Antiangiogenesis by Chemotherapeutic Agents

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Abstract: Many tumors are not curable because current treatments primarily target the tumor cells. Intratumoral endothelial cells, on the other hand, proliferate rapidly and are sensitive to the cytotoxic effects of chemotherapeutic agents. This review summarizes the literature concerning the antiangiogenic effects of these agents when administered alone or in combination with other angiogenesis inhibitors.

Keywords: Angiogenesis, antiangiogenesis, chemotherapeutic agents, endothelial cells, tumor progression.

INTRODUCTION

Angiogenesis occurs during embryo development and in postnatal life in wound repair and cyclically in the female reproductand time and the result of an equilibrium between activator and inhibitor systems, that together keep the microcirculation in a quiescent state with very low proliferation and turnover of endothelial cells.

Tumor angiogenesis is unlimited in time and characterized by a 30- to 40-fold proliferative activity of endothelial cells [1]. It is essential for tumor growth, invasion and metastasis. The progression from *in situ* to invasive and metastatic solid tumors is accompanied by the transition from a avascular to a vascular phase [2]. Substantial laboratory and indirect clinical evidence of the central role of angiogenesis in the progression of bladder, brain, breast, cervical, colon, lung, prostate and testis tumors, and hematological malignancies, including acute myeloid leukemia, acute lymphoblastic leukemia and multiple myeloma, has been obtained in the last 20 years [3, 4].

ANTIANGIOGENESIS AS A THERAPEUTIC STRATEGY IN THE TREATMENT OF TUMORS

Many solid and hematologic tumors are not curable because current anticancer treatments primarily target the tumor cells, whose genetic instability results in multiple genetic alterations that facilitate tumor progression and metastasis. Cell clones with diverse biological aggressiveness may coexist within the same tumor. These two properties lead to resistance to cytotoxic agents, which is still the main cause of treatment failure. By contrast, endothelial cells are normal diploid cells that do not acquire mutations and should not become resistant.

The existence of specific angiogenesis inhibitors was first postulated by Folkman in 1971 [5]. The term 'antiangiogenesis' was introduced to describe treatments designed to prevent the induction of new blood vessels and perhaps reduce the number of those already present.

Several approaches inhibit tumor angiogenesis [6-9] and more than 60 antiangiogenic compounds have been clinically evaluated. Because tumor-associated angiogenesis develops in a physiological context, its inhibition should not induce resistance and should potentiate the oncostatic effect, because each neovessel supplies hundreds of tumor cells. Inhibitors may be synthetic or semi-synthetic agents, endogenous inhibitors, or biological antagonists of the angiogenic cascade. By contrast, vascular targeting focused on specific molecular determinants of neovasculature is used for local delivery of a toxic effect that leads to vascular damage and tumor necrosis.

THE CHEMOTHERAPEUTICAL ANTIANGIOGENESIS MODEL (TABLE 1)

Animal studies in tumor-bearing mice were the first to demonstrate direct damage to endothelial cells or to tumor endothelium by chemotherapeutic anticancer agents [10, 11]. Angiogenesis can also be suppressed by cytotoxic agents, particularly at low and frequent doses. Intratumoral vascular endothelial cells, in contrast to the endothelium of quiescent mature blood vessels of normal adult tissues, proliferate rapidly and this renders them sensitive to the cytotoxic effects of such drugs in a manner similar to that of dividing bone marrow cells [12].

The dose-schedule chemotherapy regimens are conventionally administered at the maximum tolerated dose (MTD), followed by a treatment-free interval to allow recovery of bone marrow and gastrointestinal tract cells. For this reason they cannot provide the sustained blockade of angiogenesis achieved by angiogenesis inhibitors. Moreover, the long interval between courses allows microvascular endothelial cells to resume their proliferation and support tumor regrowth [13].

