

## BIOIMMUNOTHERAPEUTIC TARGETS ON ANGIOGENETIC BLOOD VESSELS IN SOLID MALIGNANCIES

Michele Maio, Maresa Altomonte, Luana Calabrò, Ester Fonsatti

*Cancer Bioimmunotherapy Unit, Centro di Riferimento Oncologico, Istituto Nazionale di Ricovero e Cura a Carattere Scientifico, 33081 Aviano, Italy*

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Vascular endothelial growth factor (VEGF)
4. Vascular endothelial growth factor receptors (VEGFR)
5. Matrix metalloproteinases (MMP)
6.  $\alpha$  v beta 3 integrin (CD51/CD61)
7. Endostatin
8. Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31)
9. Endoglin (CD105)
10. Conclusions and future directions
11. Acknowledgements
12. References

### 1. ABSTRACT

Physiological angiogenesis is a tightly regulated process that occurs mainly during reproduction, development and wound healing. Although angiogenesis is a continuous process, different consecutive steps can be identified, including: i) release of pro-angiogenic factors; ii) release of proteolytic enzymes; iii) endothelial cell migration, morphogenesis and proliferation. Angiogenesis is also a hallmark of malignant diseases, and an inverse correlation between tumor vascularity and survival was demonstrated. Thus, strategies aimed at interfering with tumor blood supply by targeting tumor vasculature, presently represent promising new approaches for the treatment of solid malignancies. In fact, at least 30 angiogenic inhibitors, utilized alone or in combination with other therapeutic agents, are currently being tested in clinical trials in humans. In this paper, we will review current knowledge on selected molecules expressed by endothelial cells and involved in distinct steps of the angiogenic process, that represent potential targets for bioimmunotherapeutic approaches in human malignancies.

### 2. INTRODUCTION

Angiogenesis is a complex process that leads to new blood vessels development from pre-existing microvessels, and involves sequential events including proteolysis and remodeling of the extracellular matrix, as well as proliferation and migration of endothelial cells (1). In the adult, with the exception of the reproductive cycle in women, angiogenesis occurs in response to pathological

conditions such as inflammation, wound healing and hypoxia (2). Furthermore, excessive or insufficient vascularization has been associated with several non-malignant diseases (2-6), and it has long been established that angiogenesis plays a crucial role in tumor growth and metastasis (7). In this regard, it has been demonstrated that microvascular density correlates with distant metastasis and prognosis in solid malignancies of different histotype (8-13), and in hematological malignancies (14-15). Recent progresses in identifying and characterizing physiological regulators of blood vessels development, prompted several pre-clinical studies designed to block tumor vessel growth in order to interrupt blood supply to neoplastic cells. In light of these pre-clinical data, a variety of angiogenic inhibitors are currently being tested in clinical trials aiming to target specific molecules involved in blood vessel neoformation, or to directly inhibit specific biologic functions of endothelial cells or their response to angiogenic stimuli (16).

Due to their active involvement in angiogenesis, targeting of proliferating endothelial cells presents several advantages compared to conventional treatment of human malignancies; in fact, it allows: i) easy accessibility of therapeutic agents to endothelial cells through the blood stream; ii) suitability of this therapeutic strategy to solid tumors of different histotype; iii) targeting of a genetically stable cell population, thereby reducing the possibility of acquiring drug resistance. In addition, targeting of proliferating endothelia potentially amplifies the killing of

transformed cells since each blood capillary sustains the growth of a great number of malignant cells (17).

Although anti-angiogenetic therapy currently represents one of the most promising approaches for cancer treatment, a number of limitations must be taken into account when anti-angiogenetic therapies are carried out in humans. In fact, angiogenesis is highly regulated by a balance between positive and negative stimuli, that are tightly coordinated (1). Additionally, the mechanism of action of several angiogenetic inhibitors is poorly understood yet (1). Furthermore, cytokines and pro-angiogenetic molecules secreted by cancer and immune cells can modulate the phenotypic profile of tumor endothelia (1). Finally, the quantification of angiogenesis in response to angiogenetic inhibitors remains, to date, impractical in metastatic diseases; thus, the identification of reliable soluble markers of angiogenesis is required to monitor the effectiveness of anti-vascular therapies. In this regard, recent findings suggested that measurement of serum vascular cell adhesion molecule (VCAM)-1 might help in the assessment of anti-angiogenetic drugs currently in clinical trials (18).

### 3. VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

VEGF is a disulphide-linked dimeric glycoprotein, that represents a key mediator of vasculogenesis and angiogenesis (19-21), and presents at least 5 isoforms (VEGF<sub>121</sub>, VEGF<sub>145</sub>, VEGF<sub>165</sub>, VEGF<sub>189</sub>, VEGF<sub>206</sub>) generated by alternative splicing of a single gene (19-20). These different isoforms show similar biological activities, but differ for their binding to heparin and to the extracellular matrix (22). The smaller isoforms are secreted in a soluble form, whereas the larger ones remain cell-associated and their availability is regulated by proteolysis (22). Many different cell types, including cancer cells, are able to produce VEGF that exerts its biological activity predominantly on endothelial cells (19-20). *In vivo*, it induces both vascular permeability and angiogenesis, and contributes to vasculature maintenance (20, 23). *In vitro*, VEGF promotes endothelial cell proliferation and it modulates the expression of adhesion molecules such as VCAM-1 and ICAM-1 on endothelial cells (20). Additionally, it has been recently demonstrated that VEGF prolongs the survival of human dermal microvascular endothelial cells by inducing the expression of the anti-apoptotic protein Bcl-2 (24).

Increased levels of serum VEGF and of VEGF expression have been found in different angiogenesis-related diseases including malignancies of different histotype (25-26), and anti-VEGF monoclonal antibodies (mAb) strongly inhibited the growth of human tumor xenografts transplanted subcutaneously in nude or SCID mice (27-30). Taken together, these studies demonstrated that treatment with anti-VEGF mAb inhibited tumor neovascularization in animal models, and interfered with tumor vasculature maintenance, malignant ascites fluid formation, and metastatic spreading (27-30). However, tumor growth resumed upon cessation of the mAb

treatment, suggesting that it may not be sufficient for complete tumor eradication (27, 31). Thus, curative therapy in cancer patients may necessitate a combination of both anti-angiogenetic agents such as anti-VEGF mAb and cytotoxic agents, to disrupt both tumor and endothelial cells (27). Humanized forms of anti-VEGF mAb, which retain the same affinity and efficacy of murine mAb, have been generated and are being tested in humans (16, 32-34, URL: <http://cancertrials.nci.nih.gov>). Results emerging from Phase I clinical trials with anti-VEGF mAb, administered alone or in association with chemotherapy, showed that these treatments are well tolerated; thus, human anti-VEGF mAb can be safely combined with chemotherapy without apparent synergistic toxicity (33-34). Phase II clinical trials showed objective responses, including one complete response, in breast cancer patients treated with anti-VEGF mAb (33, 35). In addition, treatment of patients affected by advanced non-small cell lung carcinoma or colorectal cancer with anti-VEGF mAb in combination with chemotherapy, increased the clinical response rate and prolonged the time-to-disease progression compared to chemotherapy alone (33, 36-37).

