Neovastat—a novel antiangiogenic drug for cancer therapy

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Neovastat (Æ-941) is an antiangiogenic drug isolated from marine cartilage. It interferes with several steps associated with the development of angiogenesis through its ability to induce endothelial cell apoptosis, and to inhibit matrix metalloproteinase activities and vascular endothelial growth factor-mediated signaling pathways, suggesting that Neovastat behaves as a multifunctional antiangiogenic drug. Neovastat is orally bioavailable, and shows significant antitumor and antimetastatic properties in animal models. An excellent safety profile with few side effects has been monitored in more than 800 patients who have been exposed to Neovastat, some of whom for more than 4 years. This indicates that Neovastat is suitable for long-term use, either alone or in combination with other anticancer therapies. Accordingly, Neovastat is currently under evaluation in three pivotal clinical studies with two phase III clinical trials in patients with lung and renal carcinoma, and a phase II clinical trial in patients with multiple myeloma is ongoing. *Anti-Cancer Drugs* **14:91–96**

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Introduction

The current view of the cellular and molecular events involved in the development of cancer identify six major alterations in cell physiology that collectively dictate malignant growth: self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of apoptosis, unlimited replicative potential, tissue invasion and sustained angiogenesis [1]. All of these processes are highly complex, but, in each case, several key regulatory mechanisms governing the development of malignancy have been unveiled, providing novel drug targets amenable for therapeutic intervention [1]. Among these events, the targeting of tumor-induced angiogenesis as a means of blocking tumor progression has generated a growing interest in recent years [2]. This interest stems from several observations that tumor cells cannot grow significantly in the absence of blood vessels [3] and that molecules interfering with angiogenesis have potent antitumor properties in animal models [4]. Moreover, since endothelial cells (ECs) are genetically stable, inhibitors specific to these cells should not induce resistance in tumors in contrast to cytotoxic compounds (antimitotic, antimetabolites and alkylating agents) for which resistance is commonly observed [5].

Extensive studies on the cellular and molecular processes underlying angiogenesis have identified key events associated with tumor-induced neovascularization: (i) stimulation of ECs by tumor-derived angiogenic cytokines, such as the vascular endothelial growth factor (VEGF), resulting in increased EC proliferation and migration, (ii) secretion of matrix-degrading enzymes, such as matrix metalloproteinases and plasminogen activators, resulting in the digestion of the surrounding extracellular matrix (ECM), and (iii) formation of a three-dimensional capillary network in the vicinity of tumor cells, allowing their sustained growth by providing oxygen and essential nutrients. These cellular and molecular steps all represent attractive antiangiogenic targets, and have led to the identification and development of a variety of compounds targeting either EC proliferation, migration or vessel formation [6]. At the present time, almost 300 angiogenesis inhibitors have been identified and developed, and more than 75 are under clinical evaluation, from which only three are under investigation in phase III clinical trials.

Neovastat

Cartilage was the first tissue reported to contain biological inhibitor(s) of angiogenesis, based on experiments showing that fragments of cartilage grafted onto the chick embryo chorioallantoic membrane were resistant to invasion by the vascularized mesenchyme of the host [7]. Aqueous extraction of cartilage removed this antiangiogenic activity, suggesting that cartilage contains biologically active hydrosoluble inhibitors of angiogenesis [8]. These observations contributed to the development of a proprietary manufacturing process for Neovastat, a

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naturally occurring antiangiogenic agent obtained from dogfish cartilage that is developed and prepared by Æterna Laboratories (Québec, Canada) [9].

Neovastat is prepared by homogenization of the cartilage in purified water, followed by purification of a molecular fraction whose components have molecular weights of less than approximately 500 kDa [9]. As such, Neovastat is not cartilage, but an extract from cartilage from which more than 95% of insoluble material has been discarded [10]. Neovastat is produced and tested by state-of-the-art facilities that are managed according to current Good Manufacturing Practice (cGMP) criteria. The quality controls for Neovastat are based on commonly used attributes, e.g. physico-chemical characteristics, microbiology, chemical identity of the components, molecular fingerprints and biological activities.

