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TNP-470 (AGM-1470): Mechanisms of Action and Early Clinical Development

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INTRODUCTION

ANGIOGENESIS DEFINES the complex phenomenon that leads to the genesis of new blood vessels from the existing vasculature [1, 2]. Essential for growth and development, angiogenesis is restricted to some reproductive organs and in wound healing in adults. Folkman, the pioneer of the field, was the first to insist on the importance of angiogenesis in cancer progression and dissemination [3–5]. Today, the concept that tumour angiogenesis is an essential phenomenon to sustain tumour growth over a few millimetres is unanimously recognised. The search for angiogenesis inhibitors as potential new anticancer agents has been one of the most challenging objectives of cancer research. One exciting aspect of angiogenesis inhibition therapy is its putative selectivity. The turnover of endothelial cells in adult tissues is very slow (within a year) compared to the turnover of endothelial cells engaged in tumour angiogenesis (4 days) [6]. Therefore, the specific inhibition of endothelial cell proliferation could affect the malignant lesions with no or minor consequences for the host. Among several inhibitors characterised within the last decade, TNP-470, a synthetic analogue of fumagillin, is one of the most promising angio-inhibitors.

FUMAGILLIN AND TNP-470: INITIAL DISCOVERY

It is the accidental contamination of an endothelial cell culture flask that led to the identification of fumagillin as an angiogenesis inhibitor [7]. A fungal contamination of a capillary endothelial cell culture awakened the interest of Ingberg, one of Folkman's fellows, because it produced a local gradient of endothelial cell rounding. The fungus was isolated and identified as *Aspergillus fumigatus* fresenius. The molecule responsible for this effect was identified in conditioned medium from fungal culture as fumagillin (Figure 1), a known antibiotic used to treat amoebiasis [8]. Fumagillin inhibits endothelial cell proliferation and angiogenesis in the chorioallantoid membrane model (CAM) [7]. The antibiotic significantly reduces tumour growth *in vivo* [7]. However, the induction of a severe weight reduction in the treated animals limited its development as a new anticancer drug. In collaboration with Takeda Chemical Industries Ltd, Folkman decided to produce fumagillin analogues with the hope of generating a derivative that would be an angio-inhibitor as potent as fumagillin but without its toxic effects. From over 100 synthetic derivatives, TNP-470 (AGM-1470) (Figure 1) was identified as a potent angio-inhibitor [7]. This compound which is O-(chloroacetylcarbamoyl)-fumagillol is 50 times more active in inhibiting endothelial cell proliferation than fumagillin [7, 9]. TNP-470 significantly inhibited the growth of various experimental murine tumours with no major side-effects, including no major weight lost. The antitumour effect was due to an inhibition of tumour angiogenesis rather than a direct effect on cancer cells. TNP-470 was, therefore, one of the first angio-inhibitors which could reach clinical trials based on its efficacy as an angiogenesis inhibitor and its lack of major side-effects. After successfully completing all classical preclinical toxicology tests, phase I clinical trials with TNP-470 were initiated even though its exact mechanisms of actions were not completely elucidated.

EFFECTS ON TNP-470 ON ENDOTHELIAL CELLS AND ANGIOGENESIS

Several studies have been conducted to determine the effect(s) of TNP-470 on endothelial cells and angiogenesis. The drug was tested on several endothelial cells including human umbilical vein endothelial cells (HUVEC), bovine aortic endothelial cells (BAEC) and rat endothelial cells from adipose tissue [9–12]. TNP-470 inhibited the proliferation of growth factors-stimulated endothelial cell dose dependently with an IC_{50} of 15 pg/ml [11]. Complete cytostatic inhibition of endothelial cell proliferation is obtained with the TNP-470 concentration ranging from 300 pg/ml to 3 μg/ml. Growth inhibition is reversible within this concentration range of drug, and endothelial cell proliferation resumes 4 days after withdrawal of TNP-470 from the culture medium. Above 30 μg/ml, TNP-470 was found to be cytotoxic (reduction of the cell number below the initial plating number). At cytostatic concentrations, TNP-470 specifically inhibits DNA synthesis in endothelial cells, indicating that the molecule affects one or several step(s) of the cell cycle [11]. TNP-470 prevents the entry of both HUVEC and BAEC into the G1 phase of the cell cycle, inducing an accumulation of cells in the G0 phase [12]. Interestingly, this effect was not observed when transformed

