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Review Article

Fumagillin class inhibitors of methionine aminopeptidase-2

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CONTENTS

Abstract

The growth of solid tumors and the formation of metastases are critically dependent on neovascularization. This dependence on neovascularization, however, is not limited to cancer but is also a major contributing factor in the pathology of autoimmune diseases such as rheumatoid arthritis. Hence, antiangiogenic therapy was recognized as a potentially powerful therapeutic approach in the treatment of these devastating diseases. The discovery of fumagillin and its potent antiangiogenic and antiproliferative activities provided the rationale for the development of fumagillin analogues as a novel class of antiproliferative agents. Molecules of the fumagillin class inhibit the enzymatic activity of methionine aminopeptidase-2 (MetAP-2), and this inhibition is the first step required for the selective growth inhibition of cell types that are dependent on MetAP-2 function for growth. Mechanistically, this growth inhibition is characterized by cytostasis and arrest in the late G1 phase of the cell cycle. TNP-470 was the first MetAP-2 inhibitor of the fumagillin class to enter phase I clinical trials in cancer. Despite some encouraging results in several solid tumors in early phase I/II studies, further clinical development of this molecule was halted, primarily due to dose-limiting neurotoxicities and a poor clinical pharmacokinetic profile. More recently, fumagillin analogues specifically designed to improve upon the clinical deficiencies of TNP-470, such as PPI-2458, have advanced into phase I trials in cancer.

Introduction

Angiogenesis is the growth of new capillary blood vessels from the preexisting vasculature, and is tightly regulated in physiological processes such as the female reproductive cycle and wound healing. In cancer and autoimmune diseases, however, neovascularization has been recognized as a major factor in the pathology of disease. The concept of antiangiogenesis as a novel therapeutic strategy in cancer was first proposed by Folkman, supported by observations in animal models that the growth of solid tumors was restricted to 2-3 mm in size in the absence of neoangiogenesis (1). Subsequent clinical research has confirmed angiogenesis as a critical secondary target to the tumor cell in cancer therapy. Moreover, the potential utility of antiangiogenic therapies is not limited to cancer and has been extended to several autoimmune diseases. In rheumatoid arthritis (RA), pathological angiogenesis is known to support aggressive synovial hyperplasia, which ultimately leads to joint destruction, and in psoriasis, neovascularization is considered vital to the growth and remodeling of psoriatic plaques. Thus, antiangiogenic therapies are also thought to be an attractive therapeutic approach in autoimmune diseases.

In the late 1980s, the discovery of fumagillin and its potent antiangiogenic and antiproliferative activities initiated the development of fumagillin analogues with improved pharmacological properties as a novel class of antiproliferative agents (2). In 1992, TNP-470, a semisynthetic fumagillin analogue, appeared on the verge of realizing their great promise when it became the first molecule of this class to enter phase I clinical trials in cancer. In the late 1990s, methionine aminopeptidase-2 (MetAP-2) was identified as the molecular target of this class of molecules (3, 4), and the crystal structure of human MetAP-2 complexed with fumagillin was determined (5). Published reports of preclinical studies and clinical trials of TNP-470 further stimulated the development of additional pharmacologically improved fumagillin analogues. Although a large number of these analogues has been reported in different phases of discovery and

development, only two other molecules of this class have advanced into phase I clinical trials in cancer: CKD-732 and PPI-2458.

In this review we describe mechanism-related studies which led to the elucidation of the cytostatic G1 cell cycle arrest in sensitive cell types after the exposure to fumagillin analogues, the discovery of MetAP-2 as their common molecular target, and the inhibition of the enzymatic activity of MetAP-2 as the first pivotal step in cell growth inhibition. Moreover, we describe the preclinical development of fumagillin, CKD-732, TNP-470 and PPI-2458, and summarize the current clinical status of these molecules. Finally, we discuss the clinical potential of fumagillin analogues as a novel class of therapeutics for the treatment of hyperproliferative diseases.

Mechanism of cell cycle regulation

Most studies to elucidate the molecular mechanism of cell growth inhibition by molecules of the fumagillin class were carried out with TNP-470, following the original observation that fumagillin potently inhibited the growth of human umbilical vein endothelial cells (HUVEC) with a GI_{50} of ~1.2 nM (2). This growth inhibition followed a biphasic inhibition curve. While the first phase was characterized by a cytostatic, completely reversible mechanism of growth inhibition (complete inhibition range: 750 pM to 7 μM, GI_{50}: 37 pM), irreversible growth arrest and cytotoxicity were observed during the second phase at concentrations of 70 μM (6). The ability of fumagillin analogues to induce growth arrest at low concentrations, however, appeared to be restricted to a limited number of sensitive cell types (6-8), while direct cytotoxicity at high concentrations extended over a wide range of different cell types (6).

In HUVEC, fumagillin analogues were shown to block cell cycle progression into S phase by arresting the cells in the late G1 phase of the cell cycle, while early and mid-G1 events such as the expression of the immediate early genes *c-fos* and *c-myc* and protein tyrosyl phosphorylation were not affected (6, 9). This late G1 cell cycle block is most likely triggered through the activation of the p53 pathway and the accumulation of the cell cycle inhibitor protein $p21^{CIP/WAF}$. The function of the p53 signaling pathway, and particularly the critical roles of the p53 and $p21^{CIP/WAF}$ cell cycle regulators in the induction of growth inhibition in HUVEC was confirmed by the exposure of pulmonary endothelial cells from mice genetically deficient in either the p53 ($p53^{-/-}$) or the $p21^{CIP/WAF}$ ($p21^{-/-}$) genes to TNP-470. These cells were completely resistant to TNP-470-induced growth inhibition (10, 11). The mechanism of p53/$p21^{CIP/WAF}$ dependence on growth inhibition has not yet been observed in other cell types and is believed to be specific for HUVEC (10, 11).

Several other important cell cycle regulators have also been reported to be involved in the G1 arrest after the inhibition of MetAP-2 enzyme activity by fumagillin analogues. The hyperphosphorylation of the retinoblastoma (Rb) tumor suppressor protein during the late G1 phase of the cell cycle, which is essential for the release of the S phase-promoting transcription factor E2F from its stable association with the hypophosphorylated form of Rb, was shown to be inhibited, thus lending further support for the observed mechanism of a G1 cell cycle arrest. Several other mechanistic studies in HUVEC have linked the G1 arrest to the inhibition of cyclin-dependent kinase activation (CDK2, CDK4) (9) and the suppression of cyclin D1 expression during this phase of the cell cycle (12). Finally, a more recent study has shown that the exposure of HUVEC and human fibroblast-like synoviocytes from RA patients (HFLS-RA) to the fumagillin analogue PPI-2458 resulted in a marked decrease in the expression of yet another major cell cycle-regulatory protein, proliferating cell nuclear antigen (PCNA) (7). More importantly, this decrease was found to be directly proportional to the amount of MetAP-2 enzyme inhibited, thus suggesting a molecular link between the inhibition of MetAP-2 and PCNA in the induction of G1 cell cycle arrest (7).

Molecular target MetAP-2

All eukaryotic cells express two catalytically active isoforms of a class of metallopeptidases, MetAP-1 and MetAP-2 (13-15). These enzymes catalyze the cotranslational removal of the *N*-terminal initiator methionine from nascent polypeptide chains, a central step in protein maturation (13, 14). This processing step is an important prerequisite in the regulation of a number of cellular processes such as protein targeting and turnover and cell proliferation. Although both MetAPs share the same general substrate specificity, selective differences in the hydrolysis of the initiator methionine are determined by the penultimate residue, which is mostly a small and uncharged residue. MetAP-1 and MetAP-2 share a high degree of structural conservation (extended *N*-terminal domain, *C*-terminal domain with conserved metal cofactor binding residues), although they have highly divergent primary structures. The most distinguishing structural motif between both isoforms is an approximately 60-amino-acid insert of yet undetermined function in the catalytic domain of MetAP-2 which is conserved from archaebacterial to human MetAP-2 (Fig. 1). Both MetAP isoforms are further distinguished by a noncatalytic function unique for MetAP-2. The association of MetAP-2 with the eukaryotic initiation factor-2α subunit (eIF-2α) protects this factor from inhibitory phosphorylation; thus, MetAP-2 serves as a positive regulator of translation (16, 17). Genetic studies in yeast have demonstrated an essential function for MetAP-2 in the control of cell growth (18, 19), and a similar role for MetAP-2 has been suggested in the regulation of mammalian cell growth (7, 8, 20-23). A recent report which investigated the roles of MetAP-1 and MetAP-2 in the control of mammalian cell proliferation provided further experimental evidence to support a critical role for MetAP-2 in the control of mammalian cell proliferation (8).

