed in the medical press [41].

In patients receiving oral marimastat for 28 days or longer, the principle dose-limiting toxicity was musculoskeletal symptoms of pain and tenderness in muscles, tendons and joints, predominantly affecting the shoulders and hands. The majority of patients receiving marimastat at a doses of 25 or 50 mg twice daily developed musculoskeletal side-effects within 3 to 4 months of treatment. At 10 mg twice daily, the side-effects were generally milder and less frequent with approximately one third of patients experiencing discomfort after 3 to 5 months. The precise cause of the musculoskeletal pain has not been established and may be related to impairment of the normal processes governing tissue remodelling in tendons and joints. It will be of interest to establish whether this side-effect is encountered with other MMPIs as they enter clinical development.

In the course of clinical trials in patients with cancer, it has been observed that the trough plasma marimastat concentrations are approximately 2- to 3-fold higher than predicted from the phase I studies in healthy volunteers. Reduced drug clearance and increased protein binding with age and in cancer patients may account, in part, for these differences. The mean 12 h trough marimastat concentration in patients receiving 10 mg twice daily was 81.9 ng/ml, approximately 40 times the IC_{50} of marimastat for collagenase and gelatinase. A population pharmacokinetic analysis in cancer patients has given a derived elimination half-life for marimastat of 10–14 h.

CONSIDERATIONS FOR THE CLINICAL STUDY OF MMPIs IN CANCER

As with any novel agent undergoing early clinical evaluation, the primary aims of phase I studies of MMPIs are to establish safe doses and schedules which deliver the agents to the biological target at appropriate concentrations. As the MMPIs are thought to act by prevention of tumour cell

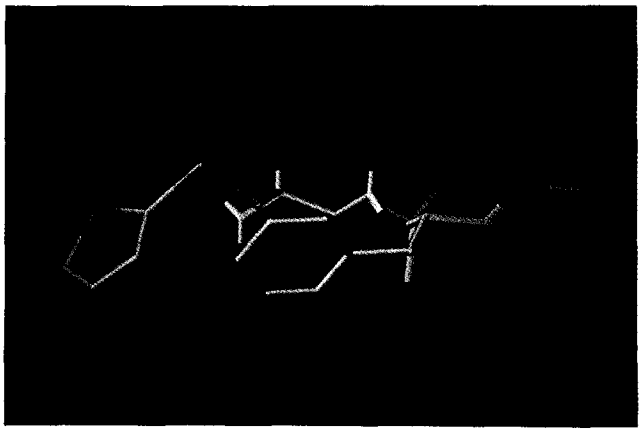

Figure 3. X-ray crystal structure of collagenase-hydroxamate MMP inhibitor complex viewed with a Connoly surface. The model structure gives information on space available for larger substitutent groups which are likely to be important in the design of selective MMP inhibitors.

invasion and metastasis and by inhibition of tumour-induced angiogenesis, rather than by cytotoxic mechanisms, the use of commonly employed clinical endpoints of cytoreduction may not be appropriate in single agent studies. Within the limitations of toxicity, the goal in clinical evaluation of this class of agent should be to ensure that appropriate pharmacodynamic endpoints are established. Preclinical investigation has confirmed that the MMP family of enzymes are involved in normal tissue remodelling processes, including wound healing and bone fracture repair. Although little opportunity exists in evaluating these processes in phase I studies in cancer patients, measurement of urinary products of bone turnover, laser doppler probes to determine capillary blood flow, PET scanning to quantify angiogenesis and zymographic determination of intratumour ratios of the activated pro-enzymatic forms of MMP-2 and 9 are being employed to monitor the effects *in vivo* in current clinical trials of MMPIs. Sensitive assays of individual MMPIs are essential in determining the pharmacokinetic parameters of these agents and have been important in demonstrating the prolonged plasma half-life of batimastat following intrapleural or intraperitoneal administration and confirming the good bioavailability of marimastat and CGS 27023A.

It should be anticipated that, by virtue of their mode of action, the toxicity profile of the MMPIs would differ markedly to cytotoxic drugs. Monitoring of toxicity should reflect this by the introduction of methods for assessing adverse effects predicted from the known biochemical and physiological interactions of the agent and grading unusual toxicities as they arise. Inhibitory activity against other biologically important zinc-dependent MMPs, such as angiotensin converting enzyme and neural endopeptidase 24.11, which functions in the degradation of naturetic factor, has usually been assessed during drug development of MMPIs for cancer therapy. Other interactions that may be of concern involve the inhibition of cytokine processing enzymes by MMP inhibitors. Since these have only been partially defined, it is possible that an, as yet, unidentified enzyme will be inhibited causing a change in cytokine concentrations. Routine biochemical and haematological clinical monitoring should be sufficient to detect any significant changes that might result.

CONCLUSION

The increase in tissue concentrations of metalloproteinase immunoreactivity occurring in a wide range of malignant diseases including stomach, head and neck, prostate, breast, ovarian, non-small cell lung cancer, colon and melanoma clearly marks these enzymes as worthy targets for therapeutic intervention. Early clinical trials have established the safety of a number of MMPIs and the next stage will include phase II studies in a wide range of tumour types and appropriate placebo-controlled randomised studies. As the MMPIs do not exhibit cytotoxic activity *per se*, their role in combination with standard antitumour agents should be evaluated in randomised comparisons of cytotoxic chemotherapy in combination with MMPI against chemotherapy alone. Marimastat, for example, is currently being studied in a randomised comparison with gemcitabine in patients with advanced pancreatic cancer and is due to commence double-blind placebo-controlled studies in other malignancies.

The physiological mechanisms by which MMPIs exert their effect in tumour angiogenesis suggest they may have a potential role to play early in the natural history of malignancy, perhaps in the adjuvant setting or for the management of low-bulk disease. The well established role that matrix metalloproteinases play in angiogenesis could be exploited in the treatment of highly vascular tumours. The indications from studies of experimental animal haemangiomas, for example, indicate that MMPIs are able to reduce growth *in vivo*, probably by blockade of endothelial cell recruitment in angiogenesis [31]. The focus in recent years has been in the design of specific inhibitors of MMPs, yet therapy in the future may include the specific downregulation of tumour metalloproteinase activity by antisense oligonucleotides or by transfection of cDNA of tissue inhibitors of MMP-2, a process shown to be possible *in vivo* by retroviral gene transfer [42]. These avenues await clinical evaluation.

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