### 4. VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTORS (VEGFR)

The main receptors that initiate signal transduction cascades in response to VEGF comprise a family of closely related receptor tyrosine-kinases VEGFR-1, VEGFR-2 and VEGFR-3. Among these, VEGFR-1 and VEGFR-2 expression is largely restricted to the vascular endothelium, and both receptors bind VEGF with high affinity (19-21). VEGFR-2 seems to mediate the major growth and permeability actions of VEGF, whereas VEGFR-1 may have a negative role, either by acting as a decoy receptor or by suppressing signaling through VEGFR-2 (19-21). In adult human tissues, VEGFR-3 is mainly expressed in the lymphatic endothelia and in some high endothelial venules (38). Noteworthy, the mRNA for VEGFR-1 and -2 was found to be up-regulated in tumor-associated endothelial cells (26, 39); thus, VEGF receptors represent attractive targets in the aim to effectively block VEGF activity. Opposite to anti-VEGF mAb, the efficacy of SU5416, an inhibitor of the tyrosine-kinase activity of VEGFR-2, was reported to be best against slow-growing tumors, and more variable against fast-growing tumors (27). In addition, it was demonstrated that SU5416 has long-lasting effects on VEGFR-2 phosphorylation and function (40), and that it reverts tumor resistance to radiotherapy (41). Results from Phase I clinical studies indicated that anti-VEGF therapy with antibodies (Ab) or receptor kinase inhibitors is well tolerated; moreover, patients with advanced disease appeared to respond to therapy with disease stabilization or tumor shrinkage (27). In addition, among 28 patients with metastatic colorectal cancer enrolled in a Phase I/II clinical study, designed to investigate the safety of SU5416 in combination with 5-fluorouracil (FU)/leucovorin, 15 patients showed a clinical response (i.e., 1 complete response, 5 partial responses, 9 stable diseases) (42). According to these results, SU5416 is currently in Phase III clinical trials for advanced malignancies.

### 5. MATRIX METALLOPROTEINASES (MMP)

The MMP are a family of secreted and membrane-associated endopeptidases that selectively degrade components of the extracellular matrix and basement membrane, allowing endothelial cells migration and metastatic spread of cancer cells (43). These enzymes are produced by a variety of cell types, including endothelial and epithelial cells, fibroblasts, and inflammatory cells (43).

The identification of natural tissue inhibitors of MMP (TIMP), that are primarily secreted by endothelial cells, has stimulated studies focused on MMP inhibition to reduce the metastatic spreading of neoplastic cells. Among TIMP, TIMP-1, which is mainly released by endothelial cells (44), was shown to inhibit angiogenesis both *in vitro* and *in vivo* (45-46). Furthermore, TIMP-1 over-expression induced on endothelial cells by gene transfer, strongly decreased their migration and invasion of the extracellular matrix (47); furthermore, levels of TIMP-1 expression correlated with prognosis in patients with gastric carcinoma (48).

Extensive pre-clinical data generated in animal models have shown that the administration of synthetic MMP inhibitors (MMPI) reduces primary tumor growth as well as the number and size of metastatic lesions. Based on these promising results, synthetic MMPI have been developed and taken into clinical trials (49). Among these, Marimastat, BAY- 129566, CGS-27023A, Prinomastat (AG-3340), BMS-275291 and Metastat (COL-3) are in different stages of clinical development, ranging from Phase I to Phase III trials (50). Furthermore, with the aim to potentiate tumor cytotoxicity, as well as to reduce the size and number of metastatic lesions, several MMPI are being administered in clinical trials in combination with chemotherapy (49-51).

### 6. ALPHA V BETA 3 INTEGRIN (CD51/CD61)

The integrin family member alpha v beta 3 is an adhesion receptor, strongly implicated in the response of endothelial cells to angiogenetic stimuli. Its expression on angiogenetic endothelial cells is thought to facilitate their adhesion to the extracellular matrix during migration; in fact, alpha v beta 3 integrin was shown to bind directly to the MMP-2 on the surface of vascular endothelial cells during angiogenesis, suggesting a possible functional link between these endothelial cells surface proteins (52).

Furthermore, alpha v beta 3 integrin has been described as a marker for angiogenetic blood vessels, as it has been found predominantly expressed in wound healing and in tumor-associated blood vessels (53-54). Although the vasculature within apparently normal tissues also stained for alpha v beta 3 integrin, the percentage of stained vessels and their staining intensity were lower compared to neoplastic tissues (55). The relevance of this integrin in neovascularization was strongly supported by the ability of the anti-alpha v beta 3 integrin mAb LM609 to induce endothelial cells apoptosis within angiogenetic blood

vessels (56), and to promote tumor regression by inhibiting tumor angiogenesis (57).

Clinical trials utilizing a humanized version of mAb LM609 (Vitaxin) have been initiated, to evaluate its safety and pharmacokinetics in late stage cancer patients (58). Results emerging from a pilot study have shown that Vitaxin was generally well tolerated; however, no objective regressions or significant stabilizations of disease were observed in 15 patients with advanced leiomyosarcomas (59).

### 7. ENDOSTATIN

Endostatin is a 20 kDa terminal fragment of collagen XVIII, that was originally isolated as an inhibitor of endothelial cells proliferation from the culture medium of the EOMA hemangioendothelioma cell line (60). Endostatin shows a widespread distribution in blood vessel walls and basement membrane zones, and a strong association with elastic fibers of aorta and with large arteries was found in adult mouse tissues (61).