Antiangiogenic activities of Neovastat

In vitro, Neovastat inhibits the proliferation of ECs, whereas it has no significant effect on the proliferation of human skeletal muscle cells, dermal fibroblasts or several tumor cell lines [11]. Antiangiogenic activity of the compound is also supported by its inhibitory effect on neovascularization in the *ex ovo* chick chorioallantoid membrane model as well as by its inhibition of neovascularization induced *in vivo* by s.c. implants of Matrigel [11]. These studies thus indicate that Neovastat is an orally bioavailable antiangiogenic agent [11]. Extensive investigations conducted on the mechanisms by which Neovastat exerts its antiangiogenic activity have demonstrated that it has at least four mechanisms of action: it inhibits specific matrix metalloproteinases (MMP-2, -9 and -12), down-regulates the VEGF signaling pathway (VEGFR-2), induces apoptosis in ECs and stimulates tissue-type plasminogen activator (tPA) enzymatic activities.

Inhibition of the VEGF signaling pathway

New blood vessel formation during the angiogenic process happens mainly through the stimulation of EC proliferation and migration by angiogenic stimulators such as VEGF and basic fibroblast growth factor (bFGF) [12–14]. VEGF specifically binds to receptors of the tyrosine kinase family at the EC surface, the fms-like tyrosine kinase (Flt-1 or VEGFR-1) and the fetal liver kinase-1 (Flk-1/KDR or VEGFR-2) [15]. The binding of VEGF to VEGFR-2 triggers the tyrosine phosphorylation of a number of key signaling intermediates [16] that appear to account for most of the mitogenic [17], chemotactic [18], anti-apoptotic [19] and hyperpermeabilizing [20] effects of VEGF.

There is compelling evidence that Neovastat contains molecules that interfere with VEGF-mediated angiogenesis. Neovastat significantly inhibits VEGF-dependent sprouting of ECs (a phenomenon including cell proliferation and migration) in the rat aortic ring preparation, an *ex vivo* model of angiogenesis [21]. It also strongly inhibits VEGF-dependent migration of ECs, an important component of many biological processes including wound healing, inflammation and angiogenesis. *In vivo*, oral administration of Neovastat also leads to an inhibition of VEGF-induced blood vessel hyperpermeabilization [21]. These data suggest that Neovastat contains components that interfere with VEGFR-2 function, leading to the alteration of VEGF-mediated signaling pathways. Accordingly, Neovastat was shown to compete for the binding of [^{125}I]VEGF to its receptor in ECs [21]. However, it does not significantly alter the binding of [^{125}I]bFGF to its receptor, although it inhibits bFGF-dependent proliferation of ECs. The effect of Neovastat on VEGF binding was correlated with a marked reduction of VEGFR-2 tyrosine phosphorylation, whereas VEGFR-1 activity was unaffected.

Induction of EC apoptosis

A hallmark of antiangiogenic molecules is their ability to induce apoptosis of EC but not of several other normal or tumoral cell types. This induction of EC apoptosis appears to represent an essential component of their antiangiogenic effects, leading to subsequent tumor cell apoptosis [22]. In a similar manner, cell death was induced in bovine and human ECs that were exposed to Neovastat, whereas other cell types were unaffected in similar assays [23]. Neovastat-induced apoptotic cell death was markedly reduced by zVAD-fmk, a broadly active caspase inhibitor, suggesting the involvement of caspase activities. Accordingly, Neovastat was found to induce apoptotic cell death by increasing caspase-3 and -8 activities, and this was correlated with nuclei fragmentation and the release of cytochrome *c* from mitochondria to the cytoplasm [23].

Inhibition of matrix proteinase activities

The capacity of tumors to form metastatic foci appears to correlate with their ability to degrade basement membrane barriers, a process involving the activation of extracellular proteases of the MMP family [24,25]. MMPs are multidomain zinc-dependent endopeptidases that, with a few exceptions, share a basic structural organization comprising propeptide, catalytic, hinge and hemopexin-like domains [25]. About 25 members of this family have been described to date and these enzymes have been extensively characterized, mostly in terms of their specificity towards ECM proteins [4–6]. These studies have shown that collectively, MMPs are capable of degrading all molecular components of the ECM, supporting an essential function in tumor invasion and in angiogenesis [26]. In the extracellular medium, the activity of MMPs is controlled by the tissue inhibitors of matrix metalloproteinases (TIMPs), a family of proteins currently composed of four members (TIMP1–4) which act as endogenous inhibitors of MMPs and thus prevent

degradation of ECM components [27]. A large body of evidence suggests that the equilibrium existing between MMP and TIMPs determines whether or not cells invade the ECM [27].

