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Arresting Cancer Progression by VEGF Inhibitions: An Update

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Abstract
According to WHO report, more than 8 million people died globally from cancer only in year 2012 (latest year for which information is available) and problem was expected to increase with alarming rate in future. To target Vascular Endothelial growth factor in tumorous environment is an option to deprive the cancer cells from vital nutrients and hence stop the cancer progression. Vascular endothelial growth factors (VEGF) targeting agents administered alone or in association with other drugs are used to stop the cancer progression. They have been revealed for the disease improvement in the patients of advanced-stage cancers. Numbers of agents which can alter VEGF pathways are under investigation from different aspects regarding their efficacy in cancer treatment. In time, we have become gradually more aware of the fact that it is not only VEGF-Inhibited angiogenesis which blocks cancer cells growth, rather it involves different mechanisms. Currently five approaches are practiced to halt the VEGF signaling pathways which are; monoclonal ABs to target vascular endothelial growth factors and their receptors, Tyrosine kinase inhibitors of Vascular endothelial growth factor receptors, soluble receptors of VEGF acting as decoy receptors for VEGF, ribozymes which target VEGF mRNA and finally siRNA which suppress the mRNA of VEGF by RNA interference mechanism. The purpose of this article is to discuss updated reports on above mentioned approaches with possible drawbacks. A better and in depth understanding of these strategies which are used to stop VEGF pathways, will obviously revolutionize the cancer related therapeutic approaches in future.
Keywords: VEGF, Angiogenesis, Cancer, Bevacizumab, Receptors tyrosine kinase.

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INTRODUCTION

VEGF is a signaling protein developed by cells that initiates the vasculogenesis and angiogenesis (Senger *et al.,* 1983; Ferrara, 2016; Miller, 2016). Studies over the last three decades, with more than seventeen thousand papers published on the topic have revealed important insights into the process of angiogenesis by VEGF and their role in malignancies. There was a better understanding of angiogenesis process after the successful cloning of VEGF in 1989 (Keck *et al.,* 1989). Only 14 year later researcher introduced the first VEGF-Targeting agent *bevacizumab* (moAB), in association with chemotherapy which revealed remedial properties toward metastatic colorectal cancer. After that several phase III experiments have evidenced and proved the beneficial effects of *bevacizumab* and other VEGF-targeted therapies, alone or in association with other drugs (Escudier *et al.,* 2007; Miller *et al.,* 2007; Motzer *et al.,* 2007). In spite of extensive research on VEGF-targeted therapy, the detailed mechanisms of action of these moieties are not completely interpreted. Recently it is revealed that VEGF targeted approaches not merely stop cancer growth by ceasing angiogenesis rather there are different mechanisms which are responsible for the VEGF-targeted approaches. In depth understandings of underlying mechanisms are mandatory for this therapy as it is now apparent that all VEGF-targeted therapy are not necessarily fruitful for the patients with different types of cancers.

VEGFs and their receptors on cancer cells

Out of five VEGF proteins (VEGFA, VEGFB, VEGFC, VEGFD and PGF), VEGFA (also known as VEGF) is best known of this family (Ferrara, 2016). Furthermore, due to alternative splicing, VEGFA expressed with different numbers of amino acids i.e. 121-, 165-, 189-, and 206-amino acid proteins. Specifically, VEGF165 expression is much higher in human solid tumors. After the discovery of VEGF, research on its involvement in angiogenesis is much higher than any other of its function (Ferrara *et al.,* 2004; Ellis and Hicklin, 2008; Chung *et al.,* 2011).

Recent research reveals that the VEGF is not confined to new blood vessels developments (Senger, 2010), rather it can alter the immune cells function which are in tumerous environment and may also affect the fibroblasts activities in the tumour stroma (Bahce *et al.,* 2016).

VEGF RTKs and Neuropilins

Receptor tyrosine kinases are VEGFR1, VEGFR2 and VEGFR3 also known as FLT1, FLK1 and FLT4 respectively (Kowanetz and Ferrara, 2006). VEGFR2 is the main RTK that involves in VEGF pathways in endothelial cells and that drives VEGF-induced angiogenesis. Some cancer cells express VEGFR2 which shows its involvement in VEGF signaling but some of the cancerous cells are proved to be independent from these signaling. This indicates the involvements of other receptor rather than RTKs (Kowanetz and Ferrara, 2006).