Functional studies demonstrated that Endostatin inhibits endothelial cell proliferation (60) and migration (62), and that it induces endothelial cell apoptosis (63). The action of Endostatin seems to be endothelium-specific since it has no activity on fibroblasts and smooth muscle cells (60, 63-64); however, its mechanism(s) of action remain to be elucidated. It has been suggested that Endostatin inhibits the proteolytic activation of pro-MMP-2 and the catalytic activities of Membrane Type (MT)1-MMP and MMP-2 (65). In addition, most recent findings indicated that Endostatin down-regulates many genes involved in proliferation, apoptosis and migration of growing endothelial cells, resulting in a potent anti-migratory effect (66).

*In vitro*, Endostatin significantly reduced endothelial and malignant cells invasion into reconstituted basement membrane (65), while *in vivo*, it regressed established syngeneic Lewis lung carcinoma, T241 fibrosarcoma, and B16 melanoma tumors in xenograft models (60). Moreover, repeated cycles of Endostatin therapy prolonged tumor dormancy in mice, suggesting that it does not generate drug resistance (67); however, anti-angiogenetic therapy with Endostatin in tumor-bearing mice required prolonged administration and high doses of protein (60, 64). Further support to the potential usefulness of Endostatin for cancer therapy, has recently derived from the demonstration that intratumoral delivery of the Endostatin gene efficiently suppressed MCA-4 murine mammary carcinoma growth in immunodeficient mice (68). In this study, it was also demonstrated that the observed reduction of tumor growth was associated with a marked reduction in vascular density as assessed by CD31, CD105 and DiOC<sub>7</sub> staining. Noteworthy, radiation has been shown to increase the production of Endostatin; in fact, plasma levels of Endostatin were twice as high in mice that underwent tumor irradiation as compared to mice that underwent tumor resection (69). In addition, a significant tumor growth inhibition was observed in mice bearing radio-resistant tumors following combined treatment with

Endostatin and radiotherapy, compared to mice treated with irradiation alone (70). Altogether, these findings suggest that the efficacy of combined anti-angiogenic and conventional anti-cancer therapies should be further investigated for their potential implications in the treatment of human cancer. Interestingly, Ab to Endostatin were detected in the serum and in the tumor tissue of a patient with a multifocal glioblastoma, suggesting that Endostatin over-expression might induce a humoral immune response (71).

At present, Phase I clinical trials are ongoing to test the efficacy and toxicity of Endostatin in patients with advanced solid tumors (i.e., breast cancer, melanoma, head and neck cancer, colon cancer, renal carcinoma and sarcoma) for which no other standard therapy exists (URL: <http://cancertrials.nci.nih.gov>).

### 8. PLATELET ENDOTHELIAL CELL ADHESION MOLECULE-1 (PECAM-1/CD31)

CD31 is a 130 kDa glycoprotein that belongs to the immunoglobulin (Ig) superfamily (72), and that is mainly expressed on endothelial cells of large and small vessels (73). In cultured endothelial cells, and in continuous endothelia of blood vessels in human tissues, CD31 was found predominantly localized at intercellular junctions (73-74); additionally, CD31 is constitutively expressed on platelets, monocytes and leukocytes (75).

The role of CD31 in angiogenesis has not been fully clarified yet, however, several experimental findings suggest that it is involved in neovascularization. In this respect, CD31 was found to play a role in endothelial cell migration (76), endothelial cell-cell adhesion (77), and in the development of the cardiovascular system (72). Additionally, it was reported that high levels of CD31 inhibited endothelial cells morphogenesis (78), and anti-CD31 Ab inhibited tube formation in Matrigel by human umbilical vein endothelial cells (HUVEC) (79-80). *In vivo*, CD31 has proven to represent an useful immunohistochemical marker of blood vessels, and it is currently considered as the "golden standard" for the assessment of angiogenic activity in tumors (81); however, it was recently demonstrated that opposite to Endoglin, levels of CD31 expression inversely correlate with HUVEC proliferation (82).

### 9. ENDOGLIN (CD105)

CD105 is a homodimeric cell membrane glycoprotein of approximately 180 kDa, composed of disulphide-linked subunits of 95 kDa (83), which has limited species-specificity (84-85). Two different isoforms of CD105, L-CD105 and S-CD105 have been characterized (86-87). L-CD105 is predominantly expressed on endothelial cells and shares regions of sequence identity with betaglycan, a component of the Transforming Growth Factor (TGF)-beta receptor complex, that is weakly expressed or absent on endothelial cells (88).

CD105 is an accessory component of the TGF-beta receptor complex (89-90), and it binds several factors

of the TGF-beta superfamily including TGF-beta 1 and -beta 3 (90-91), activin-A, BMP-7, and BMP-2 (90). The exact role of CD105 in TGF-beta signaling remains unclear. However, CD105 over-expression on different cell types modulates several cellular responses to TGF-beta 1, including inhibition of cellular proliferation and down-regulation of c-myc mRNA, stimulation of fibronectin synthesis, cellular adhesion, platelet-endothelial cell adhesion molecule-1 phosphorylation, and homotypic aggregation (89, 92-93). On the contrary, using an antisense approach, it was shown that the inhibition of CD105 expression in cultured endothelial cells enhanced the ability of TGF-beta 1 to suppress their growth and migration (93).

Concerning its tissue distribution, CD105 was found mostly expressed on cellular lineages within the vascular system, and preferentially and strongly expressed on endothelial cells (83, 94-95). Noteworthy, highest levels of CD105 expression were identified on cultured endothelial cells with protein, RNA, and DNA levels consistent with cellular activation and proliferation (96). In agreement with this observation, a significant correlation was found between levels of CD105 expression and endothelial cells proliferation and density in culture (82, 97), as well as with markers of cell proliferation (i.e., cyclin A and Ki-67) in tumor endothelia (98). Consistently, a stronger intensity of staining for CD105 was detected on vascular endothelial cells in tissues undergoing active angiogenesis, such as regenerating and inflamed tissues or tumors (96, 98-99), compared to normal tissues. In solid malignancies of different histotype investigated, anti-CD105 mAb reacted almost exclusively with venous and arterial endothelium of both peritumoral and intratumoral vessels (96-97, 100). Additional support to the involvement of CD105 in angiogenesis derives by the demonstration that mutations in the coding region of CD105 gene are associated with hereditary hemorrhagic telangiectasia type 1 (HHT), a dominantly inherited vascular disorder characterized by multisystemic vascular dysplasia and recurrent hemorrhage (101). In addition, mice heterozygous for CD105 showed signs of HHT (102), and CD105 knockout mice died of defective vascular development at gestational day 10-11 (102-103).