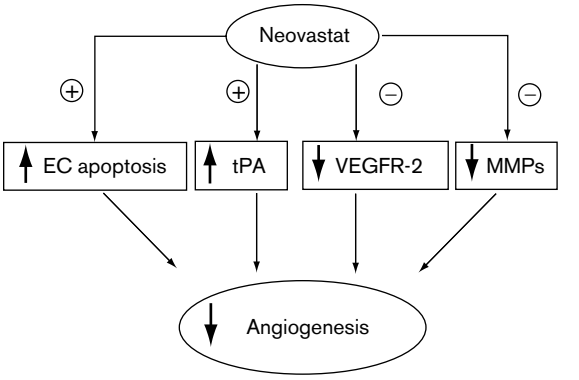
The effect of Neovastat on MMP activities was assessed by fluorimetric assays and by substrate gel zymography. In both assays, Neovastat significantly inhibited MMP-2 activity [28], whereas it had much less inhibitory activity towards MMP-1, -7, -9 and -13 activities. Although the molecular components involved in the MMP inhibitory activity remain to be biochemically identified, the inhibitory activity correlated with the presence of TIMP-like molecules within Neovastat, suggesting that these molecules may contribute to MMP inhibition [28]. Interestingly, Neovastat also showed a significant inhibitory activity towards metalloelastase (MMP-12) and neutrophil elastase, two enzymes that degrade elastin and are involved in the pathophysiology of diseases such as abdominal aneurysms and pulmonary emphysema. The contribution of this activity to the antiangiogenic effect of Neovastat remains to be determined.

Stimulation of tPA activity

The PA/plasmin system comprises two major types of PA, tPA and urokinase PA (uPA), that both specifically convert circulating plasminogen to the active proteinase plasmin by cleavage of the Arg561–Val562 peptide bond [29]. Plasmin is a trypsin-like protease with broad specificity that is capable of degrading most components of the ECM either directly or through activation of MMPs or elastases [30]. While uPA and tPA share a common substrate, there is evidence that the physiological roles of the two proteins are distinct. The binding of uPA to its cell-surface receptor (uPAR) is involved in pericellular proteolysis and is associated with cell locomotion [30,31]. This uPA/uPAR interaction has been proposed to play an important role in neovascularization based on the observation that interference with the binding of uPA to its receptor results in an inhibition of tumor angiogenesis [32]. In contrast to uPA, the role of tPA in angiogenesis remains unclear. tPA is a key enzyme in fibrinolysis due to its ability to significantly increase the cleavage of plasminogen to plasmin when bound to fibrin, leading to fibrin degradation [29]. How this fibrinolytic activity contributes to angiogenesis remains unknown. High tPA levels correlate with good prognosis in various tumors [33,34], whereas lower tPA levels were found to be associated with malignant tumors [35]. While such a positive role for tPA in tumor progression remains unexplained, it has been proposed that overstimulation of plasmin generation by tPA may induce the degradation of the proangiogenic fibrin matrix, resulting in the inhibition of angiogenesis [36].

Recent studies have suggested that Neovastat may have a positive impact on the PA system. Using recombinant tPA and uPA enzymes, we recently observed that Neovastat promoted a marked increase in tPA-dependent plasmin generation, whereas the compound had an inhibitory effect on that mediated by uPA (Gingras *et al.*, manuscript in preparation). Interestingly, this stimulatory effect of Neovastat on tPA activity is likely to occur *in vivo*, based on the observation that administration of Neovastat to animals bearing human glioblastoma results in a marked increase in the levels of EC-associated tPA (Jourdes *et al.*, submitted). The participation of this activity to the antiangiogenic effect of Neovastat is currently under extensive study.

Fig. 1



Neovastat is a multifunctional antiangiogenic agent.

In summary, these data collectively indicate that Neovastat contains several constituents with distinct mechanisms of action and further emphasizes its pleiotropic mechanism of action (see Fig. 1). It is suggested that each of these molecules might act together in synergy to produce a more complete control of angiogenesis. The biochemical identification of these molecules is underway and should provide interesting information concerning the mechanisms underlying the antiangiogenic effects of Neovastat.