Single, small, and frequent doses are more effective at targeting tumor endothelium than a large single bolus followed by long rest periods [14]. Hanahan *et al*. [15] has proposed the term 'metronomic' for regular low-doses protocols.

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Table 1. Chemotherapeutics with Reported Antiangiogenic Activity

Agent	Effect	Ref.
6-Methylmercaptopurine	Inhibition of FGF-2-induced angiogenesis *in vitro* and *in vivo*.	17
6-Thioguanine	Inhibition of FGF-2- and VEGF-induced angiogenesis *in vitro* and *in vivo*.	19
Vinblastine	Inhibition of FGF-2-induced angiogenesis *in vitro* and *in vivo* and of MMP secretion *in vitro.*	24
Paclitaxel and docetaxel	Inhibition of FGF-2-induced angiogenesis *in vitro* and *in vivo* and of MMP secretion *in vitro.*	25
Cyclophosphamide	Inhibition of angiogenesis *in vivo*.	14
Comptothecin and Topotecan	Inhibition of angiogenesis *in vitro.*	26
Daunorubicin, doxorubicin and epirubicin	Inhibition of angiogenesis *in vitro.*	27

Continuous, low-dose chemotherapy enhances the antiangiogenic and proapoptotic effects of some cytotoxic agents in both dividing tumor and endothelial cells [14, 16]. A further theoretical advantage is that it minimizes the toxic effects and allows the use of more combinations of potentially synergistic selective angiogenesis inhibitors [16].

We have demonstrated that the purine analog 6-methylmercaptopurine riboside (6-MMPR) (Fig. **1**) modulates the angiogenic activity of fibroblast growth factor-2 (FGF-2) *in vitro* and affects blood vessel formation *in vivo* in the rabbit cornea and in the chick embryo chorioallantoic membrane (CAM) models, whereas 6-methylmercaptopurine, 2-aminopurine, and adenine are devoid of antiangiogenic activity [17]. Subtle structural differences may thus determine the ability of purine analogs to affect neovascularization. The analog 6-thioguanine (6-TG) (Fig. **2**) is currently used in the management of acute myelogenous leukemia (AML) both in remission induction and in maintenance therapy [18]. Since 6-TG may be antiangiogenic and hence effective in AML, we have assessed its influence on cell proliferation, motility, endothelial cell sprouting, collagen invasion and formation of capillary-like structures induced by FGF-2 and/or vascular endothelial growth factor (VEGF) in cultured endothelial cells of different origin [19]. The effect of 6-TG on *in vivo* neovascularization has also been evaluated in the CAM assay under basal conditions or during neovascularization induced by FGF-2 or human leukemia LIK cells grafted onto the CAM. Lastly, we evaluated bone marrow neovascularization in AML patients on 6-TG maintenance therapy. We found that 6-TG inhibits different steps in the angiogenesis process *in vitro* and is potently antiangiogenic in the CAM assay. Its antiangiogenic capacity, together with its antimetabolic activity, may contribute to its action during AML maintenance therapy. Bone marrow vascularization is significantly increased in AML patients at diagnosis [20-22], and their plasma levels of various angiogenic growth factors are increased [20, 23]. In our patients, vascularization was reduced to control values after remission induced by cytosine arabinoside, daunorubicin and etoposide, and did not rise during maintenance therapy with 6-TG (up to 12 months after remission) [19]. Even though these data do not formally prove that 6-TG acts on bone marrow endothelium in AML patients, the *in vitro* and *in vivo* evidence of its antiangiogenic activity suggests that this contributes to its action in AML therapy.

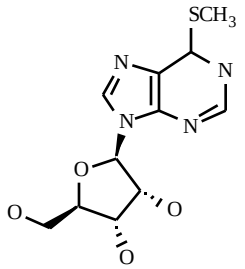


Fig. (1). Chemical structure of 6-methylmercaptopurine.