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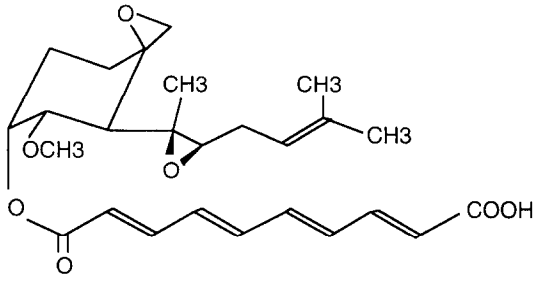
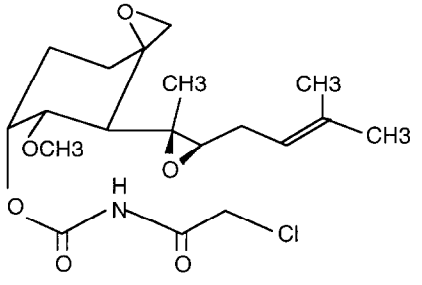


Fumagillin



TNP-470

Figure 1. Structure of fumagillin and its synthetic derivative, TNP-470.

endothelial cells Ea.hy926 or eEnd.1 were treated with the same concentration of angio-inhibitor. This suggests that TNP-470 specifically inhibits a cell cycle control pathway active in normal cells, but which could be bypassed or altered in transformed cells. Recent studies indicate that the action of TNP-470 on the cell cycle of endothelial cells is complex and could affect several key points rather than blocking the entry of the cell into the G1 phase. TNP-470 inhibits the activation of cyclin-dependent kinase cdc2 and cdk2 and the phosphorylation of RB protein, but does not affect early G1 mitotic events such as cellular protein tyrosyl phosphorylation or expression of c-fos, c-myc, cdk2, cdk4 or cyclin D1 [13]. These data suggest that the angio-inhibitory action of TNP-470 is located relatively late in the G1 phase. These are in contradiction with another report showing that TNP-470 inhibits cyclin D1 mRNA expression without altering c-myc expression [11]. Differences in the design of the experiments, including time of stimulation and time of incubation with the angio-inhibitor, could explain the discrepancies in the data and highlight the complexity of TNP-470 action. Additional studies will be necessary to identify which is or are the key activity(ies) responsible for the cytostatic inhibition of endothelial cell growth.

Several angiogenesis models have been used to study the angio-inhibitory activity of TNP-470. These include the CAM assay, the rat corneal micropocket assay, the sponge implantation assay, the rat blood vessel organ culture assay as well as *in vitro* assay of tubulogenesis [7]. In all these assays, TNP-470 inhibits angiogenesis at a concentration that inhibits cell proliferation.

EFFECTS OF TNP-470 ON HOST CELLS

To assess the therapeutic action of TNP-470 optimally and to ensure its safe use in the clinic, it was essential to examine its effect on the host tissues. Early reports showed that TNP-470 inhibits the proliferation of normal fibroblasts in the same range of concentrations that prevent endothelial cell proliferation [7, 14]. The consequence of this inhibition *in vivo* has not been specifically addressed, but the lack of major side-effects in the treated animals suggests that it does not have major consequences *in vivo*.

Several studies have reported an action of TNP-470 on cells of the immune system and it has been shown to modulate murine and human T cell functions *in vitro* [7, 15]. The effects observed were time course dependent. At early time points (from 2 to 4 days of treatment), 1 nM TNP-470 inhibits the responses of human T cells to tetanus toxoid and those of murine splenocytes to staphylococcal enterotoxin. It also partially inhibits the responses of human T cells to anti-CD3 antibodies and phytohemagglutinin. At later times, TNP-470 increases T-cell proliferation. This response is preceded by an increase of IL-2 in the supernatant. In another set of reports, TNP-470 induced a significant concurrent proliferation of both human and murine B lymphocytes [16, 17]. This effect is mediated by a direct action on T cells, requires cell contacts and is obtained at concentrations similar to those that inhibit endothelial cell proliferation. When injected subcutaneously (s.c.) or intraperitoneally (i.p.) into mice at pharmacological doses, TNP-470 induces a significant increase in size of axillary and mesenteric lymph nodes, respectively [17]. This phenomenon is mostly due to hyperplasia of the germinal centres. No effect was observed when TNP-470 was injected into T-deficient nude mice, which supports the *in vitro* demonstration that T cells play a key role in B-cell proliferation induced by the drug.