The identification of CD105 as an optimal marker of endothelial cells proliferation has encouraged studies designed to test the clinical usefulness of anti-CD105 mAb for the *in vivo* diagnosis and treatment of malignant diseases. Consistently, CD105 was shown to represent an ideal marker to quantify tumor angiogenesis (104); furthermore, microvessel density assessed by using an anti-CD105 mAb, was found to be an independent prognostic factor in breast cancer patients (104). Additionally, using *in vivo* models of spontaneous canine mammary adenocarcinoma (82) or human melanoma xenografts in C57BL/6 mice (105), it has been recently demonstrated that targeting of endothelial CD105 by radiolabeled mAb is an efficient procedure to image solid malignancies, regardless of their histological origin. Most interestingly, *in vivo* studies conducted in SCID mice bearing human breast carcinomas, demonstrated that

radiolabeled or immunotoxin-conjugated anti-CD105 mAb had a highly effective anti-tumor efficacy (106-108). In light of these findings, Phase I clinical trials have been initiated to evaluate the therapeutic efficacy and toxicity of anti-CD105 mAb in cancer patients (109).

### 10. CONCLUSIONS AND FUTURE DIRECTIONS

Agents that target the tumor vasculature by killing and/or interfering with biological functions of endothelial cells (i.e., proliferation, migration and differentiation), represent promising candidates to set up new therapeutic approaches in solid malignancies, regardless of their histotype. The pre-clinical and clinical experiences so far obtained demonstrate that a more in-depth knowledge of the endothelial cell molecules playing a role in angiogenesis, and of the molecular mechanism(s) regulating angiogenesis in tumors, may allow to design more specific and eventually more effective therapeutic approaches to cancer. Furthermore, these anti-vascular therapeutic strategies, that potentially do not induce drug resistance, might represent useful approaches for the long-term maintenance of cancer treatment, following or in association with conventional therapeutic strategies such as surgery, chemotherapy, radiotherapy and immunotherapy.

### 11. ACKNOWLEDGEMENTS

This work was supported by the Progetto Ricerca Finalizzata awarded by the Italian Ministry of Public Health and by the Associazione Italiana per la Ricerca sul Cancro.

### 12. REFERENCES

- Carmeliet P.: Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 6, 389-395 (2000)
- Carmeliet P. & R.K. Jain: Angiogenesis in cancer and other diseases. *Nature* 407, 249-257 (2000)
- Gustafsson T. & W.E. Kraus: Exercise-induced angiogenesis-related growth and transcription factors in skeletal muscle, and their modification in muscle pathology. *Front Biosci* 6, D75-89 (2001)
- Waltenberger J.: Impaired collateral vessel development in diabetes: potential cellular mechanisms and therapeutic implications. *Cardiovasc Res* 49, 554-60 (2001)
- Buschmann I. & W. Schaper: The pathophysiology of the collateral circulation (arteriogenesis). *J Pathol* 190, 338-342 (2000)
- Fleischmajer R., K. Kuroda, R. Hazan, R.E. Gordon, M.G. Lebowitz, A.N. Sapadin, F. Unda, N. Iehara & Y. Yamada: Basement membrane alterations in psoriasis are accompanied by epidermal overexpression of MMP-2 and its inhibitor TIMP-2. *J Invest Dermatol* 115, 771-777 (2000)
- Folkman J.: What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 82, 4-6 (1990)
- Jaeger T.M., N. Weidner, K. Chew, D.H. Moore, R.L. Kerschmann, F.M. Waldman & P.R. Carroll: Tumor angiogenesis correlates with lymph node metastases in invasive bladder cancer. *J Urol* 154, 69-71 (1995)
- Graham C.H., J. Rivers, R.S. Kerbel, K.S. Stankiewicz & W.L. White: Extent of vascularization as a prognostic indicator in thin (<0.76 mm) malignant melanomas. *Am J Pathol* 145, 510-514 (1994)
- Weidner N., J.P. Semple, W.R. Welch & J. Folkman: Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma. *N Engl J Med* 324, 1-8 (1991)
- Gasparini G., N. Weidner, S. Maluta, F. Pozza, P. Boracchi, M. Mezzetti, A. Testolin & P. Bevilacqua: Intratumoral microvessel density and p53 protein: correlation with metastasis in head-and neck squamous-cell carcinoma. *Int J Cancer* 55, 739-744 (1993)
- Weidner N., P.R. Carroll, J. Flax, W. Blumenfeld & J. Folkman: Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. *Am J Pathol* 143, 401-409 (1993)
- Fox S.B., R.D. Leek, J. Bliss, J.L. Mansi, B. Gusterson, K.C. Gatter & A.L. Harris: Association of tumor angiogenesis with bone marrow micrometastases in breast cancer patients. *J Natl Cancer Inst* 89, 1044-1049 (1997)
- Aguayo A., H. Kantarjian, T. Manshouri, C. Gidel, E. Estey, D. Thomas, C. Koller, Z. Estrov, S. O'Brien, M. Keating, E. Freireich & M. Albitar: Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes. *Blood* 96, 2240-2245 (2000)
- Bertolini F., P. Mancuso, A. Gobbi & G. Pruneri: The thin red line: angiogenesis in normal and malignant hematopoiesis. *Exp Hematol* 28, 993-1000 (2000)
- Oehler M.K. R. & R. Bicknell: The promise of anti-angiogenic cancer therapy. *Br J Cancer* 82, 749-752 (2000)
- Thorpe P.E. & F.J. Burrows: Antibody-directed targeting of the vasculature of solid tumors. *Breast Cancer Res Treat* 36, 237-251 (1995)
- Byrne G.J., A. Ghellal, J. Iddon, A.D. Blann, V. Venizelos, S. Kumar, A. Howell & N.J. Bundred: Serum soluble vascular cell adhesion molecule-1: role as a surrogate marker of angiogenesis. *J Natl Cancer Inst* 92, 1329-1336 (2000)
- Neufeld G., T. Cohen, S. Gengrinovitch & Z. Poltorak: Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J* 13, 9-22 (1999)
- Ferrara N.: Vascular endothelial growth factor: molecular and biological aspects. *Curr Top Microbiol*

*Immunol* 237, 1-30 (1999)

21. Yancopoulos G.D., S. Davis, N.W. Gale, J.S. Rudge, S.J. Wiegand & J. Holash: Vascular-specific growth factors and blood vessel formation. *Nature* 407, 242-248 (2000)