We have studied the antiangiogenic activity of vinblastine (Fig. **3**) and compared paclitaxel with its analog docetaxel (Fig. **4**) [24, 25]. Endothelial cell functions involved in angiogenesis, namely proliferation, chemotaxis, spreading on fibronectin, morphogenesis on Matrigel and secretion of matrix metalloproteinase-2 (MMP-2), MMP-9 and urokinase-type plasminogen activator (uPA) were studied *in vitro* upon exposure to vinblastine, docetaxel and paclitaxel, whereas the *in vivo* effect on angiogenesis was studied in the CAM assay. At noncytotoxic doses (0.1, 0.25, 0.5, 0.75 and 1.0 pmol/L for vinblastine; 0.5, 0.75, 1.0 nM for docetaxel; 2.0, 3.0, 4.0 nM for paclitaxel), all endothelial cell functions, but not protease secretion, were impacted *in vitro* in a dose-dependent fashion. Apoptosis was not induced. The antiangiogenic effect rapidly disappeared upon drug removal and was accompanied ultrastructurally by thin lesion of the cytoskeleton in the form of slight and equally reversible depolymerization and accumulation of microfilaments. *In vivo*, vinblastine (0.5, 0.75 and 1.0 pmol/L), docetaxel (1.0, 2.0 and 3.0 nM) and paclitaxel (4.0, 8.0 and 12 nM) displayed a dose-dependent antiangiogenic activity. The highest antiangiogenic dose of vinblastine in the CAM assay is equivalent to 16 μg in a 70 Kg adult subject, much lower than that used in current anticancer treatments. Moreover, vinblastine inhibits endothelial cell proliferation at doses that have no effect on NIH 3T3 fibroblasts, Burkitt lymphoma, B-cell lymphoblastic leukemia and T-cell lymphoblastic leukemia cell lines.

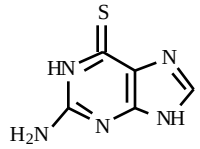


Fig. (2). Chemical structure of 6-thioguanine.

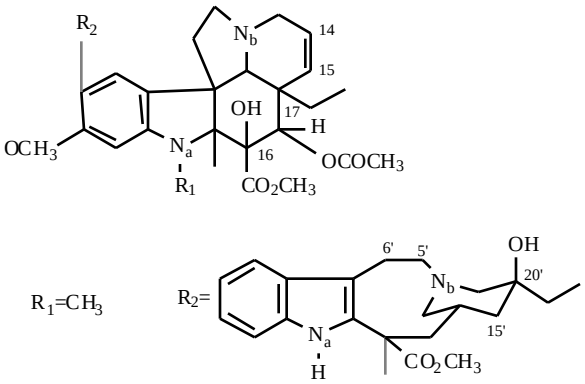


Fig. (3). Chemical structure of vinblastine.

Klement *et al*. [16] found that vinblastine at one-tenth to one-twentieth of the MTD significantly inhibited angiogenesis in subcutaneously implanted tumors. However, this treatment caused only partial tumor regression, followed by relapse and death.

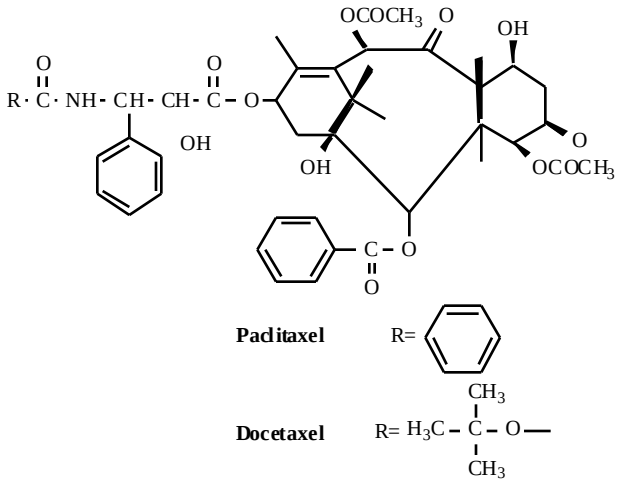


Fig. (4). Chemical structure of paclitaxel and docetaxel.