TNP-470 inhibits the induction of NK effector-cell function, indicating that the drug interacts with NK cells and can modulate IL-2-induced activation signals that lead to the acquisition of tumour killing function [18]. However, the concentration that caused half-maximal inhibition was 2 logs higher than the half-maximal cytostatic concentration for endothelial cells.

TNP-470 clearly affects cells of the immune system. The consequence of this to the host is not yet established and calls for additional investigations. These obviously should be considered in the therapeutic management of cancer patients treated with this drug.

EFFECTS OF TNP-470 ON CANCER CELL LINE PROLIFERATION

In vitro, TNP-470 inhibits tumour cell proliferation at concentrations generally much higher (around 3 logs higher) than concentrations restraining endothelial cell proliferation. Table 1 indicates the TNP-470 IC_{50} obtained for several human and non-human malignant cells. Differences in the TNP-470 effective doses have been observed between monolayer cultures and soft agar cultures of PC-3 human prostate carcinoma cells and MDA-MB-231 human breast carcinoma cells [19]. The IC_{50} values were significantly lower when cells were grown in soft agar than in monolayer culture. Human glioblastoma cells are, to date, the only cancer cell types for which TNP-470 exhibits cytostatic and

Table 1. *Effect of TNP-470 on tumour cell proliferation* in vitro

Tumour type	IC_{50}	[Ref.]
Syrian hamster pancreatic carcinoma	60 pg/ml	[21]
Murine renal carcinoma	600 ng/ml	[22]
Human prostate carcinoma	4.9 μg/ml in monolayer culture 0.05 ng/ml in soft agar	[19]
Human breast carcinoma	4.4 μg/ml in monolayer culture 0.47 μg/ml in soft agar	[19]
Human choriocarcinoma	0.005 μg/ml	[23]
Human ovarian carcinoma	2.3 μg/ml	[23]
Human glioblastoma	55 pg/ml	[20]

IC_{50} is the dose of TNP-470 that produces 50% inhibition of cell proliferation.

cytotoxic effects at concentrations similar to those known to be active on endothelial cells [20]. These data suggest that *in vivo*, the antitumour activities of TNP-470 should be mostly due to its angio-inhibitory activities rather than to a direct effect on malignant cell proliferation.

PHARMACOKINETICS AND METABOLISM OF TNP-470

Preliminary pharmacokinetic experiments indicated that TNP-470 has an extremely short plasma elimination half-life of approximately 5–10 min [24]. A detailed understanding of TNP-470 metabolism and disposition is critical to elucidate fully the pharmacodynamic properties of this new angio-inhibitor. To examine the fate and metabolic pathways of TNP-470, its biotransformation was examined in primary cultures of human hepatocytes and in microsomal fractions of various human tissues [25]. The drug undergoes rapid and extensive transformation into at least six metabolites. The two predominant extracellular metabolites are M-II and M-IV (Figure 2) [26]. M-IV formation is associated with an esterase-like enzymatic cleavage of TNP-470. M-IV is then further metabolised by microsomal epoxide hydrolase into M-II. The pharmacokinetics and metabolism of TNP-470 has been examined in a non-human primate

