VGX-100, a novel therapeutic monoclonal antibody targeting VEGF-C that inhibits tumor growth

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Tester, A., Phelan, D., McColl, B., Klupacs, R. and Baldwin, M.E.
Circadian Technologies Limited, Level 1, 10 Wallace Ave, Toorak, 3142, Victoria, Australia.
Email: megan.baldwin@circadian.com.au www.circadian.com.au

circadian technologies limited

Abstract

Angiogenesis and lymphangiogenesis are important contributors to the growth and metastasis of a wide variety of tumor types. Therapeutic targeting of the angiogenic vascular endothelial growth factor (VEGF) is now an FDA-approved treatment for several cancers. Vascular endothelial growth factor-C (VEGF-C) has also been demonstrated to stimulate angiogenesis and lymphangiogenesis in a number of experimental systems, and is essential for development of the lymphatic system during embryogenesis. In addition, expression of VEGF-C drives tumor progression and metastasis in mouse models of cancer, and is associated with poor prognosis in many human cancers. Targeting VEGF-C is therefore a highly promising strategy for novel therapeutics, with the potential to disrupt both angiogenesis and lymphangiogenesis.

VGX-100 is a highly specific, fully human monoclonal antibody for VEGF-C that does not bind to other members of the VEGF family. *In vitro* testing demonstrates that VGX-100 blocks binding of VEGF-C to both VEGF receptor-2 and VEGF receptor-3, and inhibits the proliferation of blood vascular (HUVEC) and lymphatic endothelial cells in response to VEGF-C stimulation. Here we demonstrate anti-tumor efficacy through the use of xenograft models of human cancer, with VGX-100 treatment significantly inhibiting tumor growth in several models. These findings demonstrate that VGX-100 has great potential for development as a new cancer therapeutic, with potential clinical utility in combination with existing therapies or in patients refractory to existing anti-angiogenic agents.

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 tumor-associated anglogenesis. The receptor binding specificity of VEGF-C and VEGF-D is distinct to that of VEGF, which binds VEGFR-2 but not VEGFR-3.

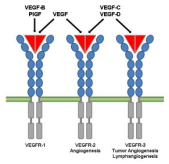


Figure 1. Receptor binding specificity of the VEGF family.

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 [1-3]. Furthermore, in some mouse tumor models, administration of small molecule inhibitors of the VEGFR tyrosine kinase activity can increase subsequent tumor invasion and metastasis [4,5]. 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 or anti-VEGFR-2 therapeutics.

Materials and Methods

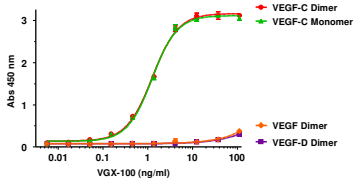
- A direct VGX-100 binding ELISA was employed. VEGF-C and -D (Circadian Technologies) or VEGF (R&D Systems) were used as capture antigens, and bound VGX-100 was detected with rabbit anti-human IgG-HRP (Abcam).
- Bioassays to measure the binding of VEGF-C to the extracellular domain of VEGFR-2 or -3 were performed with Ba/F3-VEGFR-2 or -3 / EpoR cells. Response to ligands and VGX-100 was measured by [^{3}H] thymidine incorporation following exposure for 48 hrs.
- HUVEC (Lonza) proliferation assays were conducted for 48hrs. Cell number was measured with WST-1 reagent (Roche).
- For the xenograft studies PC-3, KP4 or U87MG cells (5 x 10^6 per mouse) were implanted subcutaneously into nude mice. Mice were triaged into treatment groups when mean tumor burden reached ~125 mg. Tumor measures were recorded 2 to 3 times weekly with calipers. Animals with tumor burdens greater than 2g were euthanized. Bevacizumab (Genentech/Roche; 10 mg/kg/injection), VGX-100 (Circadian Technologies; 40 mg/kg/injection) and human IgG1 isotype control (40 mg/kg/injection) were administered by intraperitoneal injection twice weekly. Docetaxel (10 mg/kg) was administered intravenously weekly for three weeks.

References

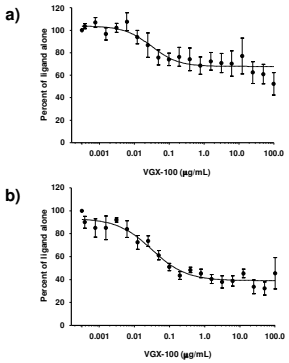
[1] Shojaei, F. et al., Nature Biotechnol., 25(8):911-20, 2007.
[2] Moffat, B. et al., Clin. Canc. Res., 1:12(5):1525-32, 2006.
[3] Grau, S. et al., Abstract 59th Ann. Meeting of the German Society of Neurosurgery, 30/5/2008.
[4] Ebos, J et al., Cancer Cell, 15(3):232-9, 2009.
[5] Paez-Ribes M., Cancer Cell, 15(3): 220-31, 2009.

