Abstract # A5

# Tivozanib, a selective VEGFR TKI, potently blocks angiogenesis and growth in tumors that express a high level of VEGF-C and are refractory to VEGF-A blockade

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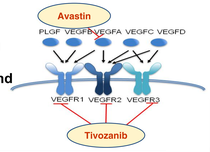
## Introduction

**Scientific understanding of the role of VEGF-A in tumor angiogenesis has led to the development of anti-angiogenic therapies, such as bevacizumab, that selectively target VEGF-A. However, clinical trials across multiple cancer types have resulted in limited positive outcomes. VEGF-C is thought to be a potent lymphangiogenic growth factor and plays a role in tumor angiogenesis through VEGFR3; it has also been shown to bind to VEGFR2, which is important in tumor angiogenesis. Nevertheless, a direct role of VEGF-C in driving tumor angiogenesis has not been established.**

**To explore the potential of VEGF-C as a driver of tumor angiogenesis and its implication in developing antiangiogenic therapies, we assessed the activity of tivozanib, a potent and selective TKI for VEGFR1, 2 and 3, and a VEGF-A targeted antibody in animal tumor models that exhibit distinct VEGF-C and VEGF-A expression.**

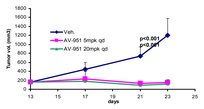



## Tivozanib (AV-951): preclinical and clinical summary

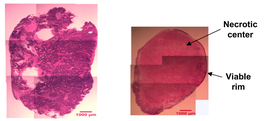
- Potent, selective ATP competitive VEGFR TKI
  - 160-240 pM pVEGFR IC50 against VEGFR1,2,3 in cell based assays
  - >8x selectivity of VEGFR1,2,3 potency/next measured kinase potency
  - 4.5 day T1/2 in human studies
- Robust efficacy in 272 patient Phase 2 RCC trial
  - ORR: 25 - 40% (all RCC independent review—cc/neph investigator review)
  - PFS: 11.7 months in overall kidney cancer population
    14.8 months in clear cell nephrectomized RCC patients (n=176)
- Safety profile consistent with on-mechanism inhibition
  - Most common AEs are Hypertension and Hoarseness
  - Low incidence of off target AEs: notably Fatigue, HFS, Mucositis, Diarrhea
- Enrollment complete for phase 3 registration study in front line RCC
  - 500 patients: Tivozanib v. sorafenib in VEGF treatment naïve patients
  - Data expected 4Q 2011 at the earliest

## Antiangiogenic mechanism of tivozanib in murine breast tumors

Complete tumor growth inhibition (BH469)



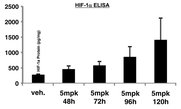

Characteristic histology changes in blockade of angiogenesis



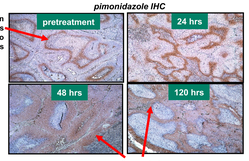

Tivozanib treatment induced hypoxia and associated tumor death

*pimonidazole IHC*

Time dependent HIF-1α induction



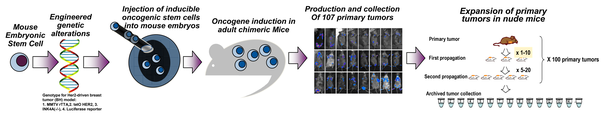

Hypoxia found in tumor cells distant to microvessels




Hypoxia extended to tumor cells adjacent to microvessels

## Population-based murine breast tumor model

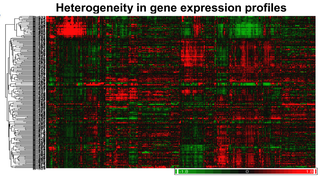
Generation of chimeric inducible murine breast tumors and expansion in nude mice



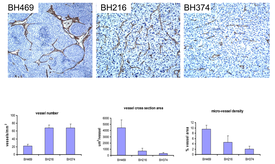

•A total of 107 tumors from chimeric mice were transplanted into and expanded in nude mice