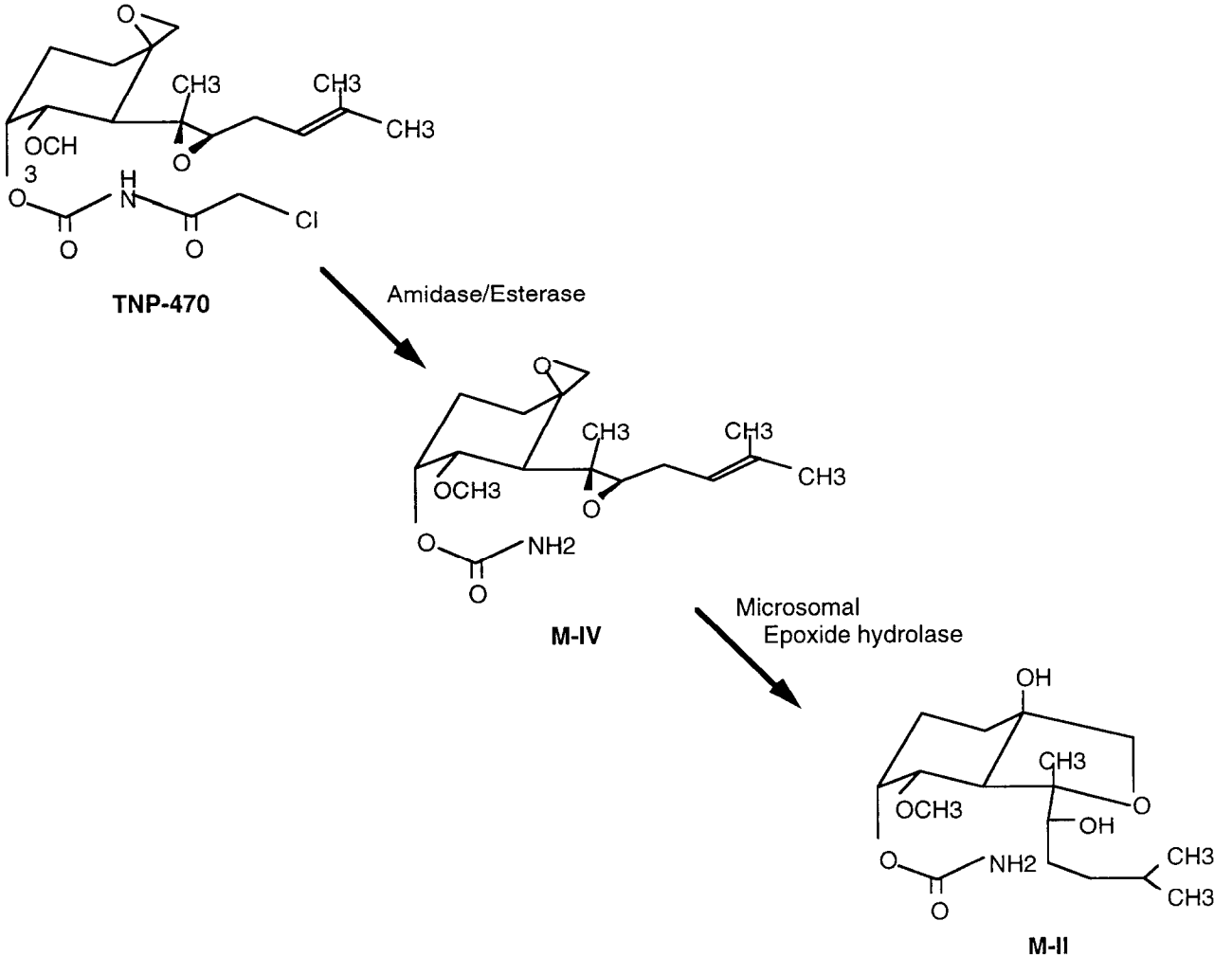


Figure 2. Putative metabolic pathways of TNP-470.

Table 2. Activity of TNP-470 on rodent tumour growth

Tumour type	Treatment schedule	Dose (mg/kg)	Tumour growth inibition (%)	[Ref].
B16BL6 melanoma	On days 1, 3, 6, 8, 10 s.c.	60	84	[31]
M5076 reticulum cell sarcoma	On days 1–12 s.c.	5	90	[31]
Walker 256 carcinoma	On days 1, 3, 5 s.c.	100	96	[31]
Lewis lung carcinoma	On days 1, 3, 5, 7 s.c.	60	83	[31]
	Every other day for 20 days s.c.	30	62	[35]
Fibrosarcoma	Every other day for 27 days s.c.	30	69	[35]
Colon adenocarcinoma	Every other day for 28 days s.c.	30	77	[35]
Pancreatic carcinoma	Three times/week for 4 weeks s.c.	30	60	[21]
Haemangioendothelioma	Every second day for 22 days s.c.	30	90	[34]

s.c., subcutaneous.

model which is highly predictive of the pharmacokinetics of various compounds in humans including anticancer and antiviral agents [27]. TNP-470 is rapidly and extensively metabolised by rhesus monkeys. These data are consistent with the rapid metabolism of the drug by human hepatocytes *in vitro*. Most of the administrated dose in monkeys is probably excreted in faeces, although a complete mass-balance study will be necessary to identify precisely the distribution of the remaining drug. The anti-angiogenic activities of the six major metabolites are, as yet, unknown and need to be determined to understand the pharmacodynamic properties of this new anticancer drug.

