The novel therapeutic monoclonal antibody VGX-100 neutralises VEGF-C and inhibits tumor growth and metastasis in subcutaneous and orthotopic models of human cancer.

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Abstract

Angiogenesis and lymphangiogenesis are important processes facilitating tumor growth and metastasis. Growth factors that stimulate blood and lymphatic proliferation within tumors are therefore potential targets for anti-cancer therapies. Proof of concept of the clinical utility of anti-angiogenic drugs was first established by the FDA/EMEA-approved drug bevacizumab (Avastin®) which blocks VEGF binding to its receptors VEGFR-1 and VEGFR-2, the latter being the key receptor signaling for angiogenesis. However, patients treated with bevacizumab may be refractory or develop resistance to bevacizumab, suggesting upregulation of alternative pro-angiogenic proteins that allow tumors to bypass the inhibition of VEGF signaling. VEGF-C is a logical candidate for inducing resistance to bevacizumab via this mechanism since it is also a ligand for the angiogenic receptor VEGFR-2 and for VEGFR-3 which is upregulated on tumor-associated vascular endothelium.

VGX-100 is a highly specific, fully human monoclonal antibody for VEGF-C that blocks VEGF-C binding to both VEGFR-2 and VEGFR-3. Here we demonstrate that VGX-100 has an additive effect in combination with docetaxel and/or anti-VEGF (bevacizumab) in several tumor models, suggesting that VEGF-C may be an important mediator of the resistance to existing anti-VEGF therapies. Further, we demonstrate that in an orthotopic model of prostate cancer, that inhibition of VEGF-C alone by VGX-100 monotherapy is sufficient to inhibit tumor growth and significantly reduce the incidence of tumor metastasis to local lymph nodes. These data indicate that VGX-100 has exciting potential as a cancer therapeutic by targeting a key factor involved in angiogenesis, lymphangiogenesis and tumor metastasis and is expected to complement chemotherapy and/or other anti-angiogenic compounds in the clinic.

Introduction

The various VEGF ligands have distinct receptor binding specificities which contribute to their diversity of function, as summarized in Figure 1. VEGF-C and VEGF-D are ligands for VEGFR-2, which signals for angiogenesis, and VEGFR-3 which mediates lymphangiogenesis and tumour-associated angiogenesis. The receptor binding specificity of VEGF-C and VEGF-D is distinct to that of VEGF, which binds VEGFR-2 but not VEGFR-3.

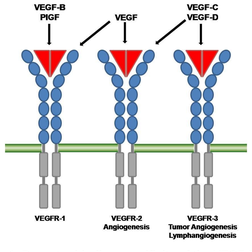


Figure 1. Receptor binding specificity of the VEGF family.

Recent publications suggest that in certain contexts, VEGF-C and VEGF-D, the alternative ligands to VEGF for VEGFR-2, can be up-regulated during VEGF blockade[1,2,3,4,5]. Furthermore, in some mouse tumor models, administration of small molecule inhibitors of the VEGFR tyrosine kinase activity can increase subsequent tumor invasion and metastasis[6,7,8]. VEGF-C and VEGF-D up-regulation during VEGF/VEGFR suppression may be a key driver of resistance to anti-VEGF/VEGFR therapies.

Expression of VEGF-C is elevated in a diverse range of tumors, including cancers of the colon, stomach, breast, ovary and prostate. Elevated levels of intra-tumoral and circulating VEGF-C frequently correlate with poor prognosis and features associated with tumor aggression (e.g. tumor depth, size, lymphatic invasion and lymph node metastasis).