A specific downstream signaling happens when ligand binds on the vascular endothelial growth factor receptor tyrosine kinases (Kowanetz and Ferrara, 2006). VEGFR1, along with its expression on the vasculature, it also express on different other kinds of cells. It binds with tenfold higher affinity to VEGF1 but exert less effect on VEGF1 signaling as compared to VEGFR2. So VEGFR1 binds to VEGF and blocks its binding to VEGFR2, in this way it can act as negative regulator for angiogenesis. VEGFR3 binds preferably with VEGFC and VEGFD on lymphatic endothelial cells (Laakkonen *et al.,* 2007). VEGFR3 has prime role in remodeling of primary vascular networking in embryo development and also effects on post-natal lymph angiogenesis (Kukk *et al.,* 1996; Alitalo and Carmeliet, 2002).

The NP1 and NP2 (neuropilins) serve as co-receptors for the VEGF, enhancing the adhering affinity of VEGF to VEGFR TKI receptors (Klagsbrun *et al.,* 2002; Soker *et al.,* 2002; Bielenberg and Klagsbrun, 2007; Batchelor, *et al.,* 2007). Interestingly, current research suggests that dual targeting of VEGF and NP1 with antibodies is more fruitful than using single target (Batchelor *et al.,* 2007). Regarding tumor angiogenesis, VEGF increase blood flow rate in tumor by enhancing endothelial cell proliferations through various mechanisms i.e. increased endothelial cell proliferation and survival, enhanced lattice network for endothelial cells (Gimbrone *et al.,* 1973; Rafii *et al.,* 2002). Apart from angiogenesis related activities, VEGF has involvements in some other vital functions like autocrine signaling of tumor cells and suppression of immune system activities (Kaplan *et al.,* 2005). Important thing is that increased blood vessels by VEGF cannot be correlated with increased smooth blood flow in the vessel. For example dilation of blood vessel can result in turbulent and improper blood flow. VGF induced enhanced vessels permeability may result in enhanced fluid pressure and narrowing of blood vessels (Sun *et al.,* 2007; Yang *et al.,* 2007).

Experimental VEGF-targeted strategies for solid tumor inhibitions

Understanding of the fact that VEGF mediated signaling is important in angiogenesis has resulted in invention of many approaches which can target and block VEGF. These include antibodies which neutralize Vascular Endothelial Growth Factors or their receptors, Receptor hybrids which block VEGFs and TKIs which also bind selectively VEGFs. Other approaches include Ribozymes and siRNA which specifically bind and block RNAs for VEGFs (Casanovas *et al.,* 2005). Currently, used drugs include *bevacizumab* which block VEGFs, *sorafenib* neutralizes VEGFR TKIs, and *sunitimib*, also blocks the VEGFRs. *Bevacizumab* was approved by FDA for clinical use for human MCC (metastatic colorectal cancer) and breast cancers alone or as a combined therapy with other drugs (Miller *et al.,* 2005; Sandler *et al.,* 2006; Kane *et al.,* 2006; Batchelor *et al.,* 2007; Goodman *et al.,* 2007; Hurwitz *et al.,* 2007).

Monoclonal antibodies targeting VEGFs

Monoclonal antibodies continue to be a good option for targeting angiogenic agents in cancer treatment (Hinoda *et al.,* 2004). A famous humanized mouse monoclonal antibody *Bevacizumab* is important in different cancers treatment. It is in the list of the WHO's Essential Medicines (Wang and Zhang 2014). It is used to target human Vascular Endothelial Growth Factor (Zondor and Medina, 2004; Presta *et al.,* 1997). It has 17 days half-life in human and is administrated inter venous in 1.5 hours after every seventeen days. It was allowed for treatment by FDA for different metastatic cancers in 2004 as alone or in combination with standard chemotherapy . It has since been approved for use in certain lung cancers, renal tumors, ovarian cancers, and brain's glioblastoma (Bergsland, 2004). Earlier *Bevasizumab* had been approved for different cancers by FDA, but that approval was withdrawn for the breast cancer in 2014, when studies showed less evidence of effectiveness in breast cancer treatment (Kodjikian *et al.,* 2014). Regarding adverse effects of bevacizumab-related therapeutics, in a controlled study, hypertension is a major documented side effect. Twenty two percent of patients who were on bevacizumab in combination with IFL compared to Eight percent of those patients taking placebo along with IFL were reported to have problem of hypertension. But all of these cases of hypertension were treated by giving oral anti depressive medications to patients without interruption or discontinuity of bevacizumab therapy. There were minor cases of gastrointestinal perforation on IFL plus bevacizumab therapy (6 out of 393 patients; 1.5 %).

