Tivozanib Biomarker Identifies Tumor-infiltrating Myeloid Cells Contributing to Tivozanib Resistance in Both Preclinical Models and Human Renal Cell Carcinoma

Jie Lin, Xiaojian Sun, Bin Feng, Feng Jiang, Richard Nicoletti, Guangmu Li, M. Isabel Chiu, Brooke Esteves, Pankaj Bhargava, Murray O. Robinson

AVEO Pharmaceuticals, Inc., Cambridge, MA, USA.

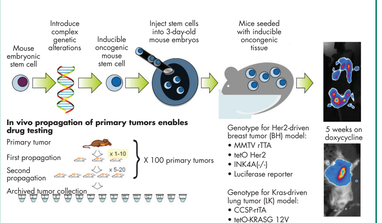
Abstract

Background: To identity biomarkers associated with tivozanib response, a population-based, genetically engineered breast tumor model comprising 107 tumors was developed, characterized, and used to test the efficacy of tivozanib, a VEGFR-1, -2, and -3 kinase inhibitor that has shown clinical activity in renal cell carcinoma (RCC) [ASCO 2009, Abstract #5032].

Results: Twenty-five tumors from the archive were treated with tivozanib, revealing both responding and non-responding tumors (40% responders, 60% resistant). Bioinformatics analysis of RNA microarray expression profiles of pretreatment tumors identified a set of 200 genes that were significantly associated with resistance. A novel coherence-based bioinformatics approach incorporating multiple human tumor datasets led to a 42-gene resistance signature representing components of hematopoietic gene expression. Immunohistochemistry (IHC) quantitation of myeloid markers in the tumors identified the presence of infiltrating myeloid cells, whose percentage composition in the tumor correlated with both the 42-gene signature and resistance to tivozanib. Examination of both the signature and myeloid infiltration in human tumor microarray datasets indicated that this resistant phenotype is present in a significant subset of all 7 human tumor types examined, including human kidney cancer. The preclinically derived IHC marker was then used to retrospectively examine the relationship between myeloid infiltration and maximum tumor shrinkage achieved in available patient samples from a phase 2 study of tivozanib in RCC [ASCO 2009, Abstract #5032]. IHC analysis of infiltrating myeloid cells in 21 patient samples demonstrated a significant correlation between the percent myeloid cell composition in the tumors and maximum tumor shrinkage by RECIST criteria.

Conclusions: These observations suggest tivozanib-sensitive and -insensitive angiogenesis mechanisms in both murine and human solid tumors, provide a candidate response biomarker for tivozanib, and demonstrate the utility of this population-based preclinical model in predicting response in humans.

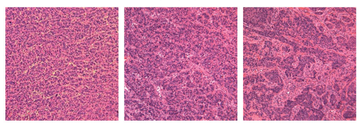
Chimeric inducible breast and lung tumor models



Diversity in the histopathological features of breast tumors

- Histological phenotypes are heritable through at least 3 serial propagations

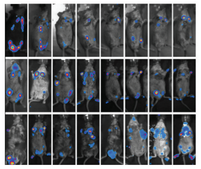
Histological subtypes of Her2+ breast adenocarcinomas after propagation

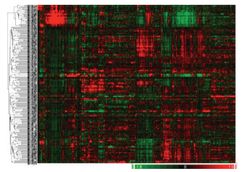


Solid sheet–like tumor morphology (well differentiated tumor cells)	Tumors with some stromal involvement (well differentiated tumor cells)	Tumors with stromal involvement showing nested pattern (poorly differentiated cells)
45% of tumors examined	40% of tumors examined	15% of tumors examined

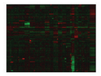
Inter-tumor variation of breast tumor archives by microarray and aCGH

- A total of 107 Her2-driven breast tumors were collected and propagated from the primary chimeric animals
- Heterogeneity in gene expression profile observed
  - All 107 tumors profiled at baseline (in duplicate)

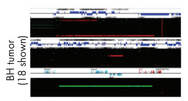




Variation in genomic profiles (aCGH)

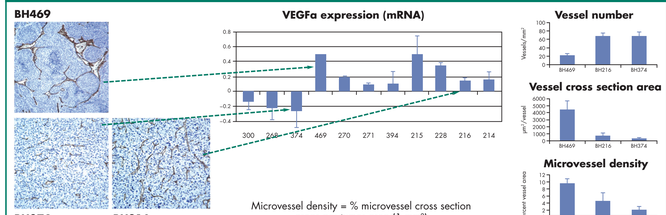


Different BH tumors exhibit distinct focal gains and losses in many human relevant genes: CDK6, Myc, PTEN



Chromosome maps showing extent and degree of amplicons (red) and deletions (green)

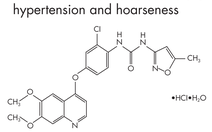
Diversity of breast archive in VEGFa expression and tumor microvasculature



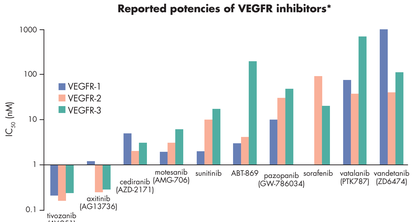
Microvessel density = % microvessel cross section areas per tumor area (1 mm²)