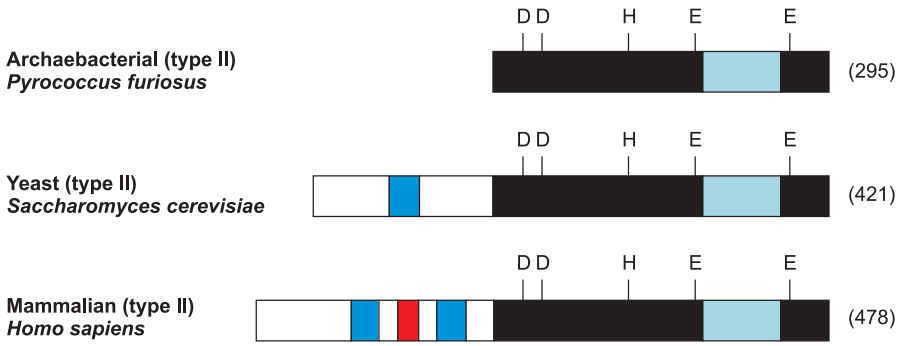

Fig. 1. Structures of methionine aminopeptidases-2 (MetAP-2). The different functional domains and structural motifs are presented in color. Catalytic domain (black), conserved 60-amino-acid insert diagnostic for MetAP-2 (*vs.* MetAP-1) (light blue), stretch of basic amino acids (dark blue) and stretch of acidic amino acids (red). The full-length protein (number of amino acids) is shown in parentheses. The DDHEE motif presents the conserved metal cofactor binding residues (corresponding to D251, D262, H331, E364 and E459 in human MetAP-2). References for MetAP-2: *Pyrococcus furiosus* (89), *Saccharomyces cerevisiae* (19) and *Homo sapiens* (13, 90, 91).

In 1997, two groups independently reported the identification of MetAP-2 as the molecular target of fumagillin and ovalicin, a natural product structurally related to fumagillin (3, 4). The first group tested the hypothesis that biologically active epoxide-containing natural products such as fumagillin are able to form covalent adducts with cellular proteins. This approach led to the identification of a 67-kDa fumagillol-binding protein from HUVEC as MetAP-2 (4). Alternatively, the second group identified MetAP-2 as a 67-kDa protein from cell lysates of bovine aortic endothelial cells (BAEC) or 14.5-day-old mouse embryos which was covalently bound to photoaffinity-labeled ovalicin (3). The crystal structure of human MetAP-2 complexed with fumagillin resolved the atomic nature of this interaction and provided an explanation for the observed binding selectivity of this class of inhibitors to MetAP-2 over the MetAP-1 isoform (5). This model revealed that the covalent bond is formed between the carbon of the reactive spiroepoxide of fumagillin and the imidazole nitrogen of histidine-231 in the catalytic site of MetAP-2 (5). The critical function of histidine-231 for catalysis was independently confirmed by site-directed mutagenesis of this residue (24). Despite a similar topology of the catalytic site in MetAP-1, relatively minor structural differences, particularly the narrowing of the specificity pocket, were found to determine the observed selectivity of fumagillin for MetAP-2 (5). These conclusions were further supported in MetAP-2 homology models and molecular dynamic simulations (25).

The discovery of MetAP-2 as the molecular target of this class of inhibitors and the critical role of this enzyme in mammalian cell growth established MetAP-2 as a potentially promising clinical target and provided the rationale for the development of molecules of the fumagillin class as novel therapeutic agents for the treatment of hyperproliferative diseases.

Preclinical development and clinical experience

The potential value of MetAP-2 as a novel therapeutic target accelerated the development of novel fumagillin analogues with pharmacologically improved properties over the parent compound. The synthesis and functional characterization of a significant number of these analogues have been reported (Table I); four molecules of this class, fumagillin, CKD-732, TNP-470 and PPI-2458, have reached clinical status (Table II). Fumagillin class inhibitors of MetAP-2 are the most potent and selective inhibitors of this enzyme; however, the development of other classes of structurally divergent inhibitors, most notably the bestatin class of pseudosubstrate-like reversible inhibitors, has also been reported. Their development will not be covered in this review (for information see reference 26).

Fumagillin

Fumagillin was first isolated in 1949 as a fungal metabolite from *Aspergillus* sp. (27). The chemical structure of this molecule is characterized by the presence of six stereogenic centers, a functionalized diol and two epoxide rings (Fig. 2). Early preclinical studies in the 1950s showed the efficacy of fumagillin against several enteric protozoan parasites, particularly *Entamoeba histolytica* (28), and clinical efficacy was subsequently shown in the treatment of patients with intestinal amebiasis (29). Moreover, several studies in the mid-1990s reported therapeutic benefit after topical treatment of microsporidial keratoconjunctivitis (30, 31). In phase I/II clinical trials of HIV-1 patients with intestinal microsporidiosis caused by chronic *Enterocytozoon bieneusi* infection, fumagillin demonstrated promise as an orally administered agent. Transient clearance of microsporidia was observed in patients receiving either 10, 20 or 40 mg/day for 14 days, while complete clearance was observed in most patients in the high-dose group (60 mg/kg/day for 14 days), with no parasitic relapse reported during a 10-12-month follow-up period (32-34). The most serious adverse events observed included thrombocytopenia and neutropenia (32-34). Fumagillin is currently in phase III clinical trials for this indication.

Table I: Efficacy of fumagillin class MetAP-2 inhibitors in animal models of cancer and arthritis.

Tumor type	Agent	Therapeutic regimen	Tumor growth inhibition	Ref.
Melanoma (B16F6), mouse	TNP-470	Dose: 10, 30, 60 mg/kg sc, days 1, 3, 6, 8, 10	Dose-dependent GI (max. 84%) on day 13	41
Reticulum cell cancer (M5076), mouse	TNP-470	Dose: 2, 5, 15 mg/kg sc qdx12	Dose-dependent GI (max. 90%) on day 13	41
Renal cell carcinoma (Renca), mouse	TNP-470	Dose: 10, 30, 90 mg/kg sc, days 1, 3, 5, 7, 9	Dose-dependent GI (max. 77%)	76
Lymphoma/leukemia (TLL-M), mouse	TNP-470	Dose: 30 mg/kg sc qod, days 1-15	Significant reduction of tumor load and tumor mass in infiltrated organs (increased apoptosis)	77
Human prostate cancer xenograft (PC-3)	TNP-470	Dose: 50, 100, 200 mg/kg sc 1x/week, start: day 19	Dose-dependent GI (max. 96%) on day 66; toxicity (deaths) at 200 mg/kg	40
Human breast cancer xenograft (MDA-MB-231)	TNP-470	Dose: 50, 100, 200 mg/kg sc 1x/week, start: day 28	Dose-dependent GI (max. 88%) on day 70; toxicity (deaths) at 200 mg/kg	40
Human pancreatic cancer xenograft (MIAPaCa-2)	TNP-470	Dose: 30 mg/kg sc qod for 14 weeks, start: day 3	Significant GI (~ 50% reduction in tumor volume) and reduction in neoangiogenesis	78
Human uterine cancer xenograft (FU-MMT-1)	TNP-470	Dose: 30 mg/kg sc 3x/week, start: day 21	Significant GI (max. 65%) on day 56	79
Human liver cancer xenograft (SNU-398)	CKD-732	Dose: 50 mg/kg sc 2x/day, start: day 12	GI (max. 50%) on day 26	36
Melanoma (B16F10), mouse	PPI-2458	Dose: 3, 10, 30, 100 mg/kg po qod, days 6-20; 100 mg/kg po qod, q3d, q4d	Significant dose- and schedule-dependent GI (~ 65%) on day 20	80
Human lymphoma xenograft (SR)	PPI-2458	Dose: 15, 30 mg/kg po or sc qdx5 for 5 weeks	Significant GI (max. 57%)	81
Tumor type and metastasis	**Agent**	**Therapeutic regimen**	**Inhibition of metastasis**	**Ref.**
Melanoma (B16F6), mouse, lung	TNP-470	Dose: 30, 60 mg/kg sc 3x/week, start: day 1	Reduction of number and size of metastatic foci on day 15; 56% extended mean survival	41
Renal cell cancer (Renca), mouse, liver and lung	TNP-470	Dose: 10, 30, 90 mg/kg sc, days 1, 3, 5, 7, 9	Dose-dependent decrease (lung: 91%, day 14; liver: 92%, day 21) and size of metastatic foci	76
Human colon cancer xenograft (TK-9), liver	TNP-470	Dose: 20, 30 mg/kg sc qod, start: day 10	Complete inhibition of metastasis (high dose) on day 42	82
Human breast cancer xenograft (MDA-MB-231), bone	TNP-470	Dose: 30 mg/kg sc 3x/week for 4 weeks, start: day 1	60-70% reduction in the number and area of osteolytic bone metastases	83
Human fibrosarcoma xenograft (HT-1080), lymph nodes	TNP-470	Dose: 10, 30, 100 mg/kg sc qod for 18 days, start: day 7	Dose-dependent inhibition of metastasis on day 27	84
Human gastric cancer xenograft (AZ-H5c), liver	TNP-470	Dose: 15, 30 mg/kg sc qod for 5 weeks, start: day 10	Complete inhibition of metastasis (high dose) on day 45	85
Tumor type	**Combination**	**Therapeutic regimen**	**Inhibition of tumor growth and metastasis**	**Ref.**
Human prostate cancer xenograft (PC-3)	TNP-470 and Cisplatin	Dose: TNP-470: 100 mg/kg sc 1x/week; Cisplatin, 5 mg/kg sc 1x/week, start: day 19	Significantly enhanced GI (day 49) compared to either single agent	40
Human neuroblastoma xenograft (CHP-134)	TNP-470 and CPP	Dose: CPP, 450 mg/kg ip over 6 days; TNP-470, 5 mg/kg, sc 1x/week, start: day 10	Significantly enhanced GI (day 30) compared to either single agent	86
Human pancreatic cancer xenograft (HPC-3H4), liver metastasis	TNP-470 and Cisplatin	Dose: TNP-470: 90 mg/kg sc qod for 4 weeks; Cisplatin: 0.25 mg/kg ip qd5/week for 4 weeks, start: day 1	Significantly enhanced GI (day 30) and reduction in the number of metastatic foci compared to either single agent	87
Human bladder cancer xenograft (KOTCC-1), lymph node metastasis	TNP-470 and Gemcitabine	Dose: TNP-470, 15 mg/kg/day sc for 21 days; Gemcitabine, 60 mg/kg ip 1x/week for 3 weeks, start: day 7	Synergistic effect on GI (day 35) and lower incidence of lymph node metastasis compared to either single agent	42