In a phase III controlled randomized study, it was observed that in patients who were given combination of bevacizumab and interferon-alfa as first line treatment, there was significant increase in progression-free survival while in patients who were given interferon alone, there was no such increase reported (Escudier *et al.,* 2007).

HuMV833 is a murine monoclonal anti-VEGF antibody. It is a humanized form of MV833 that revealed activity against multiple types of tumors. The melanoma and rhabdomyosarcoma xenografts growth were inhibited by the administration of this neutralizing antibody (Kim *et al.,* 1993). However, in phase I clinical trial (Jayso *et al.,* 2002), *HuMV833* showed variations in tissue distribution among patients, in normal organs as well as in malignant lesions. Because of this reason, it was not further investigated in upcoming trials for monoclonal antibodies action against cancers.

VEGF receptor Kinases inhibitors

Small molecule inhibitors (SMIs) are considered as one of the most effective drugs for targeted therapy of cancer. In clinical oncology, the increasing number of approved tyrosine kinase inhibitors (TKIs) indicates the improvement in attention and application of these cancer remedial tools. Many of these Kinase inhibitors, recently approved VEGFR Kinases inhibitors are in pre-clinical and clinical trials along with several side effects. Only small number of these agents has been approved for cancer therapeutics. Selective RTK–TKIs have revealed less harmful effects in contrast to multi-targeted inhibitors (Hojjat-Farsangi, 2014).

Semaxanib (SU5416) is a tyrosine-kinase inhibitor drug formulated by SUGEN (a cancer therapeutic drug discovery company) which is the potent and selective inhibitor of VEGFR-2 (O'Donnell *et al.,* 2005) and was the first drug regarding this category to reach clinical trials. This inhibitor also reveals activity in response to other VEGF receptor kinases, PDGFR and c-kit by imitating ATP and halting binding of ATP and in pre-clinical trials, it was seemed to stop growth and vascularization of tumor (Canadas *et al.,* 2010). However, it was reported later that drug has more side effects with less efficacy, which led to the discontinuation of this drug development (Hoff *et al.,* 2006). On February 2002, *Pharmacia,* the then-parent of *Sugen*, ultimately ended Phase III clinical trials of SU5416 in the treatment of advanced colorectal cancer due to discouraging results (Hoff *et al.,* 2006). However, it was approved by FDA as *sunitinib* for treatment of renal carcinoma in January 2006.

SU6668, an orally bioavailable TKI shown good pre-clinical performance but failed to prove clinical efficacy against solid tumors and hence no further improvement occurred regarding this drug (Eichhorn *et al.,* 2004). *SU11248* is a fresher oral TKI that has activity against VEGFR-1 and 2, Sixty three patients suffering from metastatic kidney cancer, who failed to respond to interferon therapy, when given this drug in clinical trials of phase II showed promising effects (Ciardiello *et al.,* 2003; Tuma, 2004).

Vatalanib is another strong, powerful & selective VEGFR-KI among 1st generation drugs, administered orally. It causes inhibitory effects on VEGFR-1 & 2 when given in µM concentrations but if given in higher concentrations it can also block tyrosine kinase like receptors of growth factors derived by platelets (Bold *et al.,* 2000).

Soluble VEGF Receptor

A decoy soluble receptor, *VEGF-Trap* was made by fusing interstitial domains of VEGFR-1 and VEGFR-2, which has strong affinity to fragment constant part of IgG1 (Konner and Dupont, 2004).

In pre-clinical studies, it revealed anti-tumor activity against several xenograft models i.e. in pancreatic cancer, preventing advancement of the primary cancer and decreasing size of tumor (Byrne *et al.,* 2003; Frischer *et al.,* 2004; Fukasawa and Korc, 2004). The *VEGF-Trap* produces its angiogenic effects by suppressing tumor vasculature, reshaping or regularizing the surviving vessels and by blocking the new vessels growth for tumor (Teng *et al.,* 2010).

