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Review

Use of angiogenesis inhibitors in tumour treatment

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Abstract

Advances in molecular biology have permitted the characterisation of mechanisms underlying angiogenesis. Angiogenesis is a crucial process in tumour pathogenesis as it sustains malignant cells with nutrients and oxygen. It is well known that tumour cells secrete various growth factors including VEGF, which triggers endothelial cells to form new capillaries. Preventing the expansion of new blood vessel networks results in reduced tumour size and metastases. Not surprisingly, numerous drugs that are currently under clinical development interfere with growth factor-derived angiogenic signals. This review aims to describe angiogenesis inhibitors and surveys their different modes of action.

Keywords: Cancer; Angiogenesis; Inhibitors; VEGF

1. Introduction

Progress in molecular biology has seen the arrival of targeted therapies and has revolutionised treatment approaches in oncology. Targeting neovascularisation offers great potential to focus on normal and genetically stable endothelial cells to hinder tumour progression (Table 1) [1]. A finely tuned equilibrium exists between physiological anti-angiogenic and pro-angiogenic factors (Table 2) [2,3]. Angiogenesis [4] is mostly mediated by vascular endothelial growth factor (VEGF) in hypoxic conditions due to tumour progression. Low intratumour pH, hypoglycemia, stress and inflammation induced by tumour proliferation also promote angiogenesis. The angiogenic tumour phenotype is characterised by high microvessel density, elevated VEGF levels and is correlated to poor prognosis. During angiogenesis, endothelial cells are stimulated by various growth factors by binding to membrane receptors such as tyrosine kinase receptors (TKRs). The TKRs that are directly involved in angiogenesis include receptors for VEGF, FGF, PDGF, Ang-1, angiopoïetin-2 (Ang-2), hepatocyte growth factor (HGF), Eph and receptors belonging to the epithelial growth factor family. VEGF is the most potent inducer of angiogenesis and there are four VEGF receptors (VEGFR). Like the other TKRs involved in angiogenesis, these receptors are expressed by endothelial cells and not by the tumour cells secreting the growth factors. TKRs are not only activated by their own ligands but also by hormones, neurotransmitters and lymphokines [5]. Large numbers of anti-angiogenic agents with various mechanisms of action are currently under clinical development and this review will focus on the most promising and advanced among them.

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Table 1
Advantages of endothelial cells as targets of anti-tumor therapeutics

- Resistance does not occur as often as it does with drugs that directly target tumor cells. Unlike tumor cells, endothelial cells are genetically stable, diploid and homogenous. Spontaneous mutations are rare and prolonged treatments are possible
- Tumor endothelial cells divide 50–100 times more rapidly than normal endothelial cells, and activated endothelial cells express markers that quiescent endothelial cells do not express and if they do, expression is much lower
- As angiogenesis is normally limited in adults, few adverse effects are expected with anti-angiogenic drugs
- Unlike tumor cells, endothelial cells are easily accessible from the blood circulation
- Numerous tumor cells depend on a single microvessel. Destroying a few microvessels amplifies anti-tumor effects

Table 2
Natural angiogenesis modulators

Inhibitors	Stimulators
Thrombospondin	Vascular endothelial growth factor (VEGF)
Angiostatin (plasminogen fragment)	Basic fibroblast growth factor (bFGF)
Endostatin (collagen XVIII fragment)	Acid fibroblast growth factor (aFGF)
AaAt (antithrombin III fragment)	Platelet-derived growth factor (PDGF)
Vasostatin (calreticulin fragment)	Platelet-derived epithelial growth factor
Prolactin (N-terminal fragment of 16-kDa)	Hepatocyte growth factor/scattered factor
Troponin 1	Epidermal growth factor
Angiopoietin-2	Insulin-like growth factor
Alpha interferon	Transforming growth factor α(TGFα)
Gamma interferon	Tarnsforming growth factor β (TGFβ)
Interleukin 12	Tumor necrosis factor α(TNFα)
Fibronectin	Placental growth factor
Tissue inhibitors of metalloproteinases	Angiopoietin-1
(TIMPs)	Angiogenin
Plasminogen activator inhibitor 1 (PAI-1)	Pleiotrophin
Platelet factor 4 (PF 4)	Interleukin 8
Pigment epithelial cell-derived factor (PEX)	Granulocyte-colony stimulating factor
Retinoic acid	(G-CSF)
2-Methoxyestradiol	Proliferin
Dopamine	Leptin