Continuation

Table I Cont.: Efficacy of fumagillin class MetAP-2 inhibitors in animal models of cancer and arthritis.

Arthritis model	Agent	Therapeutic regimen	Effect on arthritis	Ref.
Collagen-induced arthritis (CIA), rat	TNP-470	PP: Dose: 27 mg/kg sc qod, start: day 2; SP: Dose: 27 mg/kg sc qod, start: day 10	PP: Prevention of clinical arthritis (day 28) SP: Significant suppression of established disease (day 28)	46
Adjuvant-induced arthritis (AA), rat	TNP-470	PP: Dose: 27 mg/kg sc qod, start:day 2; SP: Dose: 27 mg/kg sc qod, start: day 12	PP: Significantly reduced incidence of arthritis and severity (day 28); SP: Significant reduction in the mean arthritic index	48
Collagen-induced arthritis (CIA), rat	TNP-470 and Paclitaxel	SP: Dose: TNP-470, 27.5 mg/kg, sc qod; Paclitaxel, 7.5 mg/kg ip qod, start: day 10	Significant reduction of clinical arthritis; reduction earlier and greater compared to either single agent	49
KRN/NOD transgenic mouse (spontaneous development of arthritis 27 days after birth)	TNP-470	PP: Dose: 30, 60, 90 mg/kg sc qod, days 23-41; SP: Dose: 60 mg/kg sc, after onset of disease for 20 days	PP: Dose-dependent delay of disease onset; SP: Alleviation of clinical symptoms of disease	47 47
Peptidoglycan/polysaccharide-induced arthritis (PGPS), rat	PPI-2458	SP: Dose: 0.25, 1, 5, 50 mg/kg po qod, start: day 15	Significant dose-dependent suppression of established disease (day 31)	7

For each tumor type, the tumor and/or metastasis-inducing cell line is shown in parentheses. For further review on the evaluation of these inhibitors in animal models of cancer, see references 39, 59, 60 and 97, and in animal models of arthritis, see reference 98. Abbreviations: qd, every day; qod, every other day; q3d, every third day; q4d, every fourth day; ip, intraperitoneal; po, oral; sc, subcutaneous; CPP: cyclophosphamide; GI, growth inhibition; PP, prevention protocol; SP, suppression protocol.

In 1990, the serendipitous rediscovery of fumagillin as a natural product secreted from the fungus *Aspergillus fumigatus fresenius*, and the potent antiangiogenic and antiproliferative activities found to be associated with this agent, led to the development of fumagillin analogues and structurally related molecules as a novel class of powerful antiproliferative agents. However, the development of fumagillin as a cancer therapeutic was not actively pursued due to severe toxicity observed in animal models of cancer (> 15% body weight loss) (37).

CKD-732

CKD-732 a semisynthetic fumagillin analogue, was designed by structure-based molecular modeling based on the crystal structure of human MetAP-2 complexed with fumagillin (Fig. 2) (35). Similar to other fumagillin analogues, CKD-732 has shown potent antiangiogenic activity *in vitro* (GI_{50}: 2.5 nM on HUVEC) due to a cytostatic G1 cell cycle arrest (36). CKD-732, however, significantly differs from other molecules of this class in that its growth-inhibitory activity does not follow the characteristic biphasic mode of cell growth inhibition. The exposure of HUVEC to this agent resulted in the induction of apoptosis at relatively low concentrations (ED_{50}: 25 nM), while cytotoxicity of other fumagillin analogues has only been observed at concentrations of > 10 μM. Antitumor activity of CKD-732 has recently been reported in several mouse models of cancer (Table I) (36, 37).

Acute toxicology studies in mice (10-50 mg/kg i.v., 4-h observation period postinjection) have shown minor toxicities; however, no results from chronic toxicology studies have been reported (38). CKD-732 is currently in phase I clinical trials in cancer (Table II).

TNP-470

AGM-1470, later termed TNP-470 (Fig. 2), is a semisynthetic analogue of fumagillin and was reported to possess approximately 50 times greater potency in the *in vitro* growth inhibition of endothelial cells (ECs) than its parent compound (Fig. 2) (2). The same study reported potent antitumor activity of TNP-470 in several mouse tumor models, and a significantly improved toxicity profile when these animals were treated with TNP-470 (30 mg/kg s.c. on alternate days for 100 days). Following this initial report, TNP-470 was shown to have broad antiproliferative activity *in vitro* against a wide panel of tumor cell lines of different origin (for review see 39 and references therein), and significant antitumor activity was observed in a large number of experimental rodent and human xenograft tumor models (Table I). The observed growth inhibition of primary tumors in these models, as well as the dose-dependent inhibition of metastasis formation, was predominantly attributed to the antiangiogenic activity of this agent, rather than a direct effect on the tumor cells (40). Several animal studies have also shown a survival benefit after treatment with TNP-470 (41). Finally, combination therapy of TNP-470 with conventional cytotoxic agents appeared to potentiate the efficacy of several cytotoxic therapies against primary and metastatic disease (4, 42-45); however, significant variability among different model systems has been noted.