Ribozymes

mRNA molecules are cleaved in a sequence-specific manner by Ribozymes, a RNA enzyme (Ciafre *et al.,* 2004). Various cancerous growth and proliferation was arrested by reaction of ribozymes on VEGF mRNA. Also ribozymes showed in vitro activity towards VEGF receptors (Weng and Usman, 2001). To target pre or immature mRNA, a stable ribozyme is used which cut downs the expression of both soluble and cell surface VEGFR-1. Normal volunteers well-sustained Angiozyme TM (also called RPI. 4610) (Weng and Usman, 2001). In clinical trials of phase II initial data with this drug showed some promising results but later some sever adverse side effects such as on site of injection, fatigue, anorexia, fever, constipation, vomiting, headache, abdominal pain, dyspnoea and nausea were reported. Although Angiozyme showed good safety profiles but it was ruled out from further development and advancement because of its lack in clinical effectiveness (Morrow *et al.,* 2012).

RNA Interference (RNAi) induced VEGF silencing

To shape expression of gene RNAi is specific, capable and effective mechanism. Using RNAi for gene silencing to cure certain diseases assures the development of new class of therapeutics. Former investigations have revealed that RNAi of VEGF-C effectively targets breast cancer cells and block their metastasis in lymphatic system and also RNAi of VEGF inhibits angiogenesis and blocks retinoblastoma & Ewing's sarcoma or bone cancer (Guan *et al.,* 2005; Sun *et al.,* 2008).

For examination of activity and safeness of lipid nanoparticle siRNA formulation in humans, ALN-VSP trials were started, siRNAs LNP formulation targeted kinesin spindle protein (KSP) and *VEGF,* in cancer patients. Pharmaco dynamics suggested anti-tumor action along with complete suppression of metastasis from liver to uterus or endometrial cancer. It was also revealed that IV injection of ALN-VSP twice a week was secure and well sustained. These investigations provided certification of concept that RNA interference based treatments are fruitful in cancer research (Tabernero *et al.,* 2013).

In another study RNA interference induced by siRNA was used to check that if the impairment in synthesis of VEGF can halt the development of TSP1 resistance .It was revealed that the growth of unaltered Fibro sarcoma tumor cells was reduced by systemic In-vivo administration of crude Anti-VEGF siRNA. Anti-VEGF siRNA not only retarded the growth of TSP1 resistant cancers but also halted their growth rate (Filleur *et al.,* 2003).

Still technical troubles such as stability, off target effects, immuno stimulation and delivery problems restrict the RNAi development. By optimizing the molecular structure and chemistry, investigators have tried to surpass these hurdles and improve the bioavailability and safety of RNA based therapeutics.

Challenges with Anti-VEGF therapies

First Anti-angiogenic drug has been approved by FDA since nine years i.e. monoclonal antibodies (bevacizumab) are synthesized to treat metastatic colorectal cancer. Many other anti-angiogenic drugs are under some stages of clinical investigations. Major drawbacks have been identified, related to utilization of this class of agents such as adapted resistance, by ongoing clinical and preclinical trials. Lack of validated predictive biomarker to analyze tumor progressions and therapeutic response are the major hurdles .For the above challenges, some molecular and cellular mechanisms have been provided by investigations in clinical and preclinical models (Shojaei, 2012).

For patients in which VEGF therapy was effective, it was a misfortune that the duration of activity of therapy was relatively short i.e. single-agent therapy within three to eight etargeted drug resistance mechanisms (Bergers and Hanahan, 2008). The need is to formulate better agents and to design a combined therapy to collaborate with VEGF. Pre symptomatic investigations also reveals that if the regimens targeting the VEGF ligand develops resistance against a specific drug then it might b effective to aim other VEGF family members (Batchelor *et al.,* 2007). Additionally, preclinical research has proved that therapies that target VEGF ligand and neuropilin can increase capacity of preclinical experiments (Batchelor *et al.,* 2007). Currently, about 20 different types of VEGF targeted agents are there in clinical trials. Monoclonal antibodies apply a selective targeting to VEGF components and this method has perhaps small adverse effects due to its high specificity. However TKIs with off target effects can be advantageous because of their ability to target different TKIs at same time which are involved in cancer progression and formation of blood vessels, but relatively at higher costs of toxicities. Blockage of VEGF remains an optimistic accession in the neoplasmic treatments. Combined treatment against VEGF along with chemotherapy and radiotherapy give better results in anti-tumor effects as compared to treating alone (Ziche *et al.,* 2004). RNAi therapeutics is another promising area for VEGF related cancer treatment but again we have to optimize the siRNA delivery protocols .The need is to design clinical models to explore the detailed pathways and mechanisms involved in VEGF targeting. Effects of targeting VEGF on cancer microenvironment as well as normal host tissues can be fully understood by interpretations of results taken from clinical investigations.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

Alitalo, K., Carmeliet, P., 2002. Molecular mechanisms of lymph angiogenesis in health and disease. Cancer Cell., 1(3): 219-227.