22. Houck K.A., D.W. Leung, A.M. Rowland, J. Winer & N. Ferrara: Dual regulation of vascular endothelial growth factor bioavailability by genetic and proteolytic mechanisms. *J Biol Chem* 267, 26031-26037 (1992)

23. Darland D.C. & P.A. D'Amore: Blood vessels maturation: vascular development comes of age. *J Clin Invest* 103, 157-158 (1999)

24. Nor J.E., J. Christensen, D.J. Mooney & P.J. Polverini: Vascular endothelial growth factor (VEGF)-mediated angiogenesis is associated with enhanced endothelial cell survival and induction of Bcl-2 expression. *Am J Pathol* 154, 375-384 (1999)

25. Kondo S., M. Asano, K. Matsuo, I. Ohmori & H. Suzuki: Vascular endothelial growth factor/vascular permeability factor is detectable in the sera of tumor-bearing mice and cancer patients *Biochim Biophys Acta* 1221, 211-214 (1994)

26. Brown L.F., B. Berse, R.W. Jackman, K. Tognazzi, E.J. Manseau, D.R. Senger & H.F. Dvorak: Expression of vascular permeability factor (vascular endothelial growth factor) and its receptor in adenocarcinoma of the gastrointestinal tract. *Cancer Res* 53, 4727-4735 (1993)

27. Schlaeppli J-M. & J.M. Wood: Targeting vascular endothelial growth factor (VEGF) for anti-tumor therapy, by anti-VEGF neutralizing monoclonal antibodies or by VEGF receptor tyrosine kinase inhibitors. *Cancer Metastasis Rev* 18, 473-481 (1999)

28. Kim K.J., B. Li, J. Winer, M. Armanini, N. Gillet, H.S. Phillips & N. Ferrara: Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumor growth *in vivo*. *Nature* 362, 841-844 (1993)

29. Yuan F., Y. Chen, M. Dellian, N. Safabakhsh, N. Ferrara & R.K. Jain: Time-dependent vascular regression and permeability changes in established human tumor xenograft induced by an anti-vascular endothelial growth factor/vascular permeability factor antibody. *Proc Natl Acad Sci USA* 93, 14765-14770 (1996)

30. Borgstrom P., M.A. Bourdon, K.J. Hillan, P. Sriram Rao & N. Ferrara: Neutralizing anti-vascular endothelial growth factor antibody completely inhibits angiogenesis and growth of human prostate carcinoma micro tumors *in vivo*. *Prostate* 35, 1-10 (1998)

31. Mesiano S., N. Ferrara & R.B. Jaffe: Role of vascular endothelial growth factor in ovarian cancer. *Am J Pathol* 153, 1249-1256 (1998)

32. Presta L.G., H. Chen, S.J. O'Connor, V. Chisholm,

Y.G. Meng, L. Krummen, M. Winkler & N. Ferrara: Humanization of an anti-VEGF monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer Res* 57, 4593-4599 (1997)

33. Giordano G.G., M. Muto, L. Sigalotti & M. Maio: Cancer therapies: basic and clinical perspectives in brain, prostate, and lung tumors. *J Cell Physiol* (in press)

34. Margolin K., M.S. Gordon, E. Holmgren, J. Gaudreault, W. Novotny, G. Fyfe, D. Adelman, S. Stalter & J. Breed: Phase Ib trial of intravenous recombinant humanized monoclonal antibody to vascular endothelial growth factor in combination with chemotherapy in patients with advanced cancer: pharmacologic and long-term safety data. *J Clin Oncol* 19, 851-856 (2001)

35. Sledge G., K. Miller, W. Novotny, J. Gaudreault, M. Ash & M. Colbleigh: A Phase II trial of single-agent rhumAb VEGF (recombinant humanized monoclonal antibody to vascular endothelial cell growth factor) in patients with relapsed metastatic breast cancer. *Proceedings of ASCO* 19, 3a-5C (2000)

36. DeVore R.F., L. Fehrenbacher, R.S. Herbst, C.J. Langer, K. Kelly & J. Gaudreault, E. Holmgren, W.F. Novotny & F. Kabbinavar: A randomized Phase II trial comparing rhumAb VEGF (recombinant humanized monoclonal antibody to vascular endothelial cell growth factor) plus carboplatin/paclitaxel (CP) to CP alone in patients with stage IIIB/IV NSCLC. *Proceedings of ASCO* 19, 485a-1896 (2000)

37. Bergsland E., H. Hurwitz, L. Fehrenbacher, N.J. Meropol, W.F. Novotny, J. Gaudreault, G. Lieberman & F. Kabbinavar: A randomized Phase II trial comparing rhumAb VEGF (recombinant humanized monoclonal antibody to vascular endothelial cell growth factor) plus 5-fluorouracil/leucovorin (FU/LV) to FU/LV alone in patients with metastatic colorectal cancer. *Proceedings of ASCO* 19, 242a-939 (2000)

38. Taipale J., T. Makinen, E. Arighi, E. Kukk, M. Karkkainen & K. Alitalo: Vascular endothelial growth factor receptor-3. *Curr Top Microbiol Immunol* 237, 85-96 (1999)

39. Plate K.H., G. Breier, B. Millauer, A. Ullrich & W. Risau: Up-regulation of vascular endothelial growth factor and its cognate receptors in a rat glioma model of tumor angiogenesis *Cancer Res* 53, 5822-5827 (1993)

40. Mendel D.B., R.E. Schreck, D.C. West, G. Li, L.M. Strawn, S.S. Tangiongco, S. Vasile, L.K. Shawver & J.M. Cherrington: The angiogenesis inhibitor SU5416 has long-lasting effects on vascular endothelial growth factor receptor phosphorylation and function. *Clin Cancer Res* 6, 4848-4858 (2000)

41. Geng L., E. Donnelly, G. McMahon, P.C. Lin, E. Sierra-Rivera, H. Oshinka & D.E. Hallahan: Inhibition of vascular endothelial growth factor receptor signaling leads