Antitumor and antimetastatic properties of Neovastat

Antitumor properties

Chronic administration of Neovastat significantly inhibits tumor growth *in vivo* as reflected by a significant dose-dependent inhibition of tumor volume [11]. Moreover, Neovastat-treated mice in the experimental glioblastoma model showed a significantly longer survival time compared to untreated mice [37], which correlated with a significant reduction of tumor vascularization.

Antimetastatic properties

The antimetastatic activity of Neovastat was assessed using an *in vivo* Lewis lung carcinoma (LLC) model,

which is characterized by the formation of a large number of lung metastases. Neovastat was well tolerated by the mice bearing LLC metastases with no significant effect on body weight or mortality. Importantly, a 70% inhibition of metastases invasion was observed following oral administration of Neovastat [11]. Similarly, Neovastat was shown to prevent bone metastasis *in vivo* [38]. In addition, Neovastat appears to increase the efficacy of conventional anticancer agents. Administration of Neovastat in combination with a single sub-optimal dose of cisplatin in the LLC model resulted in a significantly higher inhibition of metastases in comparison to that observed with cisplatin alone. Moreover, this combination resulted in an increased efficacy/toxicity ratio of cisplatin. These findings demonstrate a potential benefit of administering Neovastat in combination with standard chemotherapy.

Clinical trials with Neovastat

Phase I/II clinical trials

Results from two multicenter phase I/II trials in oncology in which Neovastat was given to almost 500 patients with refractory solid tumors, mostly lung, prostate, breast and kidney cancers have been recently reviewed [39–41]. Briefly, the main objectives of these trials were to establish the safety profile of Neovastat and to determine the optimal dose for phase III trials. Neovastat was well tolerated when given at doses of 30–240 ml/day and no dose-limiting toxicity, deaths or grade 3–4 laboratory abnormalities related to Neovastat have been reported. Overall, the safety profile of Neovastat is excellent.

In a study of 48 patients with refractory prostate cancer, Neovastat was well tolerated. Efficacy analyses showed that 38% of patients improved or did not have their PSA increase by 25% or more (remained stable). This benefit was found in 42% of patients receiving the highest doses and in 20% of patients receiving the lowest doses. In a study in 48 non-small cell lung cancer (NSCLC) patients, dose–response studies indicated a 33% increase in median survival time (median 6.15 versus 4.63 months; $p = 0.026$) and a more than 50% decrease in the relative risk of death in patients who received more than 2.63 ml/kg/day of Neovastat (which corresponds to a dose of approximately 180 ml/day for a 70 kg patient) compared to those who received less than 2.63 ml/kg/day of Neovastat [39].

In a second phase I/II trial in patients with various types of solid tumors, including renal cell carcinoma (RCC), a survival analysis was performed for the 22 patients with metastatic RCC refractory to standard therapies or for whom no treatment was available. The median survival time in the 14 patients receiving 240 ml/day has been found significantly higher as compared to the median survival time in the eight patients receiving 60 ml/day (16.3 versus 7.1 months; $p = 0.01$) [40].

In light of these encouraging data, two phase III trials with Neovastat were initiated in NSCLC and RCC patients [41]. Furthermore, one phase II trial was recently initiated in patients with refractory or early relapse multiple myeloma.

Phase III clinical trials

Lung cancer

The safety and efficacy of Neovastat in patients with NSCLC stage IIIA and IIIB is currently being investigated in collaboration with the US National Cancer Institute in a phase III randomized, double-blind placebo-controlled, multi-center trial. Approximately 50 investigative sites are involved in Canada and the USA, and 760 newly diagnosed stage IIIA and IIIB NSCLC patients will be randomized to receive Neovastat (240 ml/day) or a placebo, in combination with a standard platinum-based therapy and radiotherapy. Study end points of this trial are survival, progression-free survival, tumor response and tumor response duration. The recruitment started in April 2000 and the results are expected in 2005.