TNP-470: PRECLINICAL STUDIES

The antitumour effect of TNP-470 has been studied in a variety of experimental tumour models. The studies have evaluated the effect of the angio-inhibitor on tumour growth, survival and metastases. TNP-470 inhibits tumour growth and metastasis in a variety of murine and human tumour models. This antitumour effect is considered to be due mainly to the inhibition of the development of new blood vessels. No major side-effects have been found in treated animals. The effective dose generally used is 30 mg/kg every other day injected subcutaneously. The use of embolic substances (microspheres and medium-chain tryglyceride solution), in which TNP-470 is very stable, prolongs retention of the anticancer drug at the tumour site, and augments the efficacy of the antitumour therapy [28–30]. The anti-tumour effect of TNP-470 has been assessed as monotherapy or in combination with other treatment modalities.

The antitumour effect of TNP-470 in rodent tumours models

As shown in Table 2, subcutaneous treatment with TNP-470 dose-dependently inhibits tumour growth of a variety of tumours [31]. The compound was slightly less active when administered intraveneously (i.v.) compared with s.c. adminstration. Other authors have assessed the antitumour and the angio-inhibitory effect of TNP-470 in Lewis lung carcinoma and extended their studies to other murine tumours. Angiogenesis of rat pituitary tumours, induced by oestrogens, was significantly inhibited by TNP-470 [32, 33]. TNP-470 (10–20 mg/kg) decreased the growth rate and angiogenesis of Renca renal carcinoma rather than the size of the tumours [22].

TNP-470 was also effective in the treatment of a spontaneous murine haemangioendothelioma (EOMA) that retains many of the characteristics of microvascular endothelial cells [34]. TNP-470 at 30 mg/kg s.c. induced a 90% reduction in tumour size compared to the untreated controls and prolonged mice survival by 70%. No toxicity was observed, although both groups of mice (treated and controls) died from local haemorrhagic complications.

TNP-470 also prevents the development of metastases (Table 3). The drug reduced dose-dependently the number of lung metastases formed by B16BL6 melanoma cell lines at 60 mg/kg s.c. three times a week [31]. Ninety per cent inhibition and 56% better survival were obtained compared to untreated animals. The compound was also effective in decreasing the number of spontaneous liver metastases of M5076 reticulum cell sarcoma after tumour resection [31]. Complete inhibition was observed at doses of 10–30 mg/kg every day or 30 mg/kg every 3 days. Inhibition of metastasis

Table 3. Activity of TNP-470 on metastasis formation from murine tumours

Tumour type	Treatment schedule	Dose (mg/kg)	Effect on metastasis	[Ref.]
B16BL6 melanoma	6 h before cell inoculation and then three times/week for 2 weeks s.c.	60	Reduction of the number of lung metastases (90%)	[31]
M5076 reticulum cell sarcoma	Every day s.c. for 10 days	10–30	Complete inhibition of spontaneous liver metastasis	[31]
	or			
	every 3 days s.c. for 10 days	30		
LM8 osteosarcoma	Every day for 3 weeks	10–40	Complete inhibition of lung metastasis at the maximal dose in 6/9 mice	[36]
Renal carcinoma	Once every 3 days for 28 days	10–20	83% inhibition of the number of lung metastases	[22]

s.c., subcutaneous.

was also observed with LM8, an osteosarcoma cell line that metastasises to the lung [36]. TNP-470, at a dose of 10-40 mg/kg s.c. every day for 3 weeks, inhibited dose-dependently the number of pulmonary metastatic nodules and the lung wet weight of the treated mice. Significant inhibition of the number of lung metastasis was observed in renal carcinoma [22].

Antitumour effect of TNP-470 in human tumours models (Table 4)