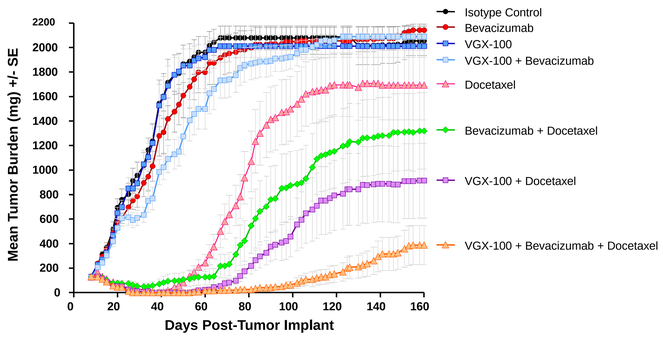
VGX-100 is a highly specific, fully human monoclonal antibody that neutralizes binding of VEGF-C to VEGFR-2 and VEGFR-3. Therefore, VGX-100 has the potential to inhibit not only primary tumor growth through its anti-angiogenic and anti-lymphangiogenic activities, but to also inhibit metastasis via the lymphatic vessels. Lymphatic metastasis is associated with poor prognosis that is not effectively blocked by anti-VEGF-A or anti-VEGFR-2 therapeutics.

We have previously reported that VGX-100 inhibits primary tumor growth as a single-agent in human pancreatic KP4 tumor xenografts, and has anti-tumor activity in combination with bevacizumab in human U87MG glioblastoma tumor xenografts. Furthermore, in the PC-3 prostate tumor model, VGX-100 significantly enhances the anti-tumor efficacy of docetaxel, and docetaxel + bevacizumab combination therapy.

Here we further demonstrate, in mouse models of human lung (H292) and ovarian (OVCAR-8) cancer, that addition of VGX-100 to chemotherapy and chemotherapy + anti-VEGF (bevacizumab) enhances efficacy and prevents the development of escape mechanisms resulting in more durable responses Furthermore, inhibition of VEGF-C significantly inhibited both primary tumor growth and metastasis of orthotopic PC-3 prostate tumors.

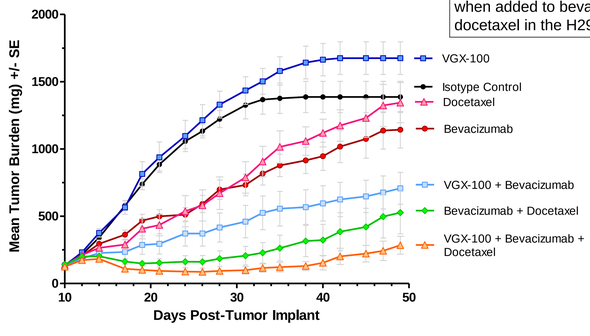
Subcutaneous Tumor Models

Prostate Carcinoma Model (PC-3)
(last observation carried forward)

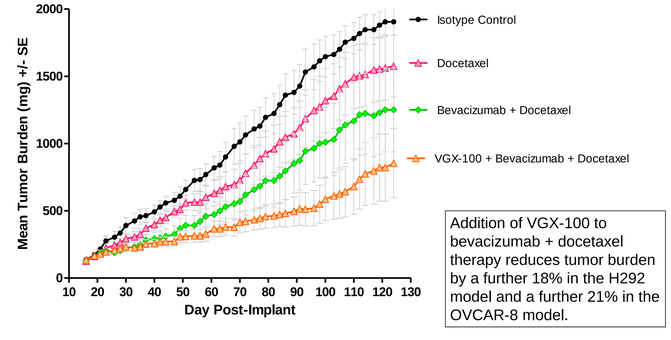


Addition of VGX-100 to bevacizumab reduces tumor burden by a further 38% compared to bevacizumab alone, and improves tumor growth inhibition by 18% when added to bevacizumab + docetaxel in the H292 model.