Kidney cancer

A phase III randomized, double-blind placebo-controlled, multi-center study in patients with progressive RCC refractory to immunotherapy is currently being conducted in Europe, Canada and the USA. Approximately 50 investigative sites are involved and 302 patients have been randomized to receive Neovastat (240 ml/day) or a placebo. The endpoints of this trial are survival, time to progression, 1-year survival rate, quality of life, overall tumor response rate and duration of response. Enrollment was completed in December 2001 and the results are expected in early 2003. Recently, the FDA granted orphan drug status to Neovastat for kidney cancer.

Phase II clinical trial in patients with multiple myeloma

As angiogenesis promotion is not only involved in the pathophysiology of solid tumors, but also that of liquid tumors, a phase II Neovastat clinical trial in patients with multiple myeloma was initiated. This multicenter, single-arm, open-label, phase II trial currently involves 35 sites across North America and Europe, and will evaluate the efficacy of Neovastat as monotherapy treatment for patients with multiple myeloma not responding to standard therapies. Approximately 120 patients are to be included and the final results of the trial are expected in 2003. The primary end point of this trial is the tumor response rate based on protein M levels, with duration of tumor response, time to progression and survival as exploratory endpoints. The recruitment of 125 patients

started in May 2001 and the final results of the trial are expected at the end of 2003.

Discussion

In recent years, remarkable progress has been made in the understanding of the cellular and molecular processes involved in the transformation, proliferation and metastasis of tumor cells [1]. These efforts have highlighted the complexity of the transformation of normal cells to malignant phenotypes, and identified a number of oncogenes, growth factors and receptors that are frequently associated with the development of various types of cancer [2]. Unfortunately, the clinical benefits of these findings have not been apparent until now and many curative treatment strategies presently in use are still ineffective. The targeting of angiogenesis represents a relatively new approach to cancer therapy that may offer several advantages over standard cytotoxic therapies. First, antiangiogenic drugs specifically target normal genetically stable cells and are thus less likely to induce resistance [5]. Second, the antiangiogenic molecules studied so far seem to induce few side effects (with the exception of non-specific MMP inhibitors and VEGF-targeted drugs). For example, over 800 patients have been treated with Neovastat over the last 4 years with no cases of clinically significant toxicity. Thirdly, the targeting of tumor blood flow should imply a greater accessibility to tumor sites. Finally, the strict dependence of both solid and liquid tumors on angiogenesis suggests a broad applicability of antiangiogenic therapies to many different tumor types.

Notwithstanding the potential clinical benefit of antiangiogenic therapy, the translation of most of the molecularly targeted angiogenesis inhibitors to the clinic is still expected. MMP inhibitors, such as AG3340 and BAY 12-9566, which showed positive effects in animals and early clinical trials, were found ineffective and even detrimental in phase III clinical trials, possibly due to their lack of specificity and their inhibition of tumor necrosis factor sheddase activity [6]. Drugs targeting the VEGF-mediated signaling pathway, such as SU5416 (VEGFR inhibitor) and the anti-VEGF antibody bevacizumab (Avastin) were recently reported to have no beneficial effects in phase III studies in patients with metastatic colorectal and advanced relapsed metastatic breast cancer, respectively, as confirmed by the information publicly released from the companies developing these drugs (see SUGEN and Genentech websites www.gene.com and www.sugen.com). Whether the failure of these trials is due to heterogeneity in the expression of VEGF by the patients or to inappropriate clinical end points remains to be determined, but it highlights the potential complexity of using agents targeting a single molecular event to treat a process that is heterogeneous in nature.

In this context, an intrinsic advantage of Neovastat over other agents may reside in its pleiotropic action against several components of the angiogenesis process (Fig. 1). Neovastat inhibits specific MMP activities, VEGF binding to ECs and its subsequent activation of intracellular signaling pathways. In addition, it has pro-apoptotic activity that specifically induces EC death. It is also a stimulator of tPA activity, which may lead to excessive proteolysis of the ECM surrounding the tumor blood vessels and to the destruction of the fibrin provisional matrix that is necessary for neovessel formation. Neovastat can thus be considered equivalent to an 'antiangiogenic cocktail', targeting several steps involved in tumor angiogenesis. We believe that the presence of several distinct activities in Neovastat may explain in part initial positive clinical trial results. Angiogenesis is not a single process, but rather a multistep process that implicates a concerted action from several proangiogenic stimuli (e.g. VEGF, MMPs, integrins). Therefore, we believe that Neovastat, by targeting different mechanisms of angiogenesis, has a greater chance of having a clinically meaningful effect on tumor angiogenesis. Moreover, isolation of the various active ingredients present in Neovastat will not only be useful for the clinic, but may also provide important scientific information about the optimal stochiometry of a cocktail of antiangiogenic agents.