- to reversal of tumor resistance to radiotherapy. *Cancer Res* 61, 2413-2419 (2001)
42. Rosen P.J., R. Amado, J.R. Hecht, D. Chang, M. Mulay, M. Parson, B. Laxa, J. Brown, G. Cropp, A. Hannah & L. Rosen: A Phase I/II study of SU5416 in combination with 5-FU/Leucovorin in patients with metastatic colorectal cancer. *Proceedings of ASCO* 19, 3a-5D (2000)
  43. Stetler-Stevenson W.G.: Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. *J Clin Invest* 103, 1237-1241 (1999)
  44. Herron G.S., M.J. Banda, E.J. Clark, J. Gavrilovic, & Z. Werb: Secretion of metalloproteinases by stimulated capillary endothelial cells. II. Expression of collagenase and stromelysin activities is regulated by endogenous inhibitors. *J Biol Chem* 261, 2814-2818 (1986)
  45. Mignatti P., R. Tsuboi, E. Robbins & D.B. Rifkin: *In vitro* angiogenesis on the human amniotic membrane: requirement for basic fibroblast growth factor-induced proteinases. *J Cell Biol* 108, 671-682 (1989)
  46. Johnson M.D., H.R. Kim, L. Chesler, G. Tsao-Wu, N. Bouck & P.J. Polverini: Inhibition of angiogenesis by tissue inhibitor of metalloproteinase. *J Cell Physiol* 160, 194-202 (1994)
  47. Fernandez H.A., K. Kallenbach, G. Seghezzi, E. Grossi, S. Colvin, R. Schneider, P. Mignatti & A. Galloway: Inhibition of endothelial cell migration by gene transfer of tissue inhibitor of metalloproteinases-1. *J Surg Res* 82, 156-162 (1999)
  48. Mimori K., M. Mori, T. Shiraishi, T. Fujie, K. Baba, M. Haraguchi, R. Abe, H. Ueo & T. Akiyoshi: Clinical significance of tissue inhibitor of metalloproteinase expression in gastric carcinoma. *Br J Cancer* 76, 531-536 (1997)
  49. Heath E.I. & L.B. Grochow: Clinical potential of matrix metalloprotease inhibitors in cancer therapy. *Drugs* 59, 1043-1055 (2000)
  50. Hidalgo M. & S.G. Eckhardt: Development of matrix metalloproteinase inhibitors in cancer therapy. *J Natl Cancer Inst* 93, 178-193 (2001)
  51. Nelson A.R., B. Fingleton, M.L. Rothenberg & L.M. Matrisian: Matrix metalloproteinases: biologic activity and clinical implications. *J Clin Oncol* 18, 1135-1149 (2000)
  52. Brooks P.C., S. Stromblad, L.C. Sanders, T.L. von Schalscha, R.T. Aimes, W.G. Stetler-Stevenson, J.P. Quigley, & D.A. Cheresh: Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. *Cell* 85, 683-693 (1996)
  53. Eatock M.M., A. Schatzlein, & S.B. Kaye: Tumour vasculature as a target for anticancer therapy. *Cancer Treat Rev* 26, 191-204 (2000)
  54. Brooks P.C., S. Stromblad, R. Klemke, D. Visscher, F.H. Sarkar, & D.A. Cheresh: Antiintegrin alpha v beta 3 blocks human breast cancer growth and angiogenesis in human skin. *J Clin Invest* 96, 1815-1822 (1995)
  55. Max R., R.R.C.M. Gerritsen, P.T.G.A. Nooijen, S.L. Goodman, A. Sutter, U. Keilholz, D.J. Ruiter & R.M. De Waal: Immunohistochemical analysis of integrin alpha v beta 3 expression on tumor-associated vessels of human carcinomas. *Int J Cancer* 71, 320-324 (1997)
  56. Brooks P.C., A.M. Montgomery, M. Rosenfeld, R.A. Reisfeld, T. Hu, G. Klier & D.A. Cheresh: Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* 79, 1157-1164 (1994)
  57. Brooks P.C., R.A.F. Clark & D.A. Cheresh: Requirement of vascular integrin alpha v beta 3 for angiogenesis. *Science* 264, 569-571 (1994)
  58. Gutheil J.C., T.N. Campbell, P.R. Pierce, J.D. Watkins, W.D. Huse, D.J. Bodkin & D.A. Cheresh: Targeted antiangiogenic therapy for cancer using Vitaxin: a humanized monoclonal antibody to the integrin alpha v beta 3. *Clin Cancer Res* 6, 3056-3061 (2000)
  59. Patel S.R., J. Jenkins, N.E. Papadopoulos, M.A. Burgess, C. Plager, C. Charnsangavej, J.U. Gutterman & R.S. Benjamin: A pilot study of an angiogenesis inhibitor Vitaxin in patients with advanced leiomyosarcomas (Leios). *Proceedings of ASCO* 19, 559a-2202 (2000)
  60. O'Reilly M.S., T. Boehm, Y. Shing, N. Fukai, G. Vasios, W.S. Lane, E. Flynn, J.R. Birkhead, B.R. Olsen & J. Folkman: Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 88, 277-285 (1997)
  61. Miosge N., T. Sasaki & R. Timpl: Angiogenesis inhibitor endostatin is a distinct component of elastic fibers in vessel walls. *FASEB J* 13, 1743-1750 (1999)
  62. Yamaguchi N., B. Anand-Apte, M. Lee, T. Sasaki, N. Fukai, R. Shapiro, I. Que, C. Lowik, R. Timpl & B.R. Olsen: Endostatin inhibits VEGF-induced endothelial cell migration and tumor growth independently of zinc binding. *EMBO J* 18, 4414-4423 (1999)
  63. Dhanabal M., R. Ramchandran, M.J.F. Waterman, H. Lu, B. Knebelmann, M. Segal & V.P. Sukhatme: Endostatin induces endothelial cell apoptosis. *J Biol Chem* 274, 11721-11726 (1999)
  64. Dhanabal M., R. Ramchandran, R. Volk, I.E. Stillman, M. Lombardo, M.L. Iruela-Arispe, M. Simons & V.P. Sukhatme: Endostatin: yeast production, mutants, and antitumor effect in renal cell carcinoma. *Cancer Res* 59, 189-197 (1999)