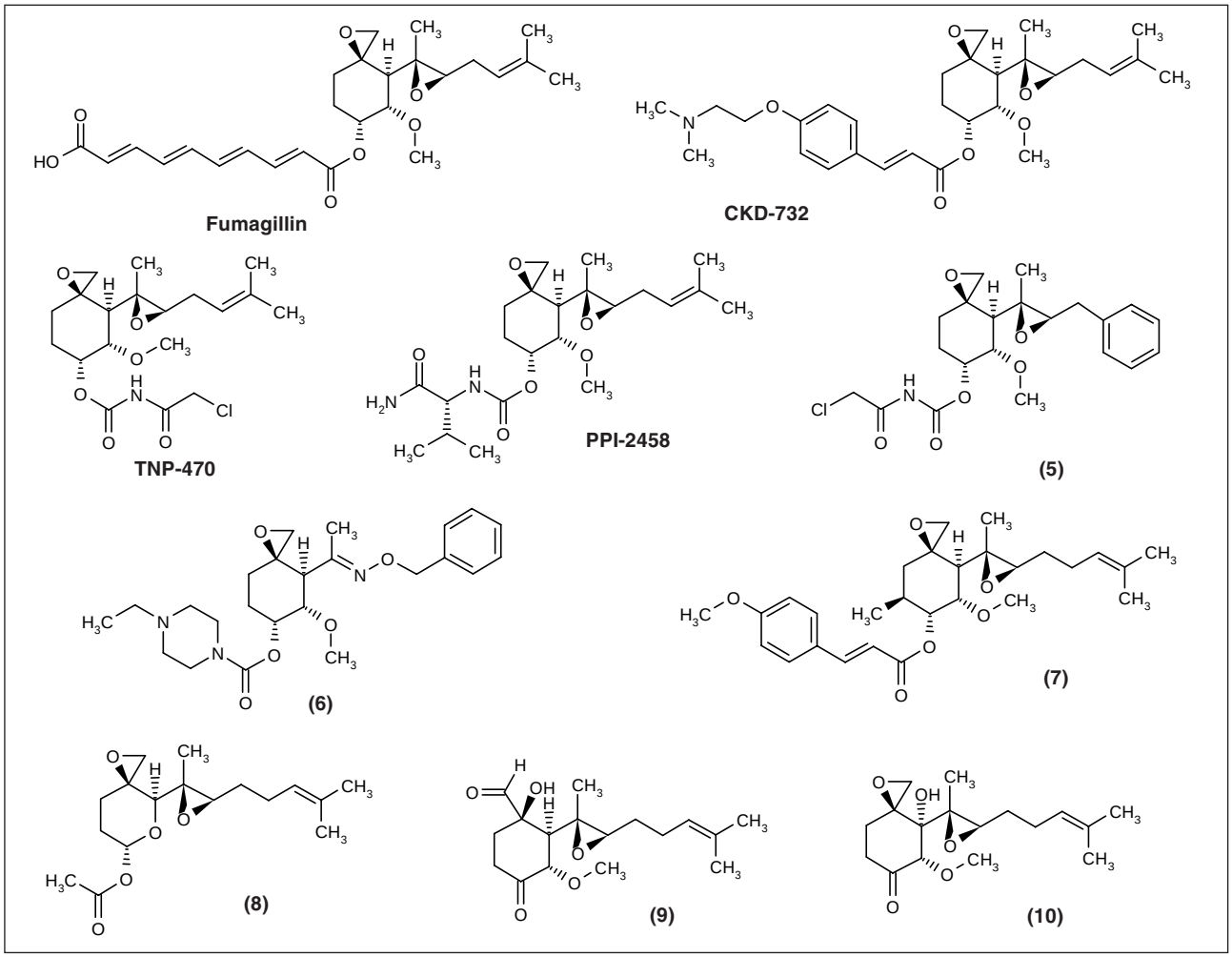

Fig. 2. Chemical structures of fumagillin class MetAP-2 inhibitors. Fumagillin, TNP-470, PPI-2458 and CKD-732 have reached clinical development status, while the inhibitors **5-10** have been reported to be in different phases of discovery and development. For more detailed information on the latter compounds, see references 59 (**5**), 92 (**6**), 93 (**7**), 94 (**8**), 95 (**9**) and 96 (**10**). Ovalicin (**10**) is a natural product which is structurally closely related to fumagillin. The inclusion of this molecule is intended to indicate that ovalicin also acts as a potent and irreversible inhibitor of MetAP-2.

The recognition of angiogenesis as an attractive target in the pathogenesis of inflammatory arthritis led to the successful evaluation of TNP-470 as the first angiogenesis inhibitor in an animal model of arthritis (46), and significant activity of this agent was later shown in multiple models of this disease (Table I). Administration of TNP-470 prior to the onset of disease in rats significantly prevented the onset of arthritis, while markedly attenuated severity of disease was observed when TNP-470 was administered to animals with established disease (46-48). Moreover, in combination therapy with ciclosporin or paclitaxel, the efficacy was significantly greater than that of either single agent in diminishing the severity of arthritis in rat models of this disease (49, 50).

The powerful antiangiogenic and antiproliferative properties of TNP-470 led to the further evaluation of this agent in a number of animal models where a critical involvement of neovascularization in the pathology of disease has been suggested. TNP-470 prevented chronic allograft vasculopathy (CAV) in a rat model of chronic allograft rejection, thus suggesting antiangiogenic therapy as a potentially new therapeutic approach in transplantation (51). In a mouse model of peritoneal fibrosis, TNP-470 suppressed the angiogenesis-dependent progression of peritoneal fibrosis, suggesting a potential therapeutic benefit to patients on long-term peritoneal dialysis (52). Finally, TNP-470 has shown potent antiproliferative activity *in vitro* against the malaria and *Leishmania* parasites *Plasmodium falciparum* and *Leishmania donovani* (53), respectively, and in a mouse model of microsporidiasis, it prolonged survival and prevented the development of ascites (54).

The importance of angiogenesis in normal physiological processes such as wound healing and the female

Table II: Clinical experience of fumagillin class MetAP-2 inhibitors in cancer.

Tumor type	Therapeutic agent	Clinical status[1]	Objective response[2]	Ref.
AIDS-related Kaposi's sarcoma	TNP-470	Phase I (39)	PR (7/39)	64, 72
AIDS-related Kaposi's sarcoma	TNP-470	Phase I (13)		71
Advanced squamous cell cancer of the cervix	TNP-470	Phase I (18)	CR (1/18) PR (3/18)	60, 69 66
Metastatic prostate cancer	TNP-470	Phase I (33)		
Solid tumors (10)[3]	TNP-470	Phase I (36)	PR (3/36)	65
Solid tumors (9)[4]	TNP-470 + paclitaxel	Phase I (32)	PR (8/32)	70
Solid tumors (4)[5]	TNP-470 + carboplatin + paclitaxel	Phase I (17)	PR (3/17)	88
Metastatic renal carcinoma	TNP-470	Phase II (33)	PR (1/33)	67
Metastatic breast cancer	TNP-470	Case study (1)	PR (1/1)	68
Non-Hodgkin's lymphoma	PPI-2458	Phase I		
NR	CKD-732	Phase I		

[1]The number of subjects enrolled in each trial is shown in parentheses. [2]Objective responses are limited to either complete or partial responses. Subjects who had stable disease or achieved other clinical benefits for some period of time are not considered. [3]Solid tumor types and number of subjects: sarcoma (12), colorectal cancer (7), melanoma (4), non-small cell lung cancer (NSCLC) (3), breast carcinoma (3), gastric adenocarcinoma (2), head and neck squamous carcinoma (2), pancreatic adenocarcinoma (1), Merkel cell carcinoma (1), spindle cell carcinoma (1). Partial responses: sarcoma (2), melanoma (1). [4]Solid tumor types and number of subjects: NSCLC (16), head and neck carcinoma (5), prostate cancer (4), kidney cancer (2), bladder cancer (1), cervical cancer (1), uterine cancer (1), breast cancer (1), sarcoma (1). Partial responses: NSCLC (6), NR (2). [5]Solid tumor types and number of subjects: NSCLC (11), head and neck cancer (3), sarcoma (2), thymoma (1). Partial responses: NSCLC (2), NR (1). PR: partial response; CR: complete response; NR: not reported.

reproductive system led several investigators to study the hypothesis that antiangiogenic therapy may adversely affect these processes. In several experimental mouse models of wound healing, conflicting results on the activity of TNP-470 were reported. While TNP-470 prevented the recurrence of liver metastasis after partial hepatectomy without suppressing liver regeneration or wound healing (55), significant, dose-dependent impairment of wound healing was observed in the dorsal excisional wound model (56). Similarly, different experimental outcomes have been reported on the activity of this agent on the female reproductive cycle. The administration of TNP-470 to pregnant mice between days 1 and 7 of gestation resulted in complete failure of embryonic growth (57). This effect, however, was completely reversible and functional recovery of the reproductive system was observed after 6-8 weeks (57). The ovulatory cycles of macaques and marmosets, which more closely approximate the potentially adverse effects of drug treatment in humans, were not affected by exposure of these animals to TNP-470 (58).

Toxicology studies in rats and dogs with TNP-470 administered intravenously (*i.e.*, clinical route of administration) for 90 days, followed by a 13-week recovery period, suggested a maximum tolerated dose (MTD) of 3 mg/kg (8 mg/m^2) and 0.5 mg/kg (10 mg/m^2) for rats and dogs, respectively (59, 60). The administration of doses higher than the MTD resulted in toxicities which included body weight loss in rats and body weight loss, convulsions, ataxia, tremors, cataracts, leukocytopenia and anemia in dogs. After 13 weeks of recovery, most toxicological findings were resolved in dogs, while recovery in rats was complete. In an effort to avoid drug-related toxicities while maintaining potent antiproliferative activity, two recent studies reported that TNP-470, when conjugated to a synthetic polymer, was as potent in a mouse tumor model as free TNP-470. Moreover, conjugated TNP-470 yielded no clinical signs of toxicity previously associated with the administration of free TNP-470 observed in this model (61, 62).