Pancreatic cancers. TNP-470 has been tested in an interesting transgenic mouse model that represents a tool for studying the development and progression of human epithelial cancers, such as breast, prostate and bladder carcinoma [37]. These mice express the simian virus 40 large T antigen (SV40Tag) oncogene in the beta cells of the Langerhan's islet under control of the insulin promoter. The Tag expression elicits tumorigenesis proceeding through four distinct stages (normality, hyperplasia, angiogenic switch and tumour formation). The mice received 30 mg/kg of TNP-470 s.c. every other day, beginning at 6 weeks of age at the hyperproliferative stage of the islet cells. The initial switch to the angiogenic phenotype during tumorigenesis was not completely blocked, but neovascularisation and tumour burden was significantly reduced in treated mice (40 and 89%, respectively, compared to controls). The antitumour effect of TNP-470 has been tested in the syrian hamster pancreatic cancer cell line HPD-NR, another *in vivo* model of pancreatic cancer which closely resembles its human counterpart [21]. The drug injected s.c. at a dose of 30 mg/kg/day three times weekly for 4 weeks showed a significant growth-inhibitory effect on HPD-NR tumour inoculated s.c. in hamsters. Histological analysis revealed that tumour vascularity was decreased and the necrotic portion tended to be increased in the treatment group. The only side-effect observed in treated animals was a 10% loss in body weight compared with non-treated animals.

Nervous system and brain tumours. Glioblastomas are the most malignant primary brain tumour characterised by hypervascularisation and expression of many angiogenic growth factors, including fibroblast growth factor (FGF) and endothelial cell growth factor [20]. The effect of TNP-470 has been evaluated in nude mice with subrenally implanted glioblastomas or with intracranial glioblastomas. TNP-470 (30 mg/kg) was injected s.c. three times per week.

At this dose, TNP-470 had a significant inhibitory effect on tumour growth and on the vascularisation of tumours implanted beneath the subrenal capsule of the kidney. It prolonged the survival of the mice with intracerebral glioblastomas [20]. However, in another study TNP-470 had no effect on the progression of rat intracranial gliosarcoma, with an i.p. 30 mg/kg dose which induced a significant weight loss in the treated animals [38]. It has been demonstrated that TNP-470 is useful for the treatment of human benign tumours of the nervous system, such as schwannomas and neurofibromas [39]. Injected s.c. or i.p. at a dose of 30 mg/kg three times a week, TNP-470 inhibited the neovascularisation of both tumour types implanted beneath the subrenal capsule of nude mice. There were no significant differences with the two routes of administration. Moreover, there was no significant difference in the death rate between the mice treated with TNP-470 and controls. The same treatment was found to inhibit both the neovascularisation and the growth of malignant neurofibrosarcoma [39].

Hormone-independent breast and prostate carcinomas. TNP-470 has been tested on hormone-independent human breast (MDA-MB-231) and prostate (PC-3) carcinoma cell lines [19]. These tumours cannot be controlled by hormonal therapy and produce growth and angiogenic factors. TNP-470 inhibited tumour growth dose-dependently (50–200 mg/kg a week s.c.) of both tumours inoculated s.c. in immunodeficient mice. The maximal dose of 200 mg/kg completely inhibited the growth of PC-3 prostate tumour and the tumour volume of MDA-MB-231 was reduced to only 12% (treated versus controls). An inhibitory effect of TNP-470 (30 mg/kg three times a week) on tumour growth has been obtained in untreated or tamoxifen-treated mice s.c. inoculated with FGF1 or FGF4-transfected MCF7 cells [40, 41].

Urogenital cancers. TNP-470 has also been tested on cell lines of choriocarcinomas, endometrial cancer, ovarian cancer and uterine endometrial cancer inoculated s.c. in nude mice, and at a dose of 30 μg/ml, choriocarcinomas were the most sensitive to the drug as expected from *in vitro* results [23].

Oesophageal and gastric cancers. A TNP-470 dose of 30 mg/kg three times a week for 2 weeks had an antitumour effect on human oesophageal and gastric cancers transplanted in nude mice. The antitumour effect was enhanced by a combination treatment with hyperthermia [42].

Metastasis. TNP-470 diminished the number and size of lung metastases of the choriocarcinoma cell line at a dose of 30 mg/kg [23] (Table 5). In contrast, at the same dose, it had no effect on the metastasis formation of MCF7 breast carcinoma cells transfected with FGF [41]. The antimetastatic effect of TNP-470 was investigated in three colon cancer cell lines implanted into the caecal wall of

Table 4. Activity of TNP-470 on human tumour growth

Tumour type	Treatment schedule	Dose (mg/kg)	Tumour growth inhibition (%)	[Ref.]
Glioblastoma	Three times/week for 3 weeks s.c.	30	94.5	[20]
Neurofibrosarcoma	Three times/week for 6 weeks s.c. or i.p.	30	91.5	[39]
Breast carcinoma	Once a week for 6 weeks s.c.	50–200	88 maximal inhibition	[19]
Prostate carcinoma	Once a week for 6 weeks s.c.	50–200	96 maximal inhibition	[19]
Choriocarcinoma	Once every other day for 5 weeks s.c.	30	60	[23]
Ovarian cancer	Once every other day for 5 weeks s.c.	30	No effect	[23]
Oesophageal and gastric cancer	Three times/week for 2 weeks s.c.	30	27	[42]

s.c., subcutaneous; i.p., intraperitoneal.