Bahce, I., Yaqub, M., Smit, E.F., Lammertsma, A.A., Dongen, G.A., Hendrikse, N.H., 2016. Personalizing NSCLC therapy by characterizing tumors using TKI-PET and immuno-PET. Lung Cancer., pii: S0169-5002(16)30347-6. .

Batchelor, T.T., Sorensen, A.G., di Tomaso, E., Zhang, W.T., Duda, D.G., Cohen, K.S., Kozak, K.R., Cahill, D.P., Chen, P.J., Zhu, M., Ancukiewicz, M., Mrugala, M.M., Plotkin, S., Drappatz, J., Louis, D.N., Ivy, P., Scadden, D.T., Benner, T., Loeffler, J.S., Wen, P.Y., Jain, R.K., 2007. AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell., 11(1): 83-95.

Bergsland, E.K., 2004. Update on clinical trials targeting vascular endothelialgrowth factor in cancer. Am. J. Health. Syst. Pharm., 61: S12-S20.

Bielenberg, D.R., Klagsbrun, M., 2007. Targeting endothelial and tumor cells with semaphorins. Cancer Metastasis Rev., 26: 421-431.

Bold, G., Altmann, K.H., Frei, J., Lang, M., Manley, P.W., Traxler, P., *et al.,* 2000. New anilino phthalazines as potent and orally well absorbed inhibitors of the VEGF receptor tyrosine kinases useful as antagonists of tumor-driven angiogenesis. J. Med. Chem., 43(12): 2310-23.

Byrne, A.T., Ross, L., Holash, J., Nakanishi, M., Hu, L., Hofmann, J.I., Yancopoulos, G.D., Jaffe, R.B., 2003. Vascular endothelial growth factor-trap decreases tumor burden, inhibits ascites, and causes dramatic vascular remodeling in an ovarian cancer model. Clin. Cancer. Res., 9(15): 5721-8.

Canadas, I., Rojo, F., Arumi, M., Rovira, A., Albanell, J., Arriola, E., 2010. C-MET as a new therapeutic target for the development of novel anticancer drugs. Clin. Transl. Oncol., 12: 253-260

Casanovas, O., Hicklin, D.J., Bergers, G., Hanahan, D., 2005. Drug resistance by evasion of anti angiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell., 8(4): 299-309.

Chung, A.S., Ferrara, N., 2011. Developmental and pathological angiogenesis. Annu. Rev. Cell. Dev. Biol., 27: 563-584.

Ciafre, S.A., Niola, F., Wannenes, F., Farace, M., 2004. An anti-VEGF Ribozyme embedded within the adenoviral VAI sequence inhibits glioblastoma cell angiogenic potential in vitro. J. Vasc. Res., 41: 220-8.

Ciardiello, F., Bianco, R., Caputo, R., Damiano, V., Troiani, T., Melisi, D., De Vita, F., De Placido, S., Bianco, A.R., Tortora, G., 2004. Antitumor activity of ZD6474, a vascular endothelial growth factor receptor tyrosine kinase inhibitor, in human cancer cells with acquired resistance to anti epidermal growth factor receptor therapy. Clin. Cancer Res., 10(2): 784-93.

Eichhorn, M.E., Strieth, S., Dellian, M., 2004. Anti-vascular tumor therapy: recent advances, pitfalls and clinical perspectives. Drug. Resist. Updat., 7(2): 125-38.

Ellis, L.M., Hicklin, D.J., 2008. VEGF-targeted therapy: mechanisms of anti-tumour activity. Nat. Rev. Cancer., 8(8): 579-91.

Escudier, B., Eisen, T., Stadler, W.M., Szczylik, C., Oudard, S., Siebels, M., *et al.,* 2007. Sorafenib in advanced clear-cell renal cell carcinoma. N. Eng. J. Med., 356: 125-134.