2. Inhibition of the VEGF pathway

2.1. Tyrosine kinase inhibitors

2.1.1. Semaxanib (SU5416)

Semaxanib, the first specific synthetic inhibitor of VEGF-TKR activity, inhibits the growth of human tumours xenografted into mice (mostly slowly-growing tumours). However, despite encouraging preliminary results in pilot studies with a good safety profile, semaxanib proved disappointing in several phase II studies in different tumours and its clinical development has been stopped.

2.1.2. SU6668

SU6668 has a wide spectrum of activity and inhibits the tyrosine kinase activity of PDGFR, FGFR1 and VEGFR2. It permits the regression of human tumour xenografts in mice with complete histological response following the rapid apoptosis of tumour microvessels. A phase I study including 56 patients with various advanced cancers demonstrated good tolerance to SU6668 and resulted in a minor response (MR) and two stabilisations (SD) [6]. Another phase I study in 24 patients obtained no objective response but 10 SD (41.7%) [7].

2.1.3. SU011248

SU011248 inhibits VEGFR1, PDGFR and c-kit, a receptor of the pluripotent cell growth factor (SCF) implicated in malignant blood diseases. At higher concentrations, it inhibits another angiogenesis TKR, FGFR1. SU011248 was synergistic with radiotherapy in mouse models attaining tumour responses and sustained tumour control [8]. In a phase II study, 63 patients with renal cancer after failure of a cytokine received SU011248 that resulted in 21 (33%) partial responses (PR) and 23 (37%) stabilisations (SD) [9]. The 1-year survival was 65%. Interestingly, SU011248 has also achieved clinical activity in gastrointestinal stromal tumours (GIST). GIST are sensitive to STI-571 a specific KIT tyrosine-kinase inhibitor. After failure of STI-571, 98 patients (92 evaluated) received SU011248. Of those patients, 7 (8%) PR and 53 (58%) SD were observed [9]. Efficacy of SU011248 is higher in patients without mutation in KIT or PDGFRA, or a mutation in the exons 9, 13 and 14 of KIT or PDGFRA. The effi-

cacy of SU011248 is limited to patients carrying mutations in the exons 11 and 17.

2.1.4. ZD6474

ZD6474 is an orally administered inhibitor of VEGFR2 and to a lesser extend ErB1 (EGFR). It has a certain degree of activity against other TKRs (PDGFR > VEGFR1 > Tie-2 > FGFR1). A phase I study demonstrated a safe clinical profile but no tumour responses. Further studies are ongoing.

2.1.5. PTK787/ZK 222584

PTK/ZK inhibits the tyrosine kinase activity of VEGFR1 and VEGFR2 and is given orally [11]. Preclinical studies demonstrated activity through the inhibition of tumour vasculature, alone or in combination with chemotherapy or radiotherapy. Pre-irradiated vasculature may be more sensitive to PTK/ZK compared to vasculature not irradiated [12]. Phase I studies have shown good PTK/ZK tolerance and efficacy in various tumours. The most commonly reported adverse events were nausea (59%), fatigue (41%), vomiting (35%), dizziness (29%), and headache (24%) [13]. The first study in 20 patients with relapsing glioblastoma yielded 4 SD and one PR in the 15 tested patients [14]. Magnetic resonance imaging depicted a decrease in vascular permeability of tumours after intake of the drug that was correlated with clinical response [15]. The second study enrolled 35 patients with advanced colorectal cancer and patients were treated every 14 days with oxaliplatin, 5-FU and folinic acid in combination with continuously escalated doses of PTK/ZK (500–2000 mg/day). No increased oxaliplatin/5-FU toxicity was seen and PTK/ZK was tolerated to doses of 1500 mg/day. In 28 patients 1 CR (4%), 14 PR (50%), 9 SD (32%), and 4 progressive disease (PD) (14%) were obtained. For all patients, the median overall survival was 16.6 months (95% CI = 12.9, 21 months) [16]. Similar results were obtained when PTK/ZK was combined with irinotecan-5-FU-folinic [17]. In the third study, 45 patients (37 evaluated) demonstrated the efficacy of PTK/ZK in metastatic renal cancer. Two patients experienced dose-limiting toxicity (grade 3 headache and grade 3 hypertension). Seven patients (19%) achieved a measurable response (1 partial and 6 minor) with a median TTP of 5.5 months (95% CI = 3.7, 7.9 months), 17 (46%) achieved SD, and 5 (14%) had PD. Rapid disease progression (within 3 months) occurred in only 28% of the patients (95% CI = 12.3, 43.6%) treated with at least 1000 mg/day compared with an expected rate of 49.7% (95% CI = 43.5%, 55.9%) based on cytokine therapy in a similar patient population. One-year overall survival (OS) was 63.7% (95% CI = 41.9%, 85.5%) [13].