Table 5. Activity of TNP-470 on metastasis formation from human tumours

Tumour type	Treatment schedule	Dose (mg/kg)	Effect on metastasis	[Ref.]
Breast carcinoma	Three times/week for 6 weeks	30	No effect	[41]
Choriocarcinoma	Once every other day for 5 weeks s.c.	30	Reduction in the number and rate of metastases	[23]
Colon cancer	Every other day for 6 weeks s.c.	20–30	70–100% inhibition of the number of metastases	[43]
	On alternate days for 5 weeks s.c.	30	Decreased the number of mice with metastasis and of the number of metastatic foci	[44]

nude mice with metastasis to the liver. TNP-470 at 20–30 mg/kg inhibited dose-dependently the number of metastatic foci in the treated animals, but did not affect tumour growth [43]. Liver metastasis of another model of human colon cancer, transplanted orthotopically, developed in only two out of eight mice treated with TNP-470 s.c. at a dose of 30 mg/kg [44].

COMBINATION THERAPY

The potential therapeutic value of TNP-470 has also been evaluated in combination with different anticancer modalities including chemotherapeutic agents, cytokines or radiotherapy (Table 6). In rat pituitary tumours induced by oestrogens, combined treatment with bromocriptine was more effective than treatment with TNP-470 alone [32, 33]. In a transgenic mouse model of islet cancer, a combination of TNP-470 with angio-inhibitors, minocycline and the IFN alpha/beta, showed synergistic anti-tumour effects [37]. Effects of other combinations in other animal models are shown in Table 6.

TNP-470 used in combination with minocycline and cyclophosphamide against FSaIIC fibrosarcoma and Lewis lung carcinoma resulted in a potentiation of cyclophosphamide toxicity [46]. The same combination TNP-470/minocycline/cyclophosphamide and TNP-470/minocycline/cisplatin or TNP-470/minocycline/thiotepa did not alter the growth of EMT-6 mammary carcinoma, but increased the tumour growth delay produced by the single agents [46]. TNP-470 administrated together with IL12 had an increased antitumour effect on Lewis lung carcinoma [47]. Similarly, TNP-470 demonstrated augmented efficacy in inhibiting the growth of MCF-7 cells when mice inoculated with tumour cells were pretreated with tamoxifen [48]. Combined therapy with mitomycin, doxorubicin, cisplatin and 5-fluorouracil not only decreased tumour growth but also reduced the number of lung experimental metastasis of B16BL6 murine melanoma [49]. Tumour growth of Lewis lung carcinoma was also inhibited by TNP-470 combined with cisplatin and 5-fluorouracil [49]. Fewer studies of combination therapies have been conducted in human tumours. The antitumour activity of TNP-470 against PC-3 tumour was enhanced by combination with cisplatin (5 mg/kg s.c. every week), but the combination of TNP-470 with 5-fluorouracil and doxorubicin did not enhance the TNP-470 effect on MDA-MB-231 breast carcinoma. The antitumour effect of TNP-470 in human oesophageal and gastric cancers was enhanced by the addition of hyperthermia (43 °C for 30 min) [42]. An enhanced effect of TNP-470 with hyperthermia (44°C for 30 min) has also been observed with SCCVII murine squamous carcinoma on tumour growth and tumour angiogenesis [45].