Lung Carcinoma Model (H292)
(last observation carried forward)



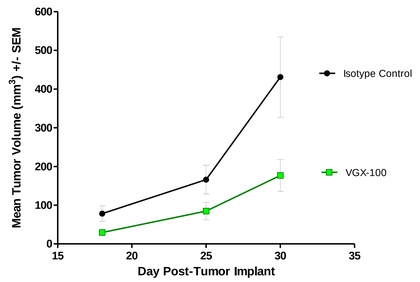
Ovarian Carcinoma Model (OVCAR-8)



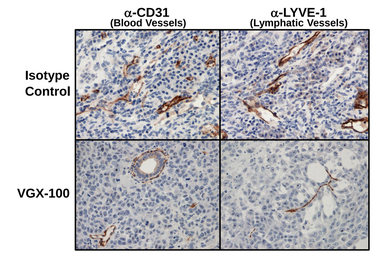
Addition of VGX-100 to bevacizumab + docetaxel therapy reduces tumor burden by a further 18% in the H292 model and a further 21% in the OVCAR-8 model.

Orthotopic Metastatic ProstateTumor Model

VGX-100 Inhibits Growth of Orthotopic PC-3 Prostate Tumors



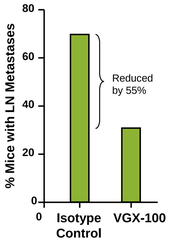
VGX-100 reduces tumor burden by 59% (at day 30) compared to control IgG.
** p=0.019 (Students t-test)*

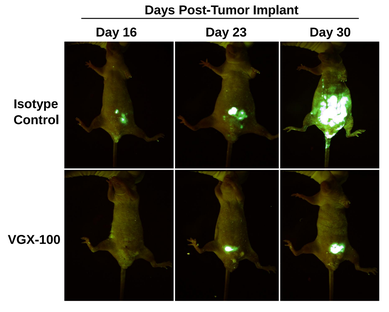


VGX-100 Reduces Lymph Node Metastasis in an Orthotopic Prostate Tumor Model

Group	# Mice	# Mice with LN Mets	% Mice with LN Mets	p value*
Isotype Antibody Control	17	12	71%	-
VGX-100	19	6	32%	0.019

** p value by Fisher exact test.*





Conclusions

- In subcutaneous mouse models of human cancer, VGX-100 has anti-tumor activity as a single-agent and in combination with chemotherapy or bevacizumab.
- VGX-100 enhances the efficacy of docetaxel and bevacizumab combination therapy in prostate, lung and ovarian cancer models.
- In an orthotopic mouse model of human prostate cancer (PC-3), single-agent VGX-100 inhibited primary tumor growth by 59% compared to an isotype control antibody, and reduced the incidence of metastasis to local lymph nodes by 55%.
- VGX-100 has the potential to affect tumor growth and invasiveness and improve patient outcomes in the clinic.
- Combination of VGX-100 with existing anti-VEGF strategies can simultaneously inhibit multiple VEGFR pathways. This may reduce redundant signalling that drives tumor resistance and limits the efficacy of currently available therapies blocking a single-target.

Materials and Methods

PC-3, H292 and OVCAR-8 subcutaneous tumor models: PC-3 (5 x 10^6), H292 (5 x 10^5) or OVCAR-8 (1 x 10^7) cells were implanted subcutaneously in nu/nu mice high in the right axilla. Mice were triaged into treatment groups (n=10/group) when the mean tumor burden was 75-175 mg. Tumor burden was estimated from caliper measurements by the formula: Tumor burden (mg) = (L x W^2)/2, where L and W are the respective orthogonal tumor length and width measurements (mm). Antibodies were administered 2x/week via intraperitoneal injection (Isotype control and VGX-100, 40 mg/kg; bevacizumab, 10 mg/kg). Docetaxel (10 mg/kg) was administered intraveneously weekly for three weeks.

Orthotopic PC-3 tumor model: PC-3-GFP human prostate cancer orthotopic MetaMouse® model was conducted by AntiCancer Inc. PC-3-GFP tumor fragments were surgically implanted between the ventral lobes of the prostate and closed by suture. Treatment was started three days after surgery (60 mg/kg, 3x/week, IP). Whole body imaging of GFP-expressing tumors was performed once a week in live animals after GFP-visible tumors were established. Primary tumor sizes were estimated once a week by caliper measurement and tumor volume (mm^3) calculated by the formula (L x W^2)/2 .

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