2.1.6. CP-547,632

CP-547,632 is an orally-administered inhibitor of the tyrosine kinase activity of VEGFR2. It binds to EGFR and also to PDGFR-β. A phase I study demonstrated good tolerance and 6 SD for more than 8 weeks and 1 for more than 6 months in 22 evaluated patients.

2.2. Monoclonal antibodies

2.2.1. Bevacizumab

Bevacizumab is a humanised antibody designed to target VEGF and not its receptor like the other agents discussed in this review. This antibody has shown tumour growth inhibition in preclinical and early clinical studies with good tolerance. Phase I studies did highlight potential risk of hypertension or thromboembolism however its clinical efficacy has since been demonstrated. A phase III trial investigated bevacizumab (5 mg/kg every two weeks) *vs.* a placebo in combination with irinotecan, 5-FU and acid folinic (IFL) as first-line therapy for metastatic colorectal cancer in 925 randomised patients. Adding bevacizumab to chemotherapy resulted in increased median survival (20.3 months *vs.* 15.6, $P = 0.00003$), progression-free survival (10.6 months *vs.* 6.24, $P < 0.00001$), a higher response rate (45% *vs.* 35%, $P = 0.0029$) and a longer duration of response (10.4 months *vs.* 7.1, $P = 0.0014$) as compared with chemotherapy plus placebo. Adding bevacizumab did not modify the toxicity of chemotherapy but only increased grade 3 hypertension (10.9 *vs.* 2.3%), which was easily managed with oral medications [18]. The inhibition of angiogenesis was demonstrated with a single infusion of bevacizumab that decreased tumour perfusion, vascular volume, microvascular density, interstitial fluid pressure and the number of viable, circulating mature and progenitor endothelial cells in patients with rectal carcinoma [19]. A randomised, double-blind, phase II trial was conducted comparing a placebo (40 patients) with bevacizumab at doses of 3 mg/kg (37 patients) and 10 mg/kg (39 patients), given every two weeks in 116 patients with metastatic renal cancer. There was a significant prolongation of TTP in the high-dose antibody group as compared with the placebo group (hazard ratio, 2.55; $P < 0.001$). The probability of having progression-free tumours for patients given the high-dose antibody, the low-dose antibody, and the placebo was 64%, 39%, and 20%, respectively, at four months and 30%, 14%, and 5%, respectively, at eight months. There were no significant differences in overall survival between the groups but primary endpoints were TTP and the response rate [20]. Another phase II trial investigated the adjunction of bevacizumab to gemcitabine as first-line therapy in 21 patients with metastatic pancreatic cancer. Among evaluated patients [16], 6 PR (38%) and 7 SD (44%) were observed. Median survival was not reached and the estimated 1-year survival rate was 54%, and TTP 5.5 months. Pretreatment VEGF levels ranged from 0 to 586 pg/ml and were not correlated with response, TTP, or survival [21].

2.2.2. IMC-1C11

IMC-1C11, a chimeric IgG1 antibody against VEGFR2 and blocks ligand-receptor binding and inhibits phosphorylation. A phase I study in 14 patients with metastatic colourectal cancer resulted in prolonged stabilisation but presence of anti-chimeric antibodies in 7 patients (2 had neutralising antibodies) was detected [22]. Antibodies against VEGFR such as IMC-1C11 may have theoretical advantages compared to antibodies against soluble ligands that are frequently over produced leading to treatment resistance.