In 1992, TNP-470 was the first MetAP-2 inhibitor of this class to enter phase I clinical trials in cancer. Kaposi's sarcoma (KS), the most prevalent neoplasm in AIDS patients, was the first oncology indication selected (Table II). The clinical presentation of this tumor shows highly vascularized dermal lesions, suggesting a prominent role of angiogenesis in the pathology of this disease (63). Seven partial responses were obtained in 39 patients administered TNP-470, with a median duration of response of 11 weeks (64). TNP-470 was further evaluated in a large number of phase I/II solid tumor trials and showed promising early results in several indications (Table II). Published outcomes included responses in metastatic squamous cell cervical cancer (65-67). In metastatic breast cancer, 1 patient demonstrated a partial response after 3 months of TNP-470 treatment, including eye and lung disease that was undectable after 3 months of treatment and an overall reduction in the size and number of other lesions (68). One patient presenting with metastatic squamous cell carcinoma of the cervix achieved a complete response after 18 weeks of TNP-470 treatment, which lasted for more than 8 months after discontinuation of therapy. This study also included 3 patients with stable disease for periods ranging from 5 to 20 months (69). Moreover, a study of TNP-470 used in combination with paclitaxel in patients with non-small cell lung cancer (NSCLC) included 6 partial responses. NSCLC patients in this trial had a median survival time of 14.1 months (70). Despite these encouraging clinical results, however, the clinical development of TNP-470

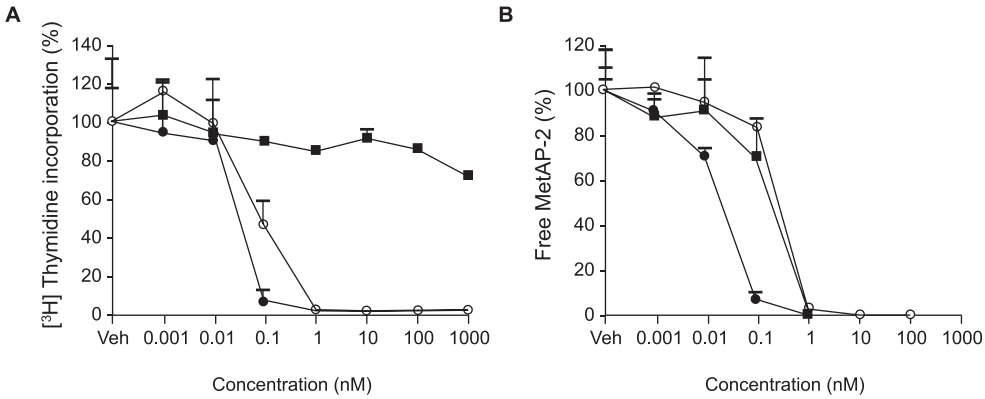

Fig. 3. PPI-2458-induced growth inhibition (A) in human fibroblast-like synoviocytes from rheumatoid arthritis patients (HFLS-RA) and human umbilical vein endothelial cells (HUVEC), but not normal human dermal fibroblasts (NHDF-Ad), is linked to MetAP-2 enzyme inhibition (B). A) HFLS-RA (●), NHDF-Ad (■) (8 x 10^3 cells) and HUVEC (○) (8 x 10^3 cells) were incubated with increasing concentrations (0.001-1000 nM) of PPI-2458 for 7 and 4 days, respectively. For the final 24 h, 1 μCi/well of [^{3}H]-thymidine was added, and proliferation was determined by [^{3}H]-thymidine incorporation. The results are representative of at least 5 independent experiments. B) The MetAP-2 assay was performed with cell lysates from HFLS-RA, NHDF-Ad and HUVEC treated exactly as described in A, except that no [^{3}H]-thymidine was added. Reproduced with permission from Ref. 7.

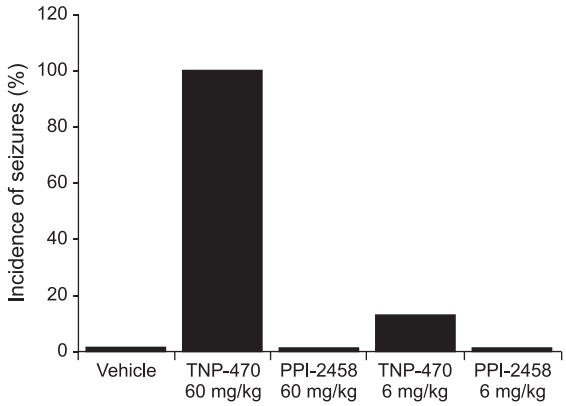

Fig. 4. Incidence of seizures after the administration of PPI-2458 and TNP-470. Sprague-Dawley rats were given PPI-2458 or TNP-470 at doses of 6 or 60 mg/kg/day i.v. for 14 consecutive days. The animals were observed daily after dosing for clinical signs of toxicity and the incidence of seizures was recorded for each treatment group (n=8; 4/sex). Reproduced with permission from Ref. 7.

was halted in phase II, primarily due to CNS toxicities (65, 66, 69) and difficulties in optimizing the dosing regimen due to its extremely short circulating half-life (65, 71, 72) and the requirement for intravenous infusion for administration. Thus, improvement of these clinical liabilities will be important for novel MetAP-2 inhibitors of this class to be successful in the clinic.

PPI-2458

PPI-2458 (Fig. 2) is a novel, orally available semisynthetic fumagillin analogue designed to maintain the potent antiproliferative activity of fumagillin class MetAP-2 inhibitors, while at the same time improving upon their toxicity profile and clinical deficiencies. PPI-2458 has shown broad antiproliferative activity against a large panel of tumor cell lines, and importantly, this *in vitro* activity has correlated with the *in vivo* activity in corresponding human xenograft models of hematological and solid tumors in mice and rodent solid tumor models (Table I). Moreover, PPI-2458 has also shown activity in multiple animal models of arthritis (7, 73, 74). This molecule is a potent inhibitor of HUVEC and HFLS-RA proliferation *in vitro* (GI_{50}: 0.2 nM and 0.04 nM, respectively) (7). Both cell types have been shown to directly contribute to the pathogenesis of RA and their activities regulate the invasive growth of the pannus that ultimately leads to joint destruction. Furthermore, the direct cytostatic growth inhibition of HUVEC and HFLS-RA was directly proportional to the amount of MetAP-2 enzyme inhibited, which suggests that MetAP-2 enzyme inhibition is the first critical step in the growth inhibition of PPI-2458-sensitive HUVEC and HFLS-RA (Fig. 3) (7). These pharmacological activities of PPI-2458 observed *in vitro* appear to translate into protective activity in animals. In the rat peptidoglycan/polysaccharide-induced (PGPS) model of arthritis PPI-2458 significantly attenuated paw swelling when administered therapeutically after the onset of chronic disease, and in a rat model of collagen-induced arthritis (CIA) PPI-2458 demonstrated significant regression of disease, as well as prevention of erosions (73, 74).

In reaction to the clinical deficiencies of TNP-470, particularly the dose-limiting neurotoxicities (65, 66, 69), PPI-2458 was designed to minimize the CNS exposure. The incidence of seizures, considered a clinical symptom of CNS toxicity, was measured in rats after administration of PPI-2458 and TNP-470 (6 or 60 mg/kg/day i.v.) for 14 consecutive days. TNP-470 at both doses demonstrated an increased incidence of seizures compared to vehicle-

treated animals. In contrast, no clinical signs of PPI-2458-associated CNS toxicity were observed (Fig. 4) (7).

PPI-2458 has advanced into phase I clinical trials in non-Hodgkin's lymphoma (NHL) (Table II). The rationale for treating NHL appears compelling as germinal center (GC) B-cells and their neoplastic counterparts express high levels of MetAP-2 (75). Moreover, animal studies have shown a concentration-dependent decrease in splenic and lymph node GC B-cells, further suggesting the potential importance of this target in NHL therapy (Cooper *et al.*, submitted).