Browder *et al*. [14] compared a metronomic schedule (170 mg/Kg cyclophosphamide every 6 days, about one-third of the MTD) with conventional, single high-dose (150 mg/kg for three days) cyclical cyclophosphamide (Fig. **5**). The metronomic schedule alone eradicated Lewis lung carcinoma and L1210 leukaemia. When transplanted Lewis lung carcinoma and EMT6 breast cancer cells were made drug-resistant before therapy, the metronomic schedule inhibited tumor growth three times more effectively.

Chemotherapeutic agents may also affect angiogenesis without killing endothelial cells. The topoisomerase inhibitors comptothecin and topotecan (Fig. **6**) inhibit endothelial cell proliferation at concentrations (50 nmol/L) far less than those required for endothelial cell death [26]. The anthracyclines daunorubicin, doxorubicin and epirubicin (Fig. **7**) inhibit tubule formation in Matrigel at doses that produce no cytotoxic effects on endothelial cells or Walker 256 carcinosarcoma cells [27].

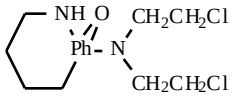


Fig. (5). Chemical structure of cyclophosphamide.

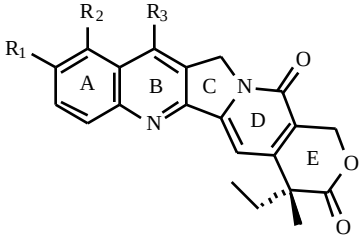
Bucci *et al*. [28] designed long-term *in vitro* assays in which human tumor cells, dermal fibroblasts and macrovascular or microvascular endothelial cells were exposed daily for up to 6 days, to various low concentrations of different chemotherapeutic drugs, including 4-hydroperoxycyclophosphamide, an oral taxane (BMS-275183), doxorubicin, epothilone B (Epo B) and its analog 5-methylpyridine EpoB. Results showed that the properties of vascular endothelial cells, including proliferation and induction of apoptosis, are preferentially affected by low concentrations of most of these drugs when exposed continuously for protracted periods of time (e.g. 6 days), but not for shorter periods of time (e.g. 24 h).

Grant *et al*. [29] compared the effect of docetaxel and paclitaxel on endothelial cell proliferation and tubule formation *in vitro* and on tumor growth *in vivo*. They found that both agents are effective at targeting endothelial cell activity. Docetaxel is more potent and its effect is primarily via increased apoptosis and inhibition of proliferation and differentiation of endothelial cells.

Lennernas *et al*. [30] studied dose-dependent effects of fluorouracil, paclitaxel, doxorubicin, cisplatin, cyclophosphamide and etoposide on VEGF-mediated angiogenesis using the rat mesenteric-window angiogenesis assay. Results demonstrated that only paclitaxel, doxorubicin and cyclophosphamide significantly suppressed the overall angiogenic response and no clear correlation was found between drug half-time, the degree of toxic effects (in terms of body weight changes) and antiangiogenic effect.

COMBINATION OF ANGIOGENIC INHIBITORS WITH CHEMOTHERAPEUTIC AGENTS

To get the best results, it may be necessary to combine antiangiogenesis drugs with each other or conventional chemotherapy. Combinations of antiangiogenic factors with chemotherapy are being studied both preclinically and in human cancer trials. Those that target multiple pathways that downregulate endothelial cell growth or promote apoptosis



	R_1	R_2	R_3
CPT	H	H	H
TPT	OH	$(CH_3)_2NHCH_2$-	H

Fig. (6). Chemical structure of comptothecin (CPT) and topotecan (TPT).

may have synergistic effects and be critical for suppressing angiogenesis in larger, more heterogeneous tumors. As a tumor grows, in fact, numerous angiogenic factors are secreted, whereas smaller, less heterogeneous tumors generate only one or two factors [31].