2.3. Other mechanisms to inhibit the VEGF pathway

2.3.1. Cellular immunotherapy

Using VEGFR-specific $CD8^+$ cytotoxic lymphocytes as opposed to antibodies is an original approach. A retroviral vector allows the transfection of VEGFR sequences in $CD8^+$ lymphocytes, which then become endowed with cytotoxic activity against VEGFR-expressing cells. These lymphocytes are able to inhibit tumour growth in mice *in vivo*. This inhibition is more powerful when administered in combination with the anti-angiogenic agent TNP-470 [23].

2.3.2. Anti-FLT-1 ribozyme

Ribozymes are catalytic RNA molecules capable of cleaving mRNA at specific sequences. Anti-FLT-1 ribozyme is a nuclease-resistant synthetic molecule targeting VEGFR1 mRNA. In a phase I/II study enrolling 28 patients with refractory solid tumours, the ribozyme was subcutaneously administered daily. Tolerance was good apart from some reactions at the inoculation site [24]. Seventeen SD lasting 1–6 months and 2 minor responses were observed. Phase II studies testing ribozyme alone or in combination with chemotherapy are awaited.

2.3.3. Aplidine

Aplidine is a cyclodepsipeptide that seems to decrease VEGFR1 expression and to induce cell cycle arrest in the G1 phase. It has demonstrated promising activity *in vitro* against several human tumours. Aplidine is currently under clinical development in phase I studies and tumour responses have been reported particularly in leukemia patients [25].

2.4. Antisense oligonucleotides

Neutralising antisense oligonucleotides for VEGF mRNA have been developed and exhibit an anti-angiogenic potential [26]. A neutralising antisense oligonucleotide for angiopoietin-1 was tested *in vitro* with HeLa tumour cell lines and showed a marked decrease in tumour growth.

2.5. VEGF Trap

VEGF Trap is a fusion protein consisting of human VEGFR1 (flt-1) segments and VEGFR2 (KDR) extracellular domains fused to the Fc portion of human IgG1. It acts by binding to and inactivating circulating VEGF that found in tissues. VEGF Trap is designed has great affinity for VEGF similar to that of the high-affinity receptor VEGFR1 (K_m = 1–5 pM). This results in approximately 100-fold tighter binding than that achieved with anti-VEGF monoclonal antibodies, while retaining pharmacokinetic properties similar monoclonal antibodies. This compound could potentially achieve greater efficacy at lower doses compared to antibodies. Furthermore, in contrast to humanised monoclonal antibodies, it contains only human amino acid sequences. A dose-escalation phase I trial tested a single subcutaneous dose of VEGF Trap in patients with relapsed or refractory tumours, followed 4 weeks later by 6 weekly injections. Fourteen patients were treated at four dose levels (25, 50, 100, and 200 mcg/kg). The VEGF Trap-VEGF complex in plasma had an apparent elimination half-life of approximately 17 days. No Grade 3 or 4 toxicities were observed and no anti-VEGF Trap antibodies were detected. Grade 1 and 2 toxicities included reversible proteinuria, fatigue, and constipation. Disease stabilisation was obtained in patients with renal or colon cancer [27].

3. COX-2 inhibition

The production of VEGF and other angiogenic factors calls into service a cascade of intermediate signals, transcription and regulation factors, and among the prostaglandins produced by cyclooxygenases (COX), the COX-2 isoform [28]. COX-2 induction has been reported in numerous solid tumours (colon, prostate, lung, breast, pancreas, skin, head and neck) but not in corresponding normal tissues. COX-2 was detected in tumour cells, tumour vascularisation and pre-existent adjacent capillaries. Tumours transplanted into *COX-2* deficient mice ($COX\text{-}2^{-/-}$) showed that COX-2 is not only crucial for the production of VEGF, but that endogenous COX-2, produced by vascular endothelium or by fibroblasts, contributed markedly to the growth of transplanted tumour cells [29]. The detection of COX-2 in neovasculature was described as a characteristic of epithelioma. In colon cancer, the number of cells expressing COX-2 was found to correlate with tumour stage, size and median survival. Pre-clinical studies have demonstrated that anti-tumour activity of selective COX-2 inhibitors have a good safety profile. The anti-tumour properties of COX-2 inhibitors may be attributed to the inhibition of angiogenesis mediated by PGE and VEGF, however COX-2 may also play a role in the migration and survival of endothelial cells, vessel