References

1 Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**:57–70.
2 Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996; **86**:353–364.
3 Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971; **285**:1182–1186.
4 Cao Y. Endogenous angiogenesis inhibitors and their therapeutic implications. *Int J Biochem Cell Biol* 2001; **33**:357–369.
5 Kerbel RS. Inhibition of tumor angiogenesis as a strategy to circumvent acquired resistance to anti-cancer therapeutic agents. *BioEssays* 1991; **13**:31–36.
6 Scappaticci FA. Mechanisms and future directions for angiogenesis-based cancer therapies. *J Clin Oncol* 2002; **20**:3906–3927.
7 Eisenstein R, Sorgente N, Soble LW, Miller A, Kuettner KE. The resistance of certain tissues to invasion: penetrability of explanted tissues by vascularized mesenchyme. *Am J Pathol* 1973; **73**:765–774.
8 Sorgente N, Kuettner KE, Soble LW, Eisenstein R. The resistance of certain tissues to invasion. II. Evidence of extractable factors in cartilage which inhibit invasion by vascularized mesenchyme. *Lab Invest* 1975; **32**:217–222.
9 Dupont E, Brazeau P, Juneau C. Extracts of shark cartilage having an antiangiogenic activity and an effect on tumor progression: process of making thereof. *US Patent 5,618,925* (1997)
10 Gingras D, Renaud A, Mousseau N, Beliveau R. Shark cartilage extracts as antiangiogenic agents: smart drinks or bitter pills? *Cancer Metast Rev* 2000; **19**:83–86.
11 Dupont E, Falardeau P, Mousa SA, Dimitriadou V, Pépin M-C, Wang T, *et al.* Antiangiogenic and antimetastatic properties of Neovastat (Æ-941), an orally active extract derived from cartilage tissue. *Clin Exp Metast* 2002; **19**:145–153.
12 Keck PJ, Hauser SD, Krivi G, *et al.* Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science* 1989; **246**:1309–1312.
13 Connolly DT, Heuvelman DM, Nelson R, *et al.* Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. *J Clin Invest* 1989; **84**:1470–1478.
14 Connolly DT, Stoddard BL, Harakas NK, Feder J. Human fibroblast-derived growth factor is a mitogen and chemoattractant for endothelial cells. *Biochem Biophys Res Commun* 1987; **144**:705–712.