Escudier, B., Pluzanska, A., Koralewski, P., Ravaud, A., Bracarda, S., Szczylik, C., *et al.,* 2007. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. Lancet. 370(9605): 2103-11.

Ferrara, N., 2016. VEGF and Intraocular Neovascularization: From Discovery to Therapy. Transl. Vis. Sci. Technol., 5(2): 10.

Ferrara, N., Hillan, K.J., Gerber, H.P., Novotny, W., 2004. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat. Rev. Drug. Discov., 3(5): 391-400.

Filleur, S., Courtin, A., Ait-Si-Ali, S., Guglielmi, J., Merle, C., Harel-Bellan, A., Clézardin, P., Cabon, F., 2003. SiRNA-mediated inhibition of vascular endothelial growth factor severely limits tumor resistance to anti angiogenic thrombospondin-1 and slows tumor vascularization and growth. Cancer Res., 63(14): 3919-22.

Frischer, J.S., Huang, J., Serur, A., Kadenhe-Chiweshe, A., McCrudden, K.W., O'Toole, K., Holash, J., Yancopoulos, G.D., Yamashiro, D.J., Kandel, J.J., 2004. Effects of potent VEGF blockade on experimental Wilms tumor and its persisting vasculature. Int. J. Oncol., 25(3): 549-53.

Fukasawa, M., Korc, M., 2004. Vascular endothelial growth factor-trap suppresses tumorigenicity of multiple pancreatic cancer cell lines. Clin. Cancer Res., 10(10): 3327-32.

Gimbrone, M. A, Leapman, S.B., Cotran, R.S., Folkman, J., 1973.Tumor angiogenesis: iris neo vascularization at a distance from experimental intraocular tumors. J. Natl. Cancer. Inst., 50(1): 219-228.

Goodman, V.L., Rock, E.P., Dagher, R., Ramchandani, R.P., Abraham, Gobburu, J.V., Booth, B.P., Verbois, S.L., Morse, D.E., Liang, C.Y., Chidambaram, N., Jiang. J.X., Tang, S., Mahjoob, K., Justice, R., Pazdur, R., 2007. Approval summary: sunitinib forthe treatment of imatinib refractory or intolerant gastrointestinal stromal tumors and advanced renal cell carcinoma. Clin. Cancer. Res., 13(5): 1367-73.

Guan, H., Zhou, Z., Wang, H., Jia, S.F., Liu, W., Kleinerman, E.S., 2005. A small interfering RNA targeting vascular endothelial growth factor inhibits Ewing's sarcoma growth in a xenograft mouse model. Clin. Cancer Res., 11(7): 2662-9.

Bergers, G., Hanahan, D., 2008. Modes of resistance to anti-angiogenic therapy. Nat. Rev. Cancer., 8(8): 592-603.

Hinoda, Y., Sasaki, S., Ishida, T., Imai, K., 2004. Monoclonal antibodies as effective therapeutic agents for solid tumors. Cancer. Sci., 95(8): 621-5.

Hoff, P.M., Wolff, R.A., Bogaard, K., Waldrum, S., Abbruzzese, J.L., 2006. A Phase I Study of Escalating Doses of the Tyrosine Kinase Inhibitor Semaxanib (SU5416) in Combination with Irinotecan in Patients with Advanced Colorectal Carcinoma. Jpn. J. Clinc. Oncol., 36(2):100-3.

Hojjat-Farsangi, M., 2014. Small-Molecule Inhibitors of the Receptor Tyrosine Kinases: Promising Tools for Targeted Cancer Therapies. Int. J. Mol. Sci., 15(8): 13768-13801.

Hurwitz, H., Fehrenbacher, L., Novotny, W., Cartwright, W., Hainsworth, J., Heim, W., Berlin. J., Baron, A., Griffing, S., Holmgren, E., Ferrara, N., Fyfe, G., Rogers, B., Ross, R., Kabbinavar, F., 2004. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N. Engl. J. Med., 350(23): 2335-42.

Jayson, G.C., Zweit, J., Jackson, A., Mulatero, C., Julyan, P., Ranson, M., 2002. Molecular Imaging and Biological Evaluation of HuMV833 Anti-VEGF Antibody: Implications for Trial Design of Antiangiogenic Antibodies. JNCI J. Natl. Cancer. Inst., 94(19): 1484-1493.