Conclusions

Fumagillin class inhibitors of MetAP-2 have emerged as a potentially new therapeutic approach with broad clinical applications for the treatment of hyperproliferative diseases. Their pharmacological properties, characterized by potent antiangiogenic and antiproliferative activity, are due to specific molecular interactions resulting in the covalent binding and irreversible inhibition of a common molecular target, MetAP-2. TNP-470 was the first fumagillin analogue to enter clinical trials and showed promising initial results. However, further clinical development of this agent was halted, primarily due to obstacles associated with safety (dose-limiting CNS toxicities) and difficulties in optimizing the dosing regimen due to its extremely short circulating half-life and the requirement for intravenous infusion for administration. The clinical experience of TNP-470 has thus provided valuable information for the development of novel fumagillin analogues with significantly improved pharmacological properties, such as the orally active molecule PPI-2458. Clinical evaluation of this agent will determine whether these changes in the pharmacological profile of PPI-2458 have translated into the anticipated clinical benefit for PPI-2458 to demonstrate the therapeutic potential of MetAP-2 inhibitors in a broad array of disease states involving hyperproliferative cells and angiogenesis.

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References

1. Folkman, J. *Tumor angiogenesis: Therapeutic implications*. New Engl J Med 1971, 285: 1182-6.

2. Ingber, D., Fujita, T., Kishimoto, S., Sudo, K., Kanamuru, T., Brem, H., Folkman, J. *Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth.* Nature 1990, 348: 555-7.

3. Griffith, E.C., Su, Z., Turk, B.E. et al. *Methionine aminopeptidase (type 2) is the common target for angiogenesis inhibitors AGM-1470 and ovalicin*. Chem Biol 1997, 4: 461-71.

4. Sin, N., Meng, L., Wang, M.Q.W., Wen, J.J., Bornmann, W.G., Crews, C.M. *The anti-angiogenic agent fumagillin covalently binds and inhibits the methionine aminopeptidase, MetAP-2.* Proc Natl Acad Sci USA 1997, 94: 6099-103.

5. Liu, S., Widom, J., Kemp, C.W., Crews, C.M., Clardy, J. *Structure of human methionine aminopeptidase-2 complexed with fumagillin.* Science 1998, 282: 1324-7.

6. Kusaka, M., Sudo, K., Kozai, Y., Marui, S., Fujita, T., Ingber, D., Folkman, J. *Cytostatic inhibition of endothelial cell growth by the angiogenesis inhibitor TNP-470 (AGM-1470).* Br J Cancer 1994, 69: 212-6.

7. Bernier, S.G., Lazarus, D.D., Clark, E., Doyle, B., Labenski, M.T., Thompson, C.D., Westlin, W.F., Hannig, G. *A methionine aminopeptidase-2 inhibitor, PPI-2458, for the treatment of rheumatoid arthritis.* Proc Natl Acad Sci USA 2004, 101: 10768-73.

8. Bernier, S.G., Taghizadeh, N., Thompson, C.D., Westlin, W.F., Hannig, G. *Methionine aminopeptidases type I and type II are essential to control cell proliferation.* J Cell Biochem 2005, in press.

9. Abe, J., Zhou, W., Taguchi, J., Kurokawa, K., Kumada, M., Takuwa, Y. *A fumagillin derivative angiogenesis inhibitor, AGM-1470, inhibits activation of cyclin-dependent kinases and phosphorylation of retinoblastoma gene product but not protein tyrosyl phosphorylation or protooncogene expression in vascular endothelial cells.* Cancer Res 1994, 54: 3407-12.

10. Yeh, J-J.J., Mohan, R., Crews, C.M. *The antiangiogenic agent TNP-470 requires p53 and p21$^{CIP/WAF}$ for endothelial cell growth arrest.* Proc Natl Acad Sci USA 2000, 97: 12782-7.

11. Zhang, Y., Griffith, E.C., Sage, J., Jacks, T., Liu, J.O. *Cell cycle inhibition by the anti-angiogenic agent TNP-470 is mediated by p53 and p21$^{WAF1/CIP1}$.* Proc Natl Acad Sci USA 2000, 97: 6427-32.

12. Hori, A., Ikeyama, S., Sudo, K. *Suppression of cyclin D1 mRNA expression by the angiogenesis inhibitor TNP-470 (AGM-1470) in vascular endothelial cells.* Biochem Biophys Res Comm 1994, 204: 1067-73.

13. Arfin, S.M., Kendall, R.L., Hall, L., Weaver, L.H., Stewart, A.E., Matthews, B.W., Bradshaw, R.A. *Eukaryotic methionyl aminopeptidases: Two classes of cobalt-dependent enzymes.* Proc Natl Acad Sci USA 1995, 92: 7714-8.

14. Bradshaw, R.A., Yi, E. *Methionine aminopeptidases and angiogenesis*. Essays Biochem 2002, 38: 65-78.

15. Keeling, P.J., Doolittle, W.F. *Methionine aminopeptidase-1: The MAP of the mitochondrion?* TIBS 1986, 21: 285-6.

16. Datta, B., Chakrabarti, D., Roy, A.L., Gupta, N.K. *Roles of a 67-kDa polypeptide in reversal of protein synthesis inhibition in heme-deficient reticulocyte lysate*. Proc Natl Acad Sci USA 1988, 85: 3324-8.

17. Datta, B., Ray, M.K., Chakrabarti, D., Wylie, D.E., Gupta, N.K. *Glycosylation of eukaryotic peptide chain initiation factor 2 (eIF-2)-associated 67-kDa polypeptide (p67) and its possible role in the inhibition of eIF-2 kinase-catalyzed phosphorylation of the eIF-2 alpha-subunit.* J Biol Chem 1989, 264: 20620-4.

18. Chang, T.H., Teichert, U., Smith, J.A. *Molecular cloning, sequencing, deletion, and overexpression of a methionine*

aminopeptidase gene from Saccharomyces cerevisiae. J Biol Chem 1992, 267: 8007-11.

19. Li, X., Chang, Y.H. *Amino-terminal protein processing in Saccharomyces cerevisiae is an essential function that requires two distinct methionine aminopeptidases*. Proc Natl Acad Sci USA 1995, 92: 12357-61.

20. Catalano, A., Romano, M., Robuffo, I., Strizzi, L., Procopio, A. *Methionine aminopeptidase-2 regulates human mesothelioma cell survival: Role of Bcl-2 expression and telomerase activity*. Am J Pathol 2001, 159: 721-31.

21. Turk, B., Griffith, E.C., Wolf, S., Biemann, K., Chang, Y.H., Liu, J.O. *Selective inhibition of amino-terminal processing by TNP-470 and ovalicin in endothelial cells.* Chem Biol 1999, 6: 823-33.

22. Wang, J., Lou, P., Henkin, J. *Selective inhibition of endothelial cell proliferation by fumagillin is not due to differential expression of methionine aminopeptidases*. J Cell Biochem 2000, 77: 465-73.

23. Wang, J., Sheppard, G.S., Lou, P. et al. *Tumor suppression by a rationally designed reversible inhibitor of methionine aminopeptidase-2.* Cancer Res 2003, 63: 7861-9.

24. Griffith, E.C., Su, Z., Niwayama, S., Ramsay, C.A., Chang, Y.H., Liu, J.O. *Molecular recognition of angiogenesis inhibitors fumagillin and ovalicin by methionine aminopeptidase 2.* Proc Natl Acad Sci USA 1998, 95: 15183-8.

25. Klein, C.D., Folkers, G. *Understanding the selectivity of fumagillin for the methionine aminopeptidase type II*. Oncol Res 2003, 13: 513-20.

26. Lowther, W.T., Matthews, B.W. *Structure and function of the methionine aminopeptidases*. Biochim Biophys Acta 2000, 1477: 157-67.

27. Hanson, F.R., Eble, T.E. *An antiphage agent isolated from Aspergillus sp*. J Bacteriol 1949, 58: 527-9.

28. McCowen, M.C., Callender, M.E., Lawlis, J.F. Jr. *Fumagillin (H-3), a new antibiotic with amebicidal properties.* Science 1951, 113: 202-3.

29. Killough, J.H., Magill, G.B., Smith, R.C. *The treatment of amebiasis with fumagillin*. Science 1952, 115: 71-2.

30. Diesenhouse, M.C., Wilson, L.A., Correnti, G.F., Visvesvara, G.S., Grossni-Klaus, H.E., Bryan, R.T. *Treatment of microsporidial keratoconjunctivitis with topical fumagillin*. Am J Ophthalmol 1993, 115: 293-8.

31. Wilkins, J.H., Joshi, N., Margolis, T.P., Cevallos, V., Dawson, D.R. *Microsporidial keratoconjunctivitis treated successfully with a short course of fumagillin.* Eye 1994, 8: 703-4.

32. Molina, J.-M., Goguel, J., Sarfati, C. et al. *Potential efficacy of fumagillin in intestinal microsporidiosis due to Enterocytozoon bieneusi in patients with HIV infection: Results of a drug screening study*. AIDS 1997, 11: 1603-10.