The population-based tumor model exhibited significant inter-tumor variations

Heterogeneity in gene expression profiles



Variation in tumor microvasculature




## Synthesis and characterization of murine VEGF-A neutralizing antibodies

- Genentech Abs B20-4.1 and G6-31 specifically bind human and mouse VEGF-A and prevent binding of ligand to VEGFR2
- B20-4.1 KD = 1.6nM and 2.0nM for human and mouse, respectively
- G6-31 KD = 0.2nM and 0.3nM for human and mouse, respectively
- Bevacizumab KD = 2.2nM for human
  *(J Biol Chem. 2006 Jan 13;281(2):951-6)*

- AVEO synthesized the above anti-murine/human VEGF-A antibodies B20-4.1 and G6-31, as surrogates of bevacizumab to investigate VEGF ligands in tumor angiogenesis

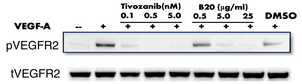
AVEO Biacore characterization of B20-4-1* and G6-31* variants

| | Human VEGF-A | | | Mouse VEGF-A** | | | Human VEGFC | Mouse VEGF-C |
|---|---|---|---|---|---|---|---|---|
| Antibody | ka ($M^{-1}s^{-1}$) | kd ($s^{-1}$) | KD (M) | ka ($M^{-1}s^{-1}$) | kd ($s^{-1}$) | KD (M) | KD (M) | KD (M) |
| Bevacizumab | 1.4E+05 | 2.3E-04 | 2.1E-09 | No Binding | | | No Binding | No Binding |
| B20-4.1 | 8.0E+04 | 2.3E-05 | 6.7E-10 | 9.3E+04 | 3.9E-05 | 4.1E-10 | No Binding | No Binding |
| G6-31 | 2.0E+06 | 2.0E-05 | 1.0E-11 | 2.6E+06 | 2.3E-06 | 8.7E-13 | No Binding | No Binding |

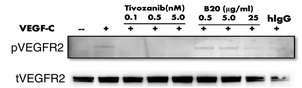
* B20-4.1 is referred as B-20 and G6-31 as G6 in the rest of this poster.
** The kd and KD were outside of the limit of detection for mouse VEGF-A.

## Tivozanib but not VEGF-A antibody B20 blocks VEGF-C stimulated VEGFR2 and VEGFR3 phosphorylation

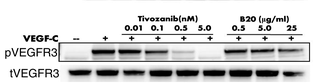
**Both tivozanib and VEGF-A antibody B20 blocks VEGFR2 phosphorylation by VEGF-A (Human umbilical vein endothelial cells in culture)**




**Tivozanib, but not VEGF-A antibody B20 blocks VEGFR2 phosphorylation by VEGF-C (Human umbilical vein endothelial cells in culture)**

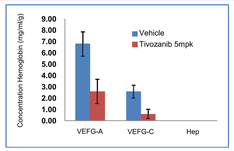



**Tivozanib, but not VEGF-A antibody B20 blocks VEGFR3 phosphorylation by VEGF-C (Human dermal lymphatic microvascular endothelial cells in culture)**




## Tivozanib blocks VEGF-C induced angiogenesis in an *in vivo* matrigel plug assay

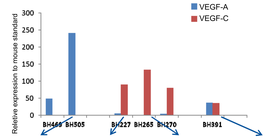
- 129 Sv/Ev mice were injected with 500uL of matrigel containing 250ng VEGF-A or 250 ng VEGF-C
- Mice were treated with tivozanib 5mpk daily
- Matrigel plugs were explanted after 7 days



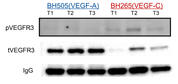

## Tivozanib exhibits potent antiangiogenesis and antitumor activity in high VEGF-C tumors in the population-based murine tumor model

### Expression of VEGF-A vs. VEGF-C in the population-based murine tumor model

A. Three distinct subtypes of tumors were identified:
- high VEGF-A and low VEGF-C
- high VEGF-C and low VEGF-A
- high VEGF-A and high VEGF-C

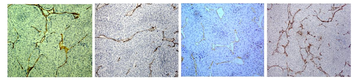



B. VEGFR3 autophosphorylation is detected in VEGF-C high tumors




--IP was performed in tumor lysates (400μg protein) with an anti-VEGFR3 antibody, followed by Western blotting using an anti-p-tyr antibody.