Tabernero, J., Shapiro, G.I., LoRusso, P.M., Cervantes, A., Schwartz, G.K., Weiss, G.J., *et al.,* 2013. First-in-Humans Trial of an RNA Interference Therapeutic Targeting VEGF and KSP in Cancer Patients with Liver Involvement. Cancer Discov., 3(4):406-17.

Kane, R.C., Farrell, A.T., Saber, H., Tang, S., Williams, G., Jee, J.M., Liang, C., Booth, B., Chidambaram, N., Morse, D., Sridhara, R., Garvey, P., Justice, R., Pazdur, R., 2006. Sorafenib for the treatment of advanced renal cell carcinoma. Clin. Cancer. Res., 12(24): 7271-8.

Kaplan, R.N., Riba, R.D., Zacharoulis, S., Bramley, A.H., Vincent, L., Costa, C., *et al.,* 2005. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature., 438(7069): 820-7.

Keck, P.J., Hauser, S.D., Krivi, G., Sanzo, K., Warren, T., Feder, J., Connolly, D.T., 1989. Vascular permeability factor, an endothelial cell mitogen related to PDGF. Sci., 246(4935):1309-12.

Kim, K.J., Li, B., Winer, J., Armanini, M., Gillett, N., Phillips, H.S., Ferrara N.,1993. Inhibition of vascular endothelial growth factor-induced angiogenesissuppresses tumour growth in vivo. Nature., 362(6423): 841-4.

Klagsbrun, M., Takashima, S., Mamluk, R., 2002. The roleof neuropilin in vascular and tumor biology. Adv. Exp. Med. Biol., 515: 33-48.

Kodjikian, L., Decullier, E., Souied, E.H., Girmens, J.F., Durand, E.E., Chapuis, F.R., Huot, L., 2014. Bevacizumab and ranibizumab for neovascular age-related macular degeneration: an updated meta-analysis of randomised clinical trials. Graefes. Arch. Clin. Exp. Ophthalmol., 252(10): 1529-37.

Konner, J., Dupont, J., 2004. Use of soluble recombinant decoy receptor vascular endothelial growth factor trap (VEGF Trap) to inhibit vascular endothelial growth factor activity. Clin. Colorectal. Cancer., Suppl 2:S81-5.

Kowanetz, M., Ferrara, N., 2006. Vascular endothelial growth factor signaling pathways: therapeutic perspective. Clin. Cancer. Res., 12(17): 5018-22.

Kukk, E., Lymboussaki, A., Taira, S., Kaipainen, A., Jeltsch, M., Joukov, V., Alitalo, K., 1996. VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development. Development 122: 3829-3837.

Laakkonen, P., Waltari, M., Holopainen, T., Takahashi, T., Pytowski, B., Steiner, P., Hicklin, D., Persaud, K., Tonra, J.R., Witte, L., Alitalo, K., 2007. Vascular endothelial growth factor receptor 3 is involved in tumor angiogenesis and growth. Cancer. Res., 67(2): 593-599.

Miller, J.W., 2016. VEGF: From Discovery to Therapy: The Champalimaud Award Lecture. Transl. Vis. Sci. Technol., 5(2): 9.

Miller, K., Wang, M., Gralow, J., Dickler, M., Cobleigh, M., Perez, E.A., Shenkier, T., Cella, D., Davidson, N.E., 2007. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N. Engl. J. Med., 357(26): 2666-76.

Miller, K.D., Chap, L.I., Holmes, F.A., Cobleigh, M.A., Marcom, P.K., Fehrenbacher, L., Dickler, M., Overmoyer, B.A., Reimann, J.D., Sing, A.P., Langmuir, R.. Rugo, H.S., 2005. Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J. Clin. Oncol., 23(4): 792-9.

Morrow, P.K., Murthy, R.K., Ensor, J.D., Gordon, G.S., Margolin, K.A., Elias, A.D., Urba, W.J., Weng, D.E., Rugo, H.S., Hortobagyi, G.N., 2012. An open-label, phase 2 trial of RPI.4610 (Angiozyme) in the treatment of metastatic breast cancer. Cancer., 118(17):4098-104.