33. Molina, J.-M., Goguel, J., Sarfati, C. et al. *Trial of oral fumagillin for the treatment of intestinal microsporidiosis in patients with HIV infection.* AIDS 2000, 14: 1341-8.

34. Molina, J.-M., Tourneur, M., Sarfati, C. et al. *Fumagillin treatment of intestinal microsporidiosis*. New Engl J Med 2002, 346: 1963-9.

35. Han, C.K., Ahn, S.K., Choi, N.S. et al. *Design and synthesis of highly potent fumagillin analogues from homology modeling for a human MetAP2.* Bioorg Med Chem Lett 2000, 10: 39-43.

36. Chun, E., Han, C.K., Yoon, J.H., Sim, T.B., Kim, Y.-K., Lee, K.-Y. *Novel inhibitors targeted to methionine aminopeptidase 2 (MetAP2) strongly inhibit the growth of cancers in xenografted nude model.* Int J Cancer 2005, 114: 124-30.

37. Kim, J.-H., Lee, S.-K., Ki, M.-H., Choi, W.-K., Ahn, S.-K., Shin, H.-J., Hong, C.I. *Development of parenteral formulation for a novel angiogenesis inhibitor, CKD-732, through complexation with hydroxypropyl-β-cyclodextrin*. Int J Pharmaceut 2004, 272: 79-89.

38. Kim, E.-J., Shin, W.-H. *General pharmacology of CKD-732, a new anticancer agent: Effects on central nervous, cardiovascular, and respiratory system*. Biol Pharm Bull 2005, 28: 217-23.

39. Castronovo, V., Belotti, D. *TNP-470 (AGM-1470): Mechanisms of action and early clinical development.* Eur J Cancer 1996, 32A: 2520-7.

40. Yamaoka, M., Yamamoto, T., Ikeyama, S., Sudo, K., Fujita, T. *Angiogenesis inhibitor TNP-470 (AGM-1470) potently inhibits the tumor growth of hormone-dependent human breast and prostate carcinoma cell lines.* Cancer Res 1993, 53: 5233-6.

41. Yamaoka, M., Yamamoto, T., Masaki, T., Ikeyama, S., Sudo, K., Fujita, T. *Inhibition of tumor growth and metastasis of rodent tumors by the angiogenesis inhibitor O-(chloroacetyl-carbamoyl)fumagillol (TNP-470; AGM-1470).* Cancer Res 1993, 53: 4262-7.

42. Muramaki, M., Miyake, H., Hara, I., Kawabata, G., Kamidono, S. *Synergistic inhibition of tumor growth and metastasis by combined treatment with TNP-470 and gemcitabine in a human bladder cancer KOTCC-1 model*. J Urol 2004, 172: 1485-9.

43. Teicher, B.A., Sotomayor, E.A., Huang, Z.D. *Antiangiogenic agents potentiate cytotoxic cancer therapies against primary and metastatic disease.* Cancer Res 1992, 5: 6702-4.

44. Teicher, B.A., Holden, S.A., Ara, G., Sotomayor, E.A., Huang, Z.D., Chen, Y.N., Brem, H. *Potentiation of cytotoxic cancer therapies by TNP-470 alone and with other anti-angiogenic agents*. Int J Cancer 1994, 57: 920-5.

45. Teicher, B.A., Holden, S.A., Ara, G., Kornbut, T., Menon, K. *Comparison of several antiangiogenic regimens alone and with cytotoxic therapies in the Lewis lung carcinoma*. Cancer Chemother Pharmacol 1996, 38: 169-77.

46. Peacock, D.J., Banquerigo, M.L., Brahn, E. *Angiogenesis inhibition suppresses collagen arthritis*. J Exp Med 1992, 175: 1135-8.

47. De Brandt, M., Grossin, M., Weber, A.J. et al. *Suppression of arthritis and protection from bone destruction by treatment with TNP-470/AGM-1470 in a transgenic mouse model of rheumatoid arthritis.* Arthritis Rheum 2000, 43: 2056-63.

48. Peacock, D.J., Banquerigo, M.L., Brahn, E. *A novel angiogenesis inhibitor suppresses rat adjuvant arthritis.* Cell Immunol 1995, 160: 174-84.

49. Oliver, S.J., Banquerigo, M.L., Brahn, E. *Suppression of collagen-induced arthritis using an angiogenesis inhibitor, AGM-1470, and a microtubule stabilizer, Taxol*. Cell Immunol 1994, 157: 291-9.

50. Oliver, S.J., Cheng, T.P., Banquerigo, M.L., Brahn, E. *Suppression of collagen-induced arthritis by an angiogenesis inhibitor, AGM-1470, in combination with cyclosporine: Reduction of vascular endothelial growth factor (VEGF).* Cell Immunol 1995, 166: 196-206.

51. Denton, M.D., Magee, C., Melter, M., Dharnidharka, V.R., Sayegh, M.H., Briscoe, D.M. *TNP-470, an angiogenesis inhibitor, attenuates the development of allograft vasculopathy.* Transplantation 78: 1218-21.

52. Yoshio, Y., Miyazaki, M., Abe, K. et al. *TNP-470, an angiogenesis inhibitor, suppresses the progression of peritoneal fibrosis in mouse experimental model*. Kidney Int 2004, 66: 1677-85.

53. Zhang, P., Nicholson, D.E., Bujnicki, J.M. et al. *Angiogenesis inhibitors specific for methionine aminopeptidase 2 as drugs for malaria and leishmaniasis*. J Biomed Sci 2002, 9: 34-40.

54. Coyle, C., Kent, M., Tanowitz, H.B., Wittner, M., Weiss, L.M. *TNP-470 is an effective antimicrosporidial agent.* J Infect Dis 1998, 177: 515-8.

55. Brem, H., Ehrlich, P., Tsakayannis, D.E., Folkman, J. *Delay of wound healing by the angiogenesis inhibitor TNP-470*. Surg Forum 1997, 48: 716.

56. Klein, S.A., Bond, S.J., Gupta, S.C., Yacoub, O.A., Anderson, G.I. *Angiogenesis inhibitor TNP-470 inhibits murine cutaneous wound healing.* J Surg Res 1999, 82: 268-74.

57. Klauber, N., Rohan, R.M., Flynn, E., D'Amato, R.J. *Critical components of the female reproductive pathway are suppressed by the angiogenesis inhibitor AGM-1470*. Nat Med 1997, 3: 443-6.

58. Fraser, H., Dickson, S., Lunn, S. *The effect of antiangiogenic agent TNP-470 on luteal establishment and function in the macaque and marmoset*. IBC Angiogenesis Conference 1998, Abst.

59. Bhargava, P., Dordal, M.S., Hawkins, M.J. *TNP-470*. In: Angiogenesis in Health and Disease: Basic Mechanisms and Future Directions. G.M. Rubayni (Ed.). Marcel Dekker Inc: New York 2000, 387-406.

60. Kruger, E.A., Figg, W.D. *TNP-470: An angiogenesis inhibitor in clinical development for cancer*. Exp Opin Invest Drugs 2000, 9: 1383-96.

61. Satchi-Fainaro, R., Puder, M, Davies, J.W. et al. *Targeting angiogenesis with a conjugate of HPMA copolymer and TNP-470.* Nat Med 2004, 10: 255-61.

62. Satchi-Fainaro, R., Mamluk, R., Wang, L. et al. *Inhibition of vessel permeability by TNP-470 and its polymer conjugate, caplostatin.* Cancer Cell 2005, 7: 251-61.

63. Biggar, R.J., Rabkin, C.S. *The epidemiology of AIDS-related neoplasms*. Hematol Clin North Am 1996, 10: 997-1010.

64. Dezube, B.J., Von Roenn, J.H., Holden-Wiltse, J. et al. *Fumagillin analog in the treatment of Kaposi's sarcoma: A phase I AIDS clinical trial group study.* J Clin Oncol 1998, 16: 1444-9.

65. Bhargava, P., Marshall, J.L., Rizvi, N. et al. *A phase I and pharmacokinetic study of TNP-470 administered weekly to patients with advanced cancer*. Clin Cancer Res 1999, 5: 1989-95.

66. Logothetis, C.J., Wu, K.K., Finn, L.D., Daliani, D., Figg, W., Ghaddar, H., Gutterman, J.U. *Phase I trial of the angiogenesis inhibitor TNP-470 for progressive androgen-independent prostate cancer*. Clin Cancer Res 2001, 7: 1198-203.