65. Kim Y.M., J.W. Jang, O.H. Lee, J. Yeon, E.Y. Choi, K.W. Kim, S.T. Lee & Y.G. Kwon: Endostatin inhibits endothelial and tumor cellular invasion by blocking the activation and catalytic activity of matrix metalloproteinase 2. *Cancer Res* 60, 5410-5413 (2000)
66. Shichiri M. & Y. Hirata: Antiangiogenesis signals by endostatin. *FASEB J* 15, 1044-1053 (2001)
67. Boehm T., J. Folkman, T. Browder & M.S. O'Reilly: Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. *Nature* 390, 404-407 (1997)
68. Ding I., J.Z. Sun, B. Fenton, W.M. Liu, P. Kimsely, P. Okunieff & W. Min: Intratumoral administration of endostatin plasmid inhibits vascular growth and perfusion in MCa-4 murine mammary carcinomas. *Cancer Res* 61, 526-531 (2001)
69. Hartford A.C., T. Gohongi, D. Fukumura & R.K. Jain: Irradiation of a primary tumor, unlike surgical removal, enhances angiogenesis suppression at a distal site: potential role of host-tumor interaction. *Cancer Res* 60, 2128-2131 (2000)
70. Hanna N.N., S. Seetharam, H.J. Mauceri, M.A. Beckett, N.T. Jaskowiak, R.M. Salloum, D. Hari, M. Dhanabal, R. Ramchandran, R. Kalluri, V.P. Sukhatme, D.W. Kufe & R.R. Weichselbaum: Antitumor interaction of short-course endostatin and ionizing radiation. *Cancer J* 6, 287-93 (2000)
71. Ratel D., V. Nasser, I. Dupre, A.L. Benabid & F. Berger: Antibodies to endostatin in a multifocal glioblastoma patient. *Lancet* 356, 1656-1657 (2000)
72. DeLisser H.M., P.J. Newman, & S.M. Albelda: Molecular and functional aspects of PECAM-1/CD31. *Immunol Today* 15, 490-495 (1994)
73. Muller W.A., C.M. Ratti, S.L. McDonnell & Z.A. Cohn: A human endothelial cell-restricted, externally disposed plasmalemmal protein enriched in intercellular junction. *J Exp Med* 170, 399-414 (1989)
74. Mazurov A.V., D.V. Vinogradov, N.V. Kabaeva, G.N. Antonova, Y.A. Romanov, T.N. Vlasik, A.S. Antonov, & V.N. Smirnov: A monoclonal antibody, VM64, reacts with a 130 kDa glycoprotein common to platelets and endothelial cells: heterogeneity in antibody binding to human aortic endothelial cells. *Thromb Haemostasis* 66, 494-499 (1991)
75. Watt S.M., S.E. Gschmeissner & P.A. Bates: PECAM-1: its expression and function as a cell adhesion molecule on hemopoietic and endothelial cells. *Leuk Lymphoma* 17, 229-244 (1995)
76. Kim C.S., T. Wang & J.A. Madri: Platelet endothelial cell adhesion molecule-1 expression modulates endothelial cell migration *in vitro*. *Lab Invest* 78, 583-590 (1998)
77. Albelda S.M., W.A. Muller, C.A. Buck & P.J. Newman: Molecular and cellular properties of PECAM-1 (endoCAM/CD31): a novel vascular cell-cell adhesion molecule. *J Cell Biol* 114, 1059-1068 (1991)
78. Sheibani N. & W.A. Frazier: Down-regulation of platelet endothelial cell adhesion molecule-1 results in thrombospondin-1 expression and concerted regulation of endothelial cell phenotype. *Mol Biol Cell* 9, 701-713 (1998)
79. Sheibani N., P.J. Newman & W.A. Frazier: Thrombospondin-1, a natural inhibitor of angiogenesis, regulates platelet-endothelial cell adhesion molecule-1 expression and endothelial cell morphogenesis. *Mol Biol Cell* 8, 1329-1341 (1997)
80. DeLisser H.M., M. Christofidou-Solomidou, R.M. Strieter, M.D. Burdick, C.S. Robinson R.S. Wexler, J.S. Kerr, C. Garlanda, J.R. Merwin, J.A. Madri & S.M. Albelda: Involvement of endothelial PECAM-1/CD31 in angiogenesis. *Am J Pathol* 151, 671-677 (1997)
81. Vermeulen P.B., G. Gasparini, S.B. Fox, M. Toi, L. Martin, P. McCulloch, F. Pezzella, G. Viale, N. Weidner, A.L. Harris, & L.Y. Dirix: Quantification of angiogenesis in solid human tumors: an international consensus on the methodology and criteria of evaluation. *Eur J Cancer* 32A, 2474-2484 (1996)
82. Fonsatti E., A.P. Jekunen, K.J.A. Kairemo, S. Coral, M. Snellman, M.R. Nicotra, P.G. Natali, M. Altomonte & M. Maio: Endoglin is a suitable target for efficient imaging of solid tumors: *in vivo* evidence in a canine mammary carcinoma model. *Clin Cancer Res* 6, 2037-2043 (2000)
83. Gougos A. & M. Letarte: Identification of a human endothelial cell antigen with monoclonal antibody 44G4 produced against a pre-B leukemic cell line. *J Immunol* 141, 1925-1933 (1988)
84. Yamashita H., H. Ichijo, S. Grimsby, A. Morén, P. ten Dijke & K. Miyazono: Endoglin forms a heteromeric complex with the signaling receptors for transforming growth factor-beta. *J Biol Chem* 269, 1995-2001 (1994)
85. Luque A., C. Cabañas, U. Raab, A. Letamendía, E. Pérez, L. Herreros, F. Sanchez-Madrid & C. Bernabeu: The use of recombinant vaccinia virus to generate monoclonal antibodies against the cell-surface glycoprotein endoglin. *FEBS Lett* 413, 265-268 (1997)
86. Gougos A. & M. Letarte: Primary structure of endoglin, an RGD-containing glycoprotein of human endothelial cells. *J Biol Chem* 265, 8361-8364 (1990)
87. Bellón T., A. Corbi, P. Lastres, C. Cales, M. Cebrian, S. Vera, S. Cheifetz, J. Massague, M. Letarte & C. Bernabeu: Identification and expression of two forms of the human transforming growth factor-beta-binding protein endoglin with distinct cytoplasmic regions. *Eur J Immunol* 23, 2340-2345 (1993)
88. Cheifetz S., T. Bellón, C. Calés, S. Vera, C. Bernabeu, J. Massagué & M. Letarte: Endoglin is a component of the transforming growth factor-beta receptor system in human



endothelial cells. *J Biol Chem* 267, 19027-19030 (1992)

89. Lastres P., A. Letamendía, H. Zhang, C. Rius, N. Almendro, U. Raab, L.A. López, C. Langa, A. Fabra, M. Letarte & C. Bernabéu: Endoglin modulates cellular responses to TGF-beta 1. *J Cell Biol* 133, 1109-1121 (1996)