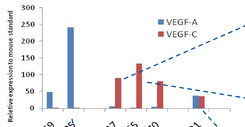
C. All 3 groups of tumors exhibited characteristic VEGF-like microvasculature.

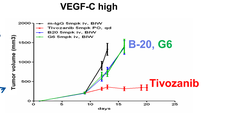


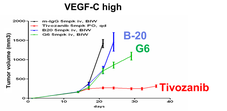
### Tumors expressing high VEGF-C are sensitive to tivozanib but resistant to VEGF-A antibodies

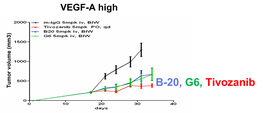
Four tumors from the population-based model were expanded in nude mice subcutaneously and treated with B20 (5mpk, b2w) or G6 (5mpk, b2w), or tivozanib (5mpk, qd).

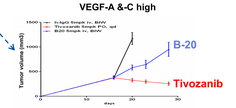
N=10 mice/arm



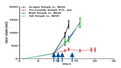



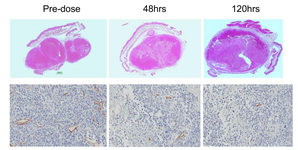




## Pharmacodynamic analysis suggested an antiangiogenic MOA of tivozanib in VEGF-C tumors

•BH227(VEGF-C) tumors were treated with 5vozanib for 0, 24, 48 and 120 hours. Tumors (n=5) were analyzed for histopathology(H&E), CD31 IHC and Ki67 IHC.





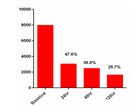

A. Tivozanib induced substantial central necrosis, characteristic of antitumor activity through antiangiogenesis mechanism.

B. Potent anti-VEGF activity on tumor microvessels was observed with CD31 IHC: substantial vessel pluming associated with tumor killing.

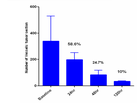
C. Time-dependent decrease in MVD and total microvessel numbers; the latter suggests a time-dependent "shrinkage" of viable tumor supported by surviving microvessels, mostly in the rim near tumor capsules.

D. No significant inhibition of tumor cell proliferation (%Ki67+) was observed at 24 and 48 hours while there was a slight decrease by 120 hrs (data not shown).

MVD in viable tumor areas

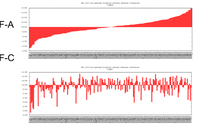



Total number of CD31+ microvessels Per tumor cross-section n=5



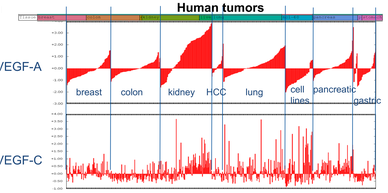

## Translational potential in human cancer

AVEO's population-based murine tumor model




- Murine breast tumors expressing high level VEGF-C are sensitive to VEGFR TKI tivozanib but resistant to VEGF-A antibodies

Human tumors




- A significant portion of human tumors express high level VEGF-C, suggesting that pan VEGFR TKIs, such as tivozanib, may have broader activity than agents that selectively target VEGF-A

## Summary

1. In a population-based murine tumor model comprising 107 individually propagated breast tumors, a sub-group exhibited high level VEGF-C expression. Immunohistochemical analysis revealed similar tumor microvasculature in high VEGF-C tumors as in high VEGF-A tumors.
2. Tivozanib, a highly selective VEGFR TKI, completely inhibited high VEGF-C tumors in mice, likely through an antiangiogenic mechanism. This is consistent with the blockade of VEGFR2 and R3 phosphorylation in tissue cultre and in vivo angiogenesis stimulated by VEGF-C, and is suggestive of a role of VEGF-C in tumor angiogenesis.
3. VEGF-A antibodies were inactive in the VEGF-C tumors and only moderately active (B20) in the VEGF-A and -C tumors.
4. These findings provide further scientific evidence that pan-VEGFR TKIs, such as tivozanib, may have broader activity than agents that selectively target VEGF-A.

### Acknowledgments

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