67. Stadler, W.M., Kuzel, T., Shapiro, C., Sosman, J., Clark, J., Vogelzang, N.J. *Multi-institutional study of the angiogenesis inhibitor TNP-470 in metastatic renal carcinoma.* J Clin Oncol 1999, 17: 2541-5.

68. Offodile, R., Walton, T., Lee, M., Stiles, A., Nguyen, M. *Regression of metastatic breast cancer in a patient treated with the anti-angiogenic drug TNP-470.* Tumori 1999, 85: 51-3.

69. Kudelka, A.P., Levy, T., Verschraegen, C.F. et al. *A phase I study of TNP-470 administered to patients with advanced squamous cell cancer of the cervix.* Clin Cancer Res 1997, 3: 1501-5.

70. Herbst, R.S., Madden, T.L., Tran, H.T. et al. *Safety and pharmacokinetic effects of TNP-470, an angiogenesis inhibitor, combined with paclitaxel in patients with solid tumors: Evidence for activity in non-small-cell lung cancer.* J Clin Oncol 2002, 20: 4440-7.

71. Figg, W.D., Pluda, J.M., Lush, R.M., Saville, M.W., Wyvill, K., Reed, E., Yarchoan, R. *The pharmacokinetic profile of TNP-470, a new angiogenesis inhibitor.* Pharmacotherapy 1997, 17: 91-7.

72. Moore, J.D., Dezube, B.J., Gill, P., Zhou, X.-J., Acosta, E.P., Sommadossi, J.-P. *Phase I dose escalation pharmacokinetics of O-(chloroacetylcarbamoyl) fumagillol (TNP-470) and its metabolites in AIDS patients with Kaposi's sarcoma.* Cancer Chemother Pharmacol 2000, 46: 173-9.

73. Brahn, E., Banquerigo, M.L., Schoettler, N.R., Lee, S. *A new angiogenesis inhibitor, PPI-2458: Regression of collagen-induced arthritis and prevention of erosions*. Arthrit Rheum 2001, 44: S271.

74. Brahn, E., Schoettler, N.R., Banquerigo, M.L., Thompson, C.D. *Involution of collagen-induced arthritis with an angiogenesis inhibitor PPI-2458.* Arthrit Rheum 2003, 48: S347.

75. Kanno, T., Endo, H., Takeuchi, K., Morishita, Y., Fukayama, M., Mori, S. *High expression of methionine aminopeptidase type 2 in germinal center B cells and their neoplastic counterparts*. Lab Invest 2002, 82: 893-901.

76. Fujioka, T., Hasegawa, M., Ogui, K., Matsushita, Y., Sato, M, Kubo, T. *Antitumor effects of angiogenesis inhibitor O-(chloroacetyl-carbamoyl) fumagillol (TNP-470) against murine renal cell carcinoma.* J Urol 1996, 155: 1775-8.

77. Noren-Nystrom, U., Eriksson, M., Eriksson, B., Roos, G., Bergh, A., Holmberg, D. *Antitumor activity of the angiogenesis inhibitor TNP-470 on murine lymphoma/leukemia cells in vivo and in vitro*. Exp Hematol 2003, 31: 143-9.

78. Hotz, H.G., Reber, H.A., Hotz, B. et al. *Angiogenesis inhibitor TNP-470 reduces human pancreatic cancer growth.* J Gastrointest Surg 2001, 5: 131-8.

79. Emoto, M., Ishiguro, M., Agr, B., Iwasaki, H., Kikuchi, M., Kawarabayashi, T. *Effect of angiogenesis inhibitor TNP-470 on the growth, blood flow, and microvessel density in xenografts of human uterine carcinosarcoma in nude mice*. Gynecol Oncol 2003, 89: 88-94.

80. Lazarus, D.D., Bernier, S.G., Labenski, M.T. et al. *Inhibition of primary tumor growth by PPI-2458, a methionine aminopeptidase-2 inhibitor: Dose and schedule responses*. 2003 AACR/NCI/EORTC Intl Conf Mol Targets Cancer Ther (Nov 17-21, Boston) 2003, Abst A174.

81. Hollingshead M.G., Kaur, G., Borgel, S.D. et al. *In vivo efficacy of PPI-2458 (NSC 720735), a novel fumagillin analog with oral bioavailability*. 2003 AACR/NCI/EORTC Intl Conf Mol Targets Cancer Ther (Nov 17-21, Boston) 2003, Abst C41.

82. Tanaka, T., Konno, H., Matsuda, I., Nakamura, S., Baba, S. *Prevention of hepatic metastasis of human colon cancer by angiogenesis inhibitor TNP-470.* Cancer Res 1995, 55: 836-9.

83. Sasaki, A., Alcalde, R.E., Nishiyama, A., Lim, D.D., Mese, H., Akedo, H., Matsumura, T. *Angiogenesis inhibitor TNP-470*

inhibits human breast cancer osteolytic bone metastasis in nude mice through the reduction of bone resorption. Cancer Res 1998, 58: 462-7.

84. Ohta, Y., Watanabe, Y., Oda, T., Hayashi, Y., Endo, Y., Tanaka, M., Sasaki, T. *Inhibition of lymph node metastasis by an anti-angiogenic agent, TNP-470.* Brit J Cancer 1997, 75: 512-6.

85. Shishido, T., Yasoshima, T., Denno, R., Sato, N., Hirata, K. *Inhibition of liver metastasis of human gastric carcinoma by angiogenesis inhibitor TNP-470.* Jpn J Cancer Res 1996, 87: 958-62.

86. Shusterman, S., Grupp, S.A., Barr, R., Carpentieri, D., Zhao, H., Maris, J.M. *The angiogenesis inhibitor TNP-470 effectively inhibits human neuroblastoma xenograft growth, especially in the setting of subclinical disease.* Clin Cancer Res 2001, 7: 977-84.

87. Shishido, T., Yasohima, T., Denno, R., Mukaiya, M., Sato, N., Hirata, K. *Inhibition of liver metastasis of human pancreatic carcinoma by angiogenesis inhibitor TNP-470 in combination with cisplatin.* Jpn J Cancer Res 1998, 89: 963-9.

88. Tran, H.T., Blumenschein, G.R. Jr., Lu C. et al. *Clinical and pharmacokinetic study of TNP-470, an angiogenesis inhibitor, in combination with paclitaxel and carboplatin in patients with solid tumors*. Cancer Chemother Pharmacol 2004, 54: 308-14.

89. Tsunasawa, S., Izu, Y., Miyagi, M., Kato, I. *Methionine aminopeptidase from the hyperthermophilic archaeon Pyrococcus furiosus: Molecular cloning and overexpression in Escherichia coli of the gene, and characteristics of the enzyme.* J Biochem 1997, 122: 843-50.

90. Li, X., Chang, Y.H. *Molecular cloning of a human complementary DNA encoding an initiation factor 2-associated protein (p67).* Biochim Biophys Acta 1995, 1260: 333-6.

91. Wu, S., Gupta, S., Chatterjee, N. et al. *Cloning and characterization of complementary DNA encoding the eukaryotic initiation factor 2-associated 67-kDa protein (p67).* J Biol Chem 1993, 268: 10796-801.

92. Pyun, H.-J., Fardis, M., Tario, J. et al. *Investigation of novel fumagillin analogues as angiogenesis inhibitors.* Bioorg Med Chem Lett 2004, 14: 1-94.

93. Rodeschini, V., Boiteau, J.-G., Van de Weghe, P., Tarnus, C., Eustache, J. *MetAP-2 inhibitors based on the fumagillin structure, side-chain modification and ring-substituted analogues*. J Org Chem 2004, 69: 357-73.

94. Balwin, J.E., Bulger, P.G., Marquez, R. *Fast and efficient synthesis of novel fumagillin analogues*. Tetrahedron 2002, 58: 5441-52.

95. Zhou, G., Tsai, C.W., Liu, J.O. *Fumagolone, a reversible inhibitor of type 2 methionine aminopeptidase and angiogenesis.* J Med Chem 2003, 46: 3452-4.

96. Sigg, H.P., Weber, H.P. *Isolation and structure elucidation of ovalicin*. Helv Chim Acta 1998, 51: 1395-408.

97. Milkowski, D.M., Weiss, R.A. *TNP-470.* In: Antiangiogenic Agents in Cancer Therapy. Teicher, B.A. (Ed.). Humana Press, Totowa, 1998, 385-98.

98. Lainer, D.T., Brahn, E. *New antiangiogenic strategies for the treatment of proliferative synovitis*. Exp Opin Invest Drugs 2005, 14: 1-17.