90. Barbara N.P., J.L. Wrana & M. Letarte: Endoglin is an accessory protein that interacts with the signaling receptor complex of multiple members of the transforming growth factor-beta superfamily. *J Biol Chem* 274, 584-594 (1999)

91. Letamendía A., P. Lastres, L.M. Botella, U. Raab, C. Langa, B. Velasco, L. Attisano & C. Bernabeu: Role of endoglin in cellular responses to transforming growth factor-beta. *J Biol Chem* 273, 33011-33019 (1998)

92. Guerrero-Esteo M., P. Lastres, A. Letamendía, M.J. Pérez-Alvarez, C. Langa, L.A. López, A. Fabra, A. García-Pardo, S. Vera, M. Letarte & C. Bernabéu: Endoglin overexpression modulates cellular morphology, migration, and adhesion of mouse fibroblasts. *Eur J Cell Biol* 78, 614-623 (1999)

93. Li C., I.N. Hampson, L. Hampson, P. Kumar, C. Bernabeu & S. Kumar: CD105 antagonizes the inhibitory signaling of transforming growth factor beta 1 on human vascular endothelial cells. *FASEB J* 14, 55-64 (2000)

94. Lastres P., T. Bellon, C. Cabañas, F. Sanchez-Madrid, A. Acevedo, A. Gougos, M. Letarte & C. Bernabéu: Regulated expression on human macrophages of endoglin, an Arg-Gly-Asp-containing surface antigen. *Eur J Immunol* 22, 393-397 (1992)

95. Rokhlin O.W., M.B. Cohen, H. Kubagawa, M. Letarte & M.D. Cooper: Differential expression of endoglin on fetal and adult hematopoietic cells in human bone marrow. *J Immunol* 154, 4456-4465 (1995)

96. Burrows F.J., E.J. Derbyshire, P.L. Tazzari, P. Amlot, A.F. Gazdar, S.W. King, M. Letarte, E.S. Vitetta & P.E. Thorpe: Up-regulation of endoglin on vascular endothelial cells in human solid tumors: implications for diagnosis and therapy. *Clin Cancer Res* 1, 1623-1634 (1995)

97. Fonsatti E., L. Del Vecchio, M. Altomonte, L. Sigalotti, M.R. Nicotra, S. Coral, P.G. Natali & M. Maio: Endoglin: an accessory component of the TGF-beta-binding receptor-complex with diagnostic, prognostic, and bioimmunotherapeutic potential in human malignancies. *J Cell Physiol* (in press)

98. Miller D.W., W. Graulich, B. Karges, S. Stahl, M. Ernst, A. Ramaswamy, H.H. Sedlacek, R. Müller & J. Adamkiewicz: Elevated expression of endoglin, a component of the TGF-beta-receptor complex, correlates with proliferation of tumor endothelial cells. *Int J Cancer* 81, 568-572 (1999)

99. Wang J.M., S. Kumar, D. Pye, N. Haboubi & L. Al-Nakib: Breast carcinoma: comparative study of tumor vasculature using two endothelial cell markers. *J Natl Cancer Inst* 86, 386-388 (1994)

100. Wang J.M., S. Kumar, D. Pye, A.J. van Agthoven, J. Krupinski & R.D. Hunter: A monoclonal antibody detects heterogeneity in vascular endothelium of tumours and normal tissues. *Int J Cancer* 54, 363-370 (1993)

101. McAllister K.A., K.M. Grogg, D.W. Johnson, C.J. Gallione, M.A. Baldwin, C.E. Jackson, E.A. Helmbold, D.S. Markel, W.C. McKinnon, J. Murrell, M.K. McCormick, M.A. Pericak-Vance, P. Heutink, B.A. Oostra, T. Haitjema, C.J.J. Westerman, M.E. Porteous, A.E. Guttmacher, M. Letarte & D.A. Marchuk: Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. *Nat Genet* 8, 345-351 (1994)

102. Bourdeau A., D.J. Dumont & M. Letarte: A murine model of hereditary hemorrhagic telangiectasia. *J Clin Invest* 104, 1343-1351 (1999)

103. Li D.Y., L.K. Sorensen, B.S. Brooke, L.D. Urness, E.C. Davis, D.G. Taylor, B.B. Boak & D.P. Wendel: Defective angiogenesis in mice lacking endoglin. *Science* 284, 1534-1537 (1999)

104. Kumar S., A. Ghellal, C. Li, G. Byrne, N. Haboubi, J.M. Wang & N. Bundred: Breast carcinoma: vascular density determined using CD105 antibody correlates with tumor prognosis. *Cancer Res* 59, 856-861 (1999)

105. Bredow S., M. Lewin, B. Hofmann, E. Marecos & R. Weissleder: Imaging of tumour neovasculature by targeting the TGF-beta binding receptor endoglin. *Eur J Cancer* 36, 675-681 (2000)

106. Seon B.K., F. Matsuno, Y. Haruta, M. Kondo & M. Barcos: Long-lasting complete inhibition of human solid tumors in SCID mice by targeting endothelial cells of tumor vasculature with antihuman endoglin immunotoxin. *Clin Cancer Res* 3, 1031-1044 (1997)

107. Matsuno F., Y. Haruta, M. Kondo, H. Tsai, M. Barcos & B.K. Seon: Induction of lasting complete regression of preformed distinct solid tumors by targeting the tumor vasculature using two new anti-endoglin monoclonal antibodies. *Clin Cancer Res* 5, 371-382 (1999)

108. Tabata M., M. Kondo, Y. Haruta, & B.K. Seon: Antiangiogenic radioimmunotherapy of human solid tumors in SCID mice using <sup>125</sup>I-labeled anti-endoglin monoclonal antibodies. *Int J Cancer* 82, 737-742 (1999)

109. Kumar S. & C. Li: Targeting of vasculature in cancer and other angiogenic diseases. *Immunol Today* 22, 129 (2001)

**Key Words:** VEGF, MMP, alpha v beta 3 integrin, endostatin, CD31, CD105, Tumor, Endothelial Cells, Angiogenesis, Review

**Send correspondence to:** Michele Maio, MD, Cancer Bioimmunotherapy Unit, Centro di Riferimento Oncologico-I.R.C.C.S., Via Pedemontana Occ.le, 12, Aviano, Italy 33081 Tel: +39-0434-659342, Fax: +39-0434-659566, E-mail: mmaio@cro.it