permeability and the suppression of immune responses [28]. Collectively, these events contribute to tumour growth and may eventually be potent therapeutic targets as assessed by several phase II studies in different tumour types. A recent trial tested the benefit of adding celecoxib (400 mg bid) to a chemotherapy cocktail containing irinotecan and capecitabine in the treatment of metastatic and unresectable colorectal cancer. Twenty-three patients were included (17 evaluated) and 9 PR (53%), 10 SD (56%) and 3 PD (17%) were observed and celecoxib appeared to decrease the toxicity of capecitabine [30]. A further study evaluated the combination of celecoxib (400 mg p.o. bid continuously) and paclitaxel (80 mg/m^2 i.v. weekly for six weeks followed by a two-week rest) in 27 patients (16 evaluated, mostly with adenocarcinomas) as second line therapy in non-small cell lung cancer (NSCLC) after failure of a first line with platinum compounds. Four PR (25%), 6 SD (37%) and 6 PD (37%) were observed with acceptable toxicity [31]. Another study evaluated the benefit afforded with celecoxib (400 mg $\times$ 2/d) combined with two preoperative cycles of paclitaxel and carboplatin in 29 patients (stages IB to IIIA). All patients completed preoperative chemotherapy, and 26 completed preoperative celecoxib. The overall clinical response rate was 65% (48% PR, 17% CR). Twenty-eight patients were explored and underwent complete resection of their tumours. No complete pathological responses were observed but seven patients (24%) had minimal residual microscopic disease [32]. Interestingly, it has recently been suggested that high *COX-2* gene expression in completely resected NSCLC is correlated with poor prognosis (RR=3.848 ; 95% CI, 1.500–9.874) and limited benefit with UFT-based adjuvant therapy. Indeed, in the setting of UFT postoperative adjuvant chemotherapy, patients with low *COX-2* gene expression have a higher 5-year survival as compared to those with high *COX-2* gene expression (93% *vs.* 67%, $P = 0.045$) [33].

A pilot study in 12 patients demonstrated the potential efficacy of celecoxib (200 mg $\times$ 2/day) in a biochemical relapse of prostate cancer (indicated by increased prostate-specific antigen (PSA) levels) after radiotherapy or radical prostatectomy. PSA levels were significantly modified in 8 patients after 3 months of treatment (declined in 5 and stabilised in 3). Of the remaining 4 patients, 3 had a marked decrease in their PSA doubling time. The short-term responses at 3 months also continued at 6 and 12 months [34].

4. Other inhibition pathways

4.1. EGFR inhibitors

EGFR inhibitors are currently under clinical development. They include small molecules such as gefitinib (ZD1839 or Iressa®) and erlotinib (OSI574 or Tarceva®) as well as monoclonal antibodies (C225, ABX-EGF). Many of these agents exhibit anti-angiogenic activity, at least in preclinical models, and this anti-angiogenic activity could partially explain their clinical efficacy [35,36].

4.2. Integrin antagonists

Vitaxin® is a humanised monoclonal antibody against the $\alpha_v\beta_3$ integrin and is currently being tested in phase I trials where it has yielded PR or SD in sarcoma. EMD 121974, an $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin antagonist, produced responses in melanoma and brain tumours in preclinical studies. A phase I trial resulted in good tolerance [37]. Another humanised IgG1 (Medi-522) targeting $\alpha_v\beta_5$ integrins was tested in a phase I study which accrued 19 patients (13 evaluated). Tolerance was acceptable and 7 patients achieved prolonged SD with 2 lasting more than 9 months [38].