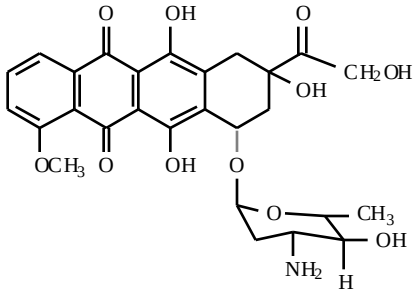


Fig. (7). Chemical structure of epirubicin.

The rationale for the beneficial effect of such combinations was based on their ability to target both the parenchymal and the stromal components of neoplasia [32]. Kakeji and Teicher [33] showed potentiation or synergism when inhibitors were combined with standard schedules of certain cytotoxic agents in *in vivo* experimental models. Synergistic activity has been reported for docetaxel together with a recombinant humanised monoclonal antibody against VEGFR-2 or 2 methoxyestradiol [34].

Browder *et al*. [14] demonstrated that coadministration of metronomic, but not conventional, cytotoxic scheduling of cyclophosphamide and TNP-470 induced endothelial cell apoptosis within tumors, an effect that precedes apoptosis of drug-resistant Lewis lung carcinomas. Xenografts of neuroblastoma cell lines were subjected to either continuous treatment with low doses of vinblastine or a monoclonal anti-vascular endothelial growth factor receptor-2 (VEGFR-2) antibody, or both agents [16]. Anti-VEGFR-2 antibody and vinblastine individually resulted in significant, but transient, xenograft regression, diminished tumor perfusion and direct inhibition of angiogenesis, while their combination resulted in full and sustained regression of tumors, without any increase in host toxicity or acquired drug resistance.

Bruns *et al*. [35] showed that coadministration of the cytotoxic agent gemcitabine and epidermal growth factor receptor (EGFR) inhibitors produced antitumor effects, mediated by antiangiogenic mechanisms. Similar results have been reported by Sirotnak *et al*.[36], who combined ZD1839, an EGF tyrosine-kinase inhibitor, and various cytotoxic agents (taxanes, anthracyclines, folate antagonists). Inoue *et al*. [37] obtained similar results by coadministration of paclitaxel and the anti-EGFR-monoclonal antibody C225.

An anti-endoglin antibody acted synergistically with cyclophosphamide in a skin tumor/severe combined immunodeficiency mouse model [38]. A combination of low-dose topotecan and anti-VEGF antibody therapy was more effective at suppressing angiogenesis in an experimental Wilm's tumor model than either agent alone [39].

Klement *et al*. [40] reported that a variety of orthotopic human breast cancer xenografts selected for high levels of P-glycoprotein and multidrug resistance respond in a significant and durable manner to different continuous low-dose chemotherapy regimens, when used in combination with an anti VEGFR-2 antibody. The Pgp substrates paclitaxel, adriamycin, and vinblastine were all effective using this type of combination treatment, although the chemotherapy protocols showed little or no effects as monotherapies.

CONCLUDING REMARKS AND PERSPECTIVES

Techniques for accurately evaluating the angiogenesis status of individual tumors are needed to identify patients for whom antiangiogenic therapy will be most effective, and elaborate combined antiangiogenic protocols and chemotherapy.

Combination therapy could be effective in advanced cases when the tumor is larger, endothelial cells are heterogeneous, several angiogenic factors are secreted and epigenetic resistance mechanisms are present.

Further investigation of combination management in preclinical studies with animal tumor models is required to identify the best modes of administration. Blocking tumor growth by antiangiogenesis requires chronic inhibition of vascular recruitment, so long-term treatment is necessary and oral administration recommended.

Inhibition of angiogenesis is an attractive way of treating cancer, but further preclinical and clinical studies are needed to define its effective application spectrum.