EFFECTS OF TNP-470 ON ANGIOGENESIS-DEPENDENT DISEASES

Arthritis

TNP-470 has been tested in rat collagen-induced arthritis (CIA) or in rat adjuvant arthritis (AA), animal models which resemble rheumatoid arthritis. TNP-470 was admi-

Table 6. Therapeutic benefit of a combination of TNP-470 with other treatment modalities in experimental models

Tumour type	Combined treatment	Additional effect	Ref.
Animal			
Lewis lung carcinoma	Interleukin-12	Increase in tumour growth inhibition	[47]
	Minocycline, cyclophosphamide	Increase in tumour growth delay, 40–50% of long-term survivors animals	[46]
FS aIIC fibrosarcoma	Minocycline, cyclophosphamide	8-fold increase in tumour cell killing	[46]
Rat pituitary tumours	Bromocriptine	Suppression of vascular formation	[32, 33]
Murine islet cancer	Interferon alfa/beta, minocycline	Reduction of tumour volume and capillary density	[37]
Rabbit VX-2 carcinoma	Doxorubicin	Antitumour effect	[30]
Murine squamous carcinoma	Hyperthermia	Tumour growth delay and reduction of vascularised area	[45]
Murine EMT-6 mammary carcinoma	Minocycline, cyclophosphamide, cisplatin	Increase in tumour growth delay	[48]
Murine melanoma	Mitomycin, doxorubicin, cisplatin 5-fluorouracil	Decrease in tumour growth and number of metastasis	[49]
Human			
PC-3 prostate cancer	Cisplatin	Increase in antitumour activity	[19]
MDA-MB-231 breast cancer	5-Fluorouracil, doxorubicin	No additional effect	[19]
	tamoxifen	Inhibition of tumour growth	[48]
Oesophageal and gastric cancer	Hyperthermia	Increased inhibition of tumour growth	[42]

nistered s.c. on alternate days using a dose of 27 mg/kg. The severity and incidence of arthritis were significantly lower in the treated animals. Moreover, this treatment schedule did not affect the immune system [50, 51]. Combination therapies with cyclosporin A or with paclitaxel were more effective than single-agent therapy [52, 53].

CLINICAL TRIALS

On the basis of the promising results obtained with TNP-470 in the *in vitro* and *in vivo* studies, this angio-inhibitor entered clinical trials for a variety of solid tumours as well as for AIDS-associated Kaposi's sarcoma (KS). A phase I pilot study is ongoing in patients with HIV-associated KS. The specific objectives of the study are (i) to determine the toxicity of TNP-470; (ii) to define the pharmacokinetic profile of the drug; and (iii) to obtain preliminary information on the activity on TNP-470 in this group of patients. No dose-limiting toxicity has been noted. No objective, measurable anti-KS responses have been seen, although 4 patients have had stable disease lasting 4–14 weeks. Reduction of tumour oedema, disappearance of KS-associated foot pain and a decrease in the size of some KS lesions have been observed in patients receiving 32.4 mg/m^2 of TNP-470 [24].

Androgen-independent prostate cancer has been selected as a target for phase I clinical trials. TNP-470 is administered as a 1 h infusion on alternate days for 4 weeks, cycled every 6 weeks, at doses of 9.3, 13.9, 21 and 31.5 mg/m^2. The only toxicity observed has been granulocytopenia in one patient treated at a dose of 13.9 mg/m^2 and a modest fatigue in patients treated with higher doses of TNP-470. Modulation of prostate-specific antigen (PSA) has been noted in one of the patients [54].

A phase I study of TNP-470 has been initiated in patients with squamous cell carcinoma of the cervix, not otherwise curable [55]. Patients without toxicities and no disease progression have been dose escalated at the subsequent cycle. The TNP-470 doses used were 9.3, 14, 31.5, 47.25 and 71.25 mg/m^2. One complete response has been observed at 71.25 mg/m^2.

Studies in newly diagnosed, previously irradiated, glioblastoma multiforme are in phase II. A phase III randomised study of TNP-470 versus synchronous radiotherapy and 5-FU–CF for locally advanced non-resectable, non-metastatic pancreatic cancer is ongoing.

CONCLUSION

TNP-470 is one of the few angio-inhibitors that have reached clinical trials. The concept of angio-inhibition therapy is elegant because it affects specifically cancer progression with theoretically no effect on the host. Preliminary data are encouraging, but it is clear that TNP-470 will not be sufficient to cure cancer by itself. Cancer is a complex multifactorial disease. It is unlikely that single treatment modalities can eradicate completely a malignant disease. The combination of several angio-inhibitors with other therapeutic modalities will probably be necessary to treat cancer effectively. However, because angio-inhibition aims at restraining the growth of a tumour by limiting its nutrient supply necessary for expansion, this new therapeutic approach could turn cancer into a chronic disease. Much more work needs to be achieved before angio-inhibition becomes routine cancer therapy. TNP-470, with a few other drugs, are on their way to reaching that stage.

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