4.3. Endogenous angiogenesis inhibitors

Endostatin and angiostatin are peptides derived from natural proteins that have been shown to exhibit anti-angiogenic properties. These agents have a short half-life and repeated, or even, continuous injections are required. Results are modest but prolonged SD was observed, particularly in patients with advanced neuroendocrine tumours who were enrolled in a phase II study. All the 41 patients (25 carcinoid and 16 pancreatic tumours) were initially treated with 60 mg/m^2/day of rhEndostatin, self-administered by subcutaneous injection in divided doses, at 12-h intervals. Minimal toxicity was observed during this treatment. Doses were escalated if the therapeutic effect was insufficient but not for progressive disease. Twenty-three (62%) SD and 12 (32%) PD were observed. The median TTP was 39 weeks and median survival was not reached [39]. Thrombospondin (TSP-1) is another natural angiogenesis inhibitor and ABT-510 is a substituted nanopeptide that mimics the anti-angiogenic activity of the endogenous protein. It was tested in a phase IB study that enrolled 36 patients (34 evaluated) with various tumours. Patients received daily subcutaneous escalating doses of ABT-510 with acceptable tolerance. One PR in a soft tissue sarcoma (STS) and 14 (41%) and 5 (15%) SD was observed in 8 and 16 weeks, respectively. Prolonged SD (>24 weeks) was seen in 1 patient with NSCLC and 1 with STS [40].

4.4. Other molecules under development

4.4.1. Squalamine

Squalamine is a natural anti-angiogenic sterol that has been proven efficient in the treatment of human

ovarian tumour xenografts in mice. It enhances the effects of cisplatin. Interestingly, as HER-2-overexpressing cells are platinum-resistant, this resistance is suppressed when cisplatin is combined with squalamine.

4.4.2. TNP-470

TNP-470 is a synthetic analog of fumagilin, a natural angiogenesis inhibitor. It acts by inhibiting the phosphorylation of methionine aminopeptidase-2, the cycline-2-dependant kinase overexpressed in tumour cells. Preliminary studies demonstrated high activity in micrometastases but with potentially limiting neurological toxicity. In phase I studies, its therapeutic activity is modest in renal and cervical cancers.

4.5. Tyrosine-phosphatase inhibitors

Once activated, VEGFR2 allows the phosphorylation of proteins involved in signal transduction through tyrosine phosphatases. Tyrosine-phosphatase inhibitors have been developed and have exhibited anti-angiogenic activity [41].

4.6. Thalidomide

Thalidomide is an inhibitor of FGF-2 or VEGF-induced angiogenesis with high clinical efficacy in myeloma despite peripheral neuropathies and somnolence [42]. A phase II study enrolling 19 patients (14 evaluated) with advanced renal cancer tested the combination of low doses of thalidomide (100 mg/day) with α IFN (3 MUI/d). Three (21.4%) PR, 7 (50%) SD were obtained. Overall survival was 17.4 months [43].

More efficient thalidomide analogs have been developed. Among these agents, CC-5013 was tested in a phase I trial in 20 patients with cerebral tumours (18 glioblastomas, 1 atypical meningioma, and 1 multiple, recurrent spinal hemangioblastoma). No sedation, constipation, peripheral neuropathy, or skin rash was observed. Three patients achieved SD for 6, 5 and 7 months, respectively [44].

4.6.1. Combretastatin A-4 phosphate (CA4P)

The phosphorylated pro-drug CA4P is a synthetic analogue of combretastatin, a tubulin-binding agent that selectively induces apoptosis in proliferating human umbilical vein endothelial cells *in vitro*, and produced up to a 90% reduction in tumour blood flow in xenografted tumour models. Phase I studies showed its potential efficacy [45], but cardiotoxicity is the major adverse side effect [46].

4.6.2. IM862

This nasally-instilled peptide inhibits angiogenesis and activates NK cells. Interesting effects were evidenced in HIV-related Kaposi's sarcoma.

4.6.3. Oltipraz

This synthtetic dithiolethione inhibits angiogenesis. Athymic mice xenografted with angiosarcoma cells were treated with oltipraz, which resulted in an 81% decrease in the tumour mass.

4.6.4. Methoxyestradiol (2ME$_2$)

2ME$_2$ is an endogenous estrogen metabolite that inhibits tumour proliferation through its potent anti-angiogenic and pro-apoptotic activities. A phase I trial in 14 patients demonstrated good tolerance, 1 PR (ovarian cancer) and 1 SD lasting 10 months (prostate cancer) [47].

