Arresting Cancer Progression by VEGF Inhibitions: An Update

Muhammad Irfan¹*, Kalsoom Sughra¹, Muhammad Naem Iqbal²³, Sajid Mehmoond⁴, Aqsa Shaheen¹, Zahid Anwar¹

¹Department of Biochemistry and Molecular Biology, University of Gujrat, HH Campus, Gujrat, Pakistan.
²The School of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China.
³Pakistan Science Mission (PSM), Noor Kot 51770, Pakistan.
⁴Islam Medical College Sialkot, Pakistan.

*Corresponding author: Muhammad Irfan; Email: muhammad.irfan@uog.edu.pk

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.

To cite this article: Irfan, M., Sughra, K., Iqbal, M.N., Mehmood, S., Shaheen, A., Anwar, Z., 2016. Arresting Cancer Progression by VEGF Inhibitions: An Update. PSM Microbiol., 01(2): 72-78.

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 tumorous 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 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 VEGF-C and VEGF-D 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 administered intravenous 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 showed 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 be 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.

**ACKNOWLEDGEMENT**

The authors would like to express thanks Dr. Umer Rashid and Dr. Nadia Zeeshan, University of Gujrat, for their kind guidance to write this manuscript.

**CONFLICT OF INTEREST**

The authors declare that they have no competing interests.

**REFERENCES**


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