ACKNOWLEDGEMENTS

Supported by Ministry for Education, the University and Research (MIUR, "Molecular Engineering-CO3" funds and Interuniversity Funds for Basic Research (FIRB), Rome), Associazione Italiana per la Ricerca sul Cancro (AIRC, Milan), and Fondazione Italiana per la Lotta al Neuroblastoma, Genoa, Italy. We would also like to thank Dr. Anthony Green for providing linguistic advice.

REFERENCES

[1] Carmeliet, P. *Nat. Med.*, **2000**, *6*, 389.
[2] Hanahan, D.; Folkman, J. *Cell*, **1996**, *86*, 353.
[3] Ribatti, D.; Vacca, A.; Dammacco, F. *Neoplasia*, **1999**, *1*, 293.
[4] (a) Ribatti, D.; Vacca, A.; Dammacco, F.; English, D. *J. Hematother. Stem Cell Res*., **2003**, *12*, 11. (b) Sirotnak, F.M.; Zakowski, M.F.; Miller, V.A.; Scher, H.I.; Kris, M.G. *Clin. Cancer Res*., **2000**, *6*, 4485.
[5] Folkman, J. *New Engl. J. Med.*, **1971**, *285*, 1182.
[6] Kerbel, R.S. *BioEssay*, **1991**, *1*, 31.
[7] Hagedon, M.; Bikfalvi, A. *Crit. Rev. Oncol. Hematol*., **2000**, *34*, 89.
[8] Burke, P.A.; De Nardo, S.J. *Crit. Rev. Oncol. Hematol*., **2000**, *39*, 155.
[9] Chao, Y. *Int. J. Biochem. Cell Biol*., **2001**, *33*, 357.
[10] Denekamp, J. *Br. J. Cancer*, **1988**, *45*, 136.
[11] Lazo, J.S. *Biochem. Pharmacol*., **1986**, *35*, 1919.
[12] Denekamp, J. *Cancer Metastasis Review*, **1990**, *9*, 267.
[13] Monestiroli, S.; Mancuso, P.; Burlini, A. *Cancer Res.*, **2001**, *61*, 4341.
[14] Browder, T.; Butterfield, C.E.; Kraling, B.M.; Shi, B.; Marshall, B.; O'Reilly, M.S.; Folkman, J. *Cancer Res.*, **2000**, *60*, 1878.
[15] Hanahan, D.; Bergers, G.; Bergsland, E. *J. Clin. Invest*., **2000**, *105*, 1045.
[16] Klement, G.; Baruchel, S.; Rak, J.; Man, S.; Clark, K.; Hicklin, D.J.; Bohlen, P.; Kerbel, R.S. *J. Clin. Invest.*, **2000**, *105*, 15.