4.6.5. Neovastat

This angiogenesis inhibitor is multifunctional. It inhibits VEGFR1 and MMP-2, 9 and 12 metalloproteases. A phase II trial accrued 144 patients with various tumour types (22 renal cancers) [48]. Neovastat was administered orally twice a day at a dose of 60 or 240 ml/d. Many patients received immunotherapy before inclusion (75% in the first group (60 ml/d) and 43% in the second). Two objective responses were observed in the second group. Median survival in the second group was 16.3 months *vs*. 7.1 months ($P = 0.01$) in the first. The 2-year survival rate was 0% in the first group *vs*. 36% in the second. A phase III study is ongoing in renal cancer [49]. A phase I/II study in 48 patients with unresectable stage IIIA, IIIB, or IV NSCLC showed a significant survival advantage with high-dose neovastst (180 ml/d) compared to lower doses (6.1 months *vs*. 4.6 months, $P = 0.026$), but no tumour responses were observed [50].

4.7. The Raf-1 pathway

The $\alpha_v\beta_3$ integrin is preferentially expressed by the endothelium of neovessels and is a potential target for a gene therapy [51]. A lipidic nanoparticle was synthesised and bound to a $\alpha_v\beta_3$-ligand. A gene could be coupled to this particle and delivered specifically to $\alpha_v\beta_3$–expressing cells. The selected gene was a mutated form of Raf that inhibits VEGF- and bFGF-induced angiogenesis. Experiments in mice bearing melanoma or human colon tumour xenografts demonstrated very high anti-angiogenic activity, specifically mediated by the mutated Raf gene delivered to $\alpha_v\beta_3$-expressing endothelial cells, with fast and dramatic tumour regressions. Nanoparticles are much less immunogenic than viral vectors and thus allow repeated and prolonged treatment.

A specific Raf-1 inhibitor has been developed : BAY 43-9006. A phase I study showed good tolerance. A phase II study included 397 patients among which 112 with a renal cell carcinoma. Among them, 65 patients

completed the treatment, 63 were evaluated with 25 (39%) PR and 16 (26%) SD [52].

4.7.1. Well-known agents: conventional cytotoxics

Conventional agents used at a low dose for prolonged periods (metronomic chemotherapy) can display clinical activity probably mediated via the inhibition of angiogenesis [53]. The efficacy of this anti-angiogenic chemotherapy was proven for 5-FU, paclitaxel and oral cyclophosphamide. A recent study showed that TSP-1 is a crucial mediator of angiogenesis inhibition by metronomic chemotherapy. Firstly, exposure of endothelial cells *in vitro* to low doses of several different anticancer agents caused marked induction of gene and protein expression of TSP-1. Secondly, increases in circulating TSP-1 were detected in the plasma of human tumour-bearing mice treated with metronomic low-dose cyclophosphamide. Thirdly, the effects of low-dose continuous cyclophosphamide were lost in TSP-1-null C57BL/6 mice [54].

5. Conclusion

During the past several years, rapid progress has been achieved in the understanding of angiogenesis including signaling pathways and their regulation. This has enabled the development of numerous potentially interesting agents. Further advances are awaited in the field. In particular, resistance to anti-angiogenic treatments has been observed to be due to mutations of the p53 protein (such is the case in 50% of human cancers) which lower tumour cell oxygen requirements and thus their dependence on neovascularisation. The induction of *bcl-2*, a gene involved in resistance to apoptosis, has also been observed. Cross activation between the different signaling pathways must also be further elucidated. Studies in animals show that VEGFR and EGFR inhibitors can be combined to enhance their efficacy [54]. One of the present challenges is to determine whether it is best to combine exclusively these new anti-angiogenic agents or to combine them with conventional cytotoxics so that the survival of patients can be increased significantly. This challenge implies new clinical development models that preferably considers the cytostatic rather than the cytotoxic nature of anti-angiogenic agents, the possibility of prolonged therapy with these agents and the rationale for combining them with other cytotoxic therapies. Ongoing clinical trials are applying these concepts with the prospect of using these anti-angiogenic therapies in clinical practice.

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