15 Ferrara N, Gerber HP. The role of vascular endothelial growth factor in angiogenesis. *Acta Haematol* 2001; **106**:148–156.
16 Matsumoto T, Claesson-Welsh L. VEGF receptor signal transduction. *Sci STKE* 2001; **112**:RE21.
17 Waltenberger J, Claesson-Welsh L, Siegbahn A, Shibuya M, Heldin CH. Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. *J Biol Chem* 1994; **269**: 26988–26995.
18 Rousseau S, Houle F, Kotanides H, *et al.* Vascular endothelial growth factor (VEGF)-driven actin-based motility is mediated by VEGFR-2 and requires concerted activation of stress-activated protein kinase 2 (SAPK2/p38) and geldanamycin-sensitive phosphorylation of focal adhesion kinase. *J Biol Chem* 2000; **275**:10661–10672.
19 Gerber HP, McMurtrey A, Kowalski J, *et al.* Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. *J Biol Chem* 1998; **273**:30336–30343.
20 Gille H, Kowalski J, Li B, *et al.* Analysis of biological effects and signaling properties of Flt-1 (VEGFR-1) and KDR (VEGFR-2): a reassessment using novel receptor-specific vascular endothelial growth factor mutants. *J Biol Chem* 2001; **276**:3222–3230.
21 Béliveau R, Gingras D, Kruger EA, *et al.* The antiangiogenic agent Neovastat (*Æ*-941) inhibits vascular endothelial growth factor-mediated biological effects. *Clin Cancer Res* 2002; **8**:1242–1250.
22 Dimmeler S, Zeiher AM. Endothelial cell apoptosis in angiogenesis and vessel regression. *Circ Res* 2000; **87**:434–439.
23 Boivin D, Gendron S, Beaulieu E, Gingras D, Béliveau R. The antiangiogenic agent Neovastat (*Æ*-941) induces endothelial cell apoptosis. *Mol Cancer Ther* 2002; **1**:795–802.
24 Forget M-A, Desrosiers RR, Béliveau R. Physiological roles of matrix metalloproteinases: implications for tumor growth and metastasis. *Can J Physiol Pharmacol* 1999; **77**:465–480.
25 Nagase H, Woessner Jr JF. Matrix metalloproteinases. *J Biol Chem* 1999; **274**:21491–21494.
26 Stetler-Stevenson WG. Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. *J Clin Invest* 1999; **103**:1237–1241.
27 Edwards DR. The tissue inhibitors of metalloproteinases (TIMPs). In: Clendeninn NJ, Appelt K (editors): *Matrix Metalloproteinase Inhibitors in Cancer Therapy.* Totowa, NJ: Humana; 2001, pp. 67–84.
28 Gingras D, Renaud A, Mousseau N, Beaulieu E, Kachra Z, Béliveau R. Matrix proteinase inhibition by *Æ*-941, a multifunctional antiangiogenic compound. *Anticancer Res* 2001; **21**:145–155.
29 Lijnen HR, Collen D. Mechanisms of physiological fibrinolysis. *Baillière's Clin Haematol* 1995; **8**:277–290.
30 Pepper MS. Role of matrix metalloproteinase and plasminogen activator-plasmin systems in angiogenesis. *Arterioscler Thromb Vasc Biol* 2001; **21**:1104–1117.
31 Gyetko MR, Todd RF, Wilkerson CC, Sitrin RG. The urokinase receptor is required for human monocyte chemotaxis *in vitro*. *J Clin Invest* 1994; **93**:1380–1398.
32 Min HY, Doyle LV, Vitt CR, Zandonella L, Stratton-Thomas JR, Shuman MA, *et al.* Urokinase receptor antagonists inhibit angiogenesis and primary tumor growth in syngeneic mice. *Cancer Res* 1996; **56**:2428–2433.
33 Ferrier CM, Suciu S, van Geloof WL, *et al.* High tPA-expression in primary melanoma of the limb correlates with good prognosis. *Br J Cancer* 2000; **83**:1351–1359.
34 Chappuis PO, Dieterich B, Sciretta V, Lohse C, Bonnefoi H, Remadi S, *et al.* Functional evaluation of plasmin formation in primary breast cancer. *J Clin Oncol* 2001; **19**:2731–2738.
35 Sawaya R, Ramo OJ, Shi ML, Mandybur G. Biological significance of tissue plasminogen activator content in brain tumors. *J Neurosurg* 1991; **74**:480–486.
36 Reijerkerk A, Voest EE, Gebbink MFBG. No grip, no growth: the conceptual basis of excessive proteolysis in the treatment of cancer. *Eur J Cancer* 2000; **36**:1695–1705.
37 Berger F, Jourdes P, Benabid AL. *Æ*-941 (Neovastat) induces the expression of angiostatin in experimental glioma. *Proc Am Soc Clin Oncol* 2001; **00**:000.
38 Weber MH, Lee J, Orr FW. The effect of Neovastat (AE-941) on an experimental metastatic bone tumor model. *Int J Oncol* 2002; **20**:299–303.
39 Falardeau P, Champagne P, Poyet P, Hariton C, Dupont E. Neovastat, a naturally occurring multifunctional antiangiogenic drug, in phase III clinical trials. *Semin Oncol* 2001; **28**:620–625.
40 Batist G, Patenaude F, Champagne P, Croteau D, Levinton C, Hariton C, *et al.* Neovastat (AE-941) in refractory renal cell carcinoma patients: report of a phase II trial with two dose levels. *Ann Oncol* 2002; **13**:1259–1263.
41 Gingras D, Batist G, Béliveau R. *Æ*-941 (Neovastat): a novel multifunctional antiangiogenic compound. *Expert Rev Anticancer Ther* 2001; **1**:341–347.