Motzer, R.J., Hutson, T.E., Tomczak, P., Michaelson, M.D., Bukowski, R.M., Rixe, O., Oudard, S., Negrier, S., Szczylik, C., Kim, S.T., Chen, I., Bycott, P.W., Baum, C.M., Figlin, R.A., 2007. Sunitinib versus interferon alfa inmetastatic renal-cell carcinoma. N Engl J Med., 356(2): 115-24.

O'Donnell, A., Padhani, A., Hayes, C., Kakkar, A., Leach, M., Trigo, J., Scurr, M., Raynaud, F., Phillips, S., Aherne, W., Hardcastle, A., Workman, P., Hannah, A., Judson, I., 2005. A Phase I study of the angiogenesis inhibitor SU5416 (semaxanib) in solid tumours, incorporating dynamic contrast MR pharmacodynamic end points. Br. J. Cancer., 93(8): 876-83.

Presta, L.G., Chen, H., O'Connor, S.J., Chisholm, V., Meng, Y.G., Krummen, L., Winkler, M., Ferrara, N., 1997. Humanization of an anti-vascular endothelial growthfactor monoclonal antibody for the therapy of solid tumors and other disorders. Cancer. Res., 57(20): 4593-9.

Rafii, S., Lyden, D., Benezra, R., Hattori, K., Heissig, B., 2002. Vascular and haematopoietic stem cells: noveltargets for anti-angiogenesis therapy? Nature. Rev. Cancer., 2(11): 826-35.

Sandler, A., Gray, R., Perry, M.C., Brahmer, J., Schiller, J.H., Dowlati, A., Lilenbaum, R., Johnson, D.H., 2006. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N. Engl. J. Med., 355(24): 2542-50.

Senger, D.R., Galli, S.J., Dvorak, A.M., Perruzzi, C.A., Harvey, V.S., Dvorak, H.F., 1983. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Sci., 219(4587): 983-985.

Senger, D.R., 2010. Vascular endothelial growth factor: much more than an angiogenesis factor. Mol. Bio. Cell., 21(3): 377-379.

Shojaei, F., 2012. Anti-angiogenesis therapy in cancer: current challenges and future perspectives. Cancer lett., 320(2): 130-7.

Soker, S., Miao, H.Q., Nomi, M., Takashima, S., Klagsbrun, M., 2002. VEGF165 mediates formation of complexes containing VEGFR-2 and neuropilin-1 that enhance VEGF165-receptor binding. J. Cell. Biochem., 85(2): 357-68.

Sun, C, Jain, R.K., Munn, L.L. 2007. Non-uniform plasma leakage affects local hematocrit and blood flow:

implications for inflammation and tumor perfusion. Ann. Biomed. Eng., 35(12): 2121-2129.

Sun, P., Gao, J., Liu, Y.L., Wei, L.W., Wu, L.P., Liu, Z.Y., 2008. RNA interference (RNAi)-mediated vascular endothelial growth factor-C (VEGF-C) reduction interferes with lymphangiogenesis and enhances epirubicin sensitivity of breast cancer cells. Mol. Cell Biochem., 308(1-2): 161-8.

Teng, L.S., Jin, K.T., He, K., Zhang, J., Wang, H.H., Cao, J., 2010. Clinical applications of VEGF-trap (aflibercept) in cancer treatment. J. Chin. Med. Assoc., 73(9): 449-56.

Tuma, R.S., 2004. Three molecularly targeted drugs tested in kidney cancer clinical trials. J. Natl. Cancer Inst., 96: 1270-1.

Wang, W., Zhang, X., 2014. Systemic adverse events after intravit real bevacizumab versus ranibizumab for age-related macular degeneration: a meta-analysis. PLoS One., 9(10):e109744.

Weng, D.E., Usman, N., 2001. Angiozyme: a novel angiogenesis inhibitor. Curr. Oncol. Rep., 3(2): 141-146.

Yang, J.C., Haworth, L., Sherry, R.M., Hwu, P., Schwartze, D.J., Topalian, S.L., Steinberg, S.M., Chen, H.X., Rosenberg, S.A., 2003. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N. Engl. J. Med., 349(5): 427-34.

Ziche, M., Donnini, S., Morbidelli, L., 2004. Development of new drugs in angiogenesis. Curr. Drug. Targets., 5(5): 485-93.

Zondor, S.D., Medina, P.J., 2004. Bevacizumab An angiogenesis inhibitorwith efficacy in colorectal and other malignancies. Ann. Pharmacother., 38(7-8): 1258-64.