[17] Presta, M.; Rusnati, M.; Belleri, M.; Morbidelli, L.; Ziche, M.; Ribatti, D. *Cancer Res.*, **1999**, *59*, 2417.
[18] Calabresi, P.; Chabner, B.A. In *The Pharmacological basis of therapeutics*, Goodman Gilman, A.; Rall, T.W.; Nies, A.S.; Taylor, P. eds. Pergamon Press, New York, **1990**, 1202-1263.
[19] Presta, M.; Belleri, M.; Vacca, A.; Ribatti, D. *Leukemia*, **2002**, *16*, 1490.
[20] Aguayo, A.; Kantarjian, H.; Mansuori, T.; Gidel, C.; Estey, E.; Thomas, D.; Koller, C.; Estrov, Z.; O'Brien, S.; Keating, M.; Freireich, E.; Albitar, M. *Blood*, **2000**, *96*, 2240.
[21] Hussing, J.W.; Rodgers, G.M.; Shami, P.J. *Blood*, **2000**, *95*, 309.
[22] Padrò, T.; Ruiz, S.; Bicker, R.; Burger, H.; Steins, M.; Kienast, J.; Buch, T.; Berdel, W.E.; Mesters, R.M. *Blood*, **2000**, *95*, 2637.
[23] Aguayo, A.; Estey, E.; Kantarjian, H.; Mansuori, T.; Gidel, C.; Keating, M.; Giles, F.; Esrtov, Z.; Barlogie, B.; Albitar, M. *Blood*, **1999**, *94*, 3717.
[24] Vacca, A.; Iurlaro, M.; Ribatti, D.; Minischetti, M.; Nico, B.; Ria, R.; Pellegrino, A.; Dammacco, F. *Blood*, **1999**, *94*, 4143.
[25] Vacca, A.; Ribatti, D.; Iurlaro, M.; Merchionne, F.; Nico, B.; Ria, R.; Dammacco, F. *J. Hematother. Stem Cell Res.*, **2002**, *11*, 103.
[26] Clements, M.K.; Jones, C.B.; Cumming, M.; Daud, S.S. *Cancer Chemother. Pharmacol*., **1999**, *44*, 411.
[27] Maragoudakis, M.E.; Peristeris, P.; Missirlis, E.; Aletras, A.; Andriopoulou, P.; Haralabopoulos, G. *Ann. N.Y. Acad. Sci.*, **1994**, *732*, 280.
[28] Bucci, G.; Nicolaou, K.C.; Kerbel, R.S. *Cancer Res.*, **2002**, *62*, 6938.
[29] Grant, D.S.; Williams, T.L.; Zahaczewsky, M.; Dicker A.P. *Int. J. Cancer*, **2003**, *104*, 121.
[30] Lennernas, B.; Albertsson, P.; Lennernas, H.; Norrby, K. *Acta Oncologica*, **2003**, *42*, 294.
[31] Relf, M.; Lejeune, S.; Scott, P.A.; Fox, S.; Smith K.; Leek, R.; Moghaddam, A.; Whitehouse, R.; Bicknell, R.; Harris, A.L. *Cancer Res*., **1997**, *57*, 963.
[32] Gasparini, G.; Harris, A.L. *Eur. J. Cancer*, **1994**, *304*, 201.
[33] Kalley, Y.; Teicher, B.A. *Invest. New Drugs*, **1997**, *15*, 39.
[34] Sweeney, C.J.; Miller, K.D.; Sissons, S.; Nozaki, S.; Heilman, D.K.; Shen, J.; Sledge, G.W. Jr. *Cancer Res*., **2001**, *61*, 3369.
[35] Bruns, C.J.; Harbison, M.T.; Davis, D.W.; Portera, C.A.; Tsan, R.; Mc Coney, D.J.; Evans, D.B.; Abbruzzese, J.L.; Hicklin, D.J.; Radinsky, R. *Clin.Cancer Res.*, **2000**, *6*, 1936.
[36] Sirotnak, F.M.; Zakowski, M.F.; Miller, V.A.; Scher, H.I.; Kris, M.G. *Clin. Cancer Res*., **2000**, *6*, 4485.
[37] Inoue, K.; Slaton, J.W.; Perrotte, P.; Davis, D.W.; Bruns, C.J.; Hicklin, D.J.; Mc Conkey, D.J.; Sweeney, P.; Radinsky, R.; Dinney, C.P. *Clin. Cancer Res.*, **2000**, *6*, 4874.
[38] Takahashi, N.; Haba, A.; Matsuno, F.; Scon, B.K. *Cancer Res.* **2001**, *61*, 7846.
[39] Soffer, S.Z.; Moore, J.T.; Kim, E.; Huang, J.; Yokoi, A.; Manley, C.; O'Toole, K.; Middlesworth, W.; Yamashiro, D.J.; Kandell, J.J. *J. Pediatr. Surg*., **2001**, *36*, 7846.
[40] Klement, G.; Huang, P.; Mayer, B.; Green, S.K.; Man, S.; Bohlen, P.; Hicklin, D.; Kerbel, R.S. *Clin. Cancer Res*., **2002**, *8*, 221.