In Vitro Characterization of VGX-100

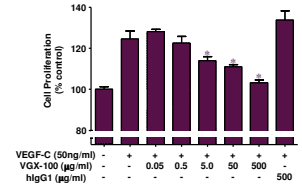
VGX-100 selectively recognizes and binds VEGF-C by ELISA with KD 1.8nM (Biacore)



VGX-100 blocks VEGF-C binding to a) VEGFR-2 and b) VEGFR-3 in Ba/F3 bioassays



VGX-100 inhibits VEGF-C stimulated HUVEC proliferation



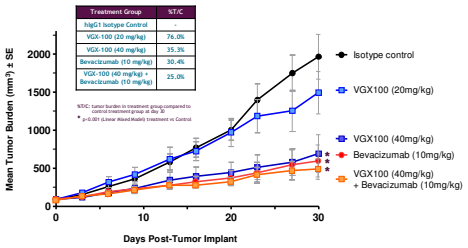
* p<0.05 (Student's t test) treatment vs. non-treatment

Anti-Tumor Efficacy of VGX-100 in Cancer Xenograft Models

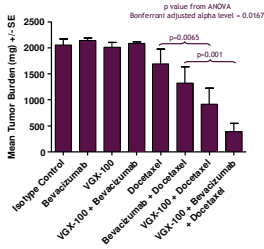
Pancreatic Carcinoma Xenograft Model (KP4)

Treatment Group	%T/C
hIgG1 Isotype Control	-
VGX-100 (20 mg/kg)	76.0%
VGX-100 (40 mg/kg)	35.3%
Bevacizumab (10 mg/kg)	30.4%
VGX-100 (40 mg/kg) + Bevacizumab (10 mg/kg)	25.0%

%T/C: tumor burden in treatment group compared to control treatment group at day 30
* p<0.001 (Linear Mixed-Models) treatment vs Control



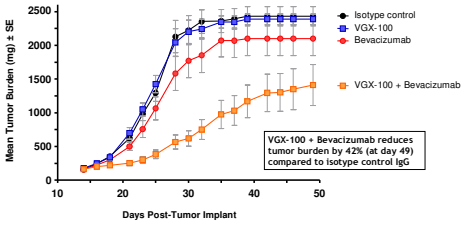
PC-3 Tumor Weight at Day 160



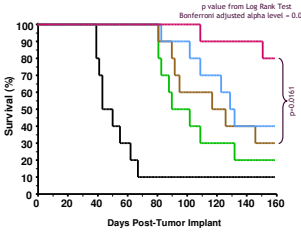
Treatment Group	%T/C
hIgG1 Isotype Control (40 mg/kg)	-
Bevacizumab (10 mg/kg)	104.2%
VGX-100 (40 mg/kg)	97.9%
VGX-100 (40 mg/kg) + Bevacizumab (10 mg/kg)	101.7%
Docetaxel (10mg/kg)	82.4%
Bevacizumab (10 mg/kg) + Docetaxel (10mg/kg)	64.2%
VGX-100 (40 mg/kg) + Docetaxel (10mg/kg)	44.5%
VGX-100 (40 mg/kg) + Bevacizumab (10 mg/kg)+ Docetaxel (10mg/kg)	16.6%

%T/C: tumor burden in treatment group compared to control treatment group at day 160

Glioblastoma Xenograft Model (U87MG)



VGX-100 Treatment in Combination with Docetaxel and Bevacizumab Enhances Survival

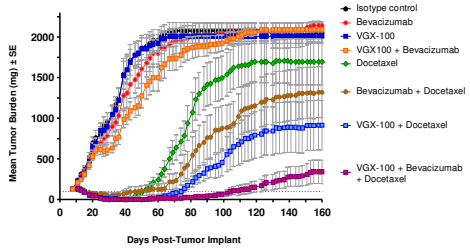


Treatment Group	Avg Survival (days)	% CR	% PR	% TFS
hIgG1 Isotype Control	59.9	0	0	0
Docetaxel (10mg/kg)	108.6	100	0	20
Bevacizumab (10 mg/kg) + Docetaxel	122.7	70	0	0
VGX-100 (40 mg/kg) + Docetaxel	131.8	100	0	20
VGX-100 (40 mg/kg) + Bevacizumab (10 mg/kg) + Docetaxel	154	100	0	40

CR: Complete Regression. Tumor burden reduced to an immeasurable volume at any point after the first treatment.
PR: Partial Regression. Tumor burden decreases to less than half of the tumor burden at first treatment.
TFS: Tumor Free Survivor. Tumor burden is immeasurable at termination of experiment (day 160).

PC-3 xenograft study was terminated at day 160

Prostate Carcinoma Xenograft Model (PC-3)



Conclusions

- In three human cancer xenograft models VGX-100 inhibits tumor growth either as a single agent or in combination with Bevacizumab and/or with standard of care chemotherapy.
- In the PC-3 prostate cancer model VGX-100 therapy combined with docetaxel significantly delays tumor growth and reduces tumor burden compared to docetaxel treatment alone.
- Addition of VGX-100 therapy to docetaxel + Bevacizumab treatment significantly reduces tumor burden and improves survival.