Tivozanib preclinical and clinical summary

- Extremely potent (~200 pM) against all 3 VEGFRs (1,2,3)
- Highly selective
- 4.5-day $t_{1/2}$ in human studies
- Robust efficacy in 272-patient phase 2 RCC trial
  - ORR: 27%-40% (27% in all RCC, independent radiology review; 40% in clear cell RCC/nephrectomy, investigator review)
  - PFS: 14.8 months in patients with clear cell RCC who had undergone nephrectomy (n = 176)
- Safety profile consistent with on-mechanism inhibition
- Most common adverse events are hypertension and hoarseness

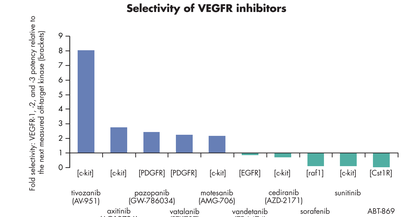


Reported potencies of VEGFR inhibitors*



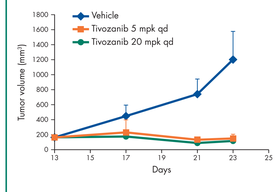
*Either biochemical or cell based IC_{50}; cell-based data shown when available.

Selectivity of VEGFR inhibitors

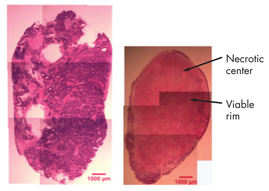


Tivozanib mechanism of action in preclinical tumors

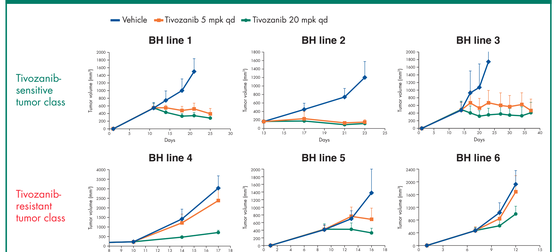
Complete tumor growth inhibition



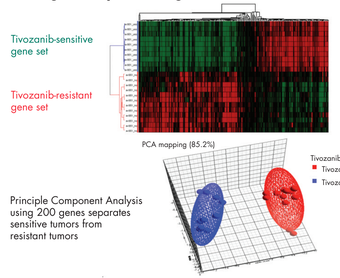
Typical histologic change for blockade of angiogenesis



Tivozanib efficacy studies across the tumor population enabled the development of a signature



200 most significantly different genes between sensitive and resistant tumors



Supervised clustering using 200 genes identified by T test

Principle Component Analysis using 200 genes separates sensitive tumors from resistant tumors

Summary

- **We have generated a population-based, engineered mouse tumor model of Her2-driven breast cancer**
- **Significant inter-tumor variation in histology, gene expression, and/or DNA copy number as well as angiogenesis was observed across the tumor population**
- **Twenty-five tumor lines were tested for sensitivity to the VEGFR tyrosine kinase inhibitor tivozanib to understand the mechanism of resistance and to explore predictive biomarkers**
- **A tivozanib biomarker, comprising infiltrating myeloid cells, was discovered. Retrospective analysis of 21 tumor samples from a phase 2 clinical trial in RCC showed significant correlation between the biomarker and response**

Acknowledgements

This study was supported by AVEO Pharmaceuticals, Inc., Cambridge, MA.
Editorial assistance was provided by Kimberly Brooks, PhD, of MedErgy.

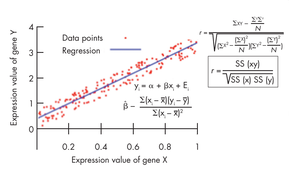
Correlation matrix identifies a set of coherently expressed resistance genes

- A key measure of the robustness of a signature is whether it retains consistent behavior across platforms
- Correlation heat maps were created to assess correlation among genes in any dataset

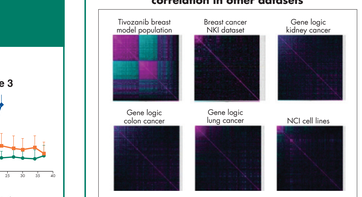
Correlation heat map approach

1. Input a gene list (signature, pathway gene set, biologically related genes, etc)
2. Use linear regression to calculate correlation of the expression values of each gene in the list within a given dataset (eg, Pearson, Spearman)
3. Plot the correlation values as a matrix (correlation heat map) to quantify the degree of correlation of each gene to each other (coherence). Each dataset has its own correlation matrix
4. High-quality gene lists exhibit coherence across multiple datasets

Expression correlations among a list of genes represented as a two-dimensional matrix

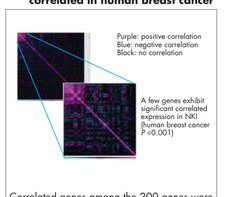


200-gene signature does not retain high correlation in other datasets



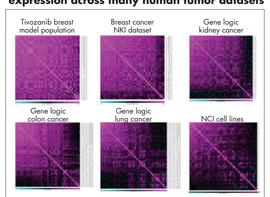
Standard statistical approaches to gene signatures typically perform poorly across tumor types

A subset of the signature genes are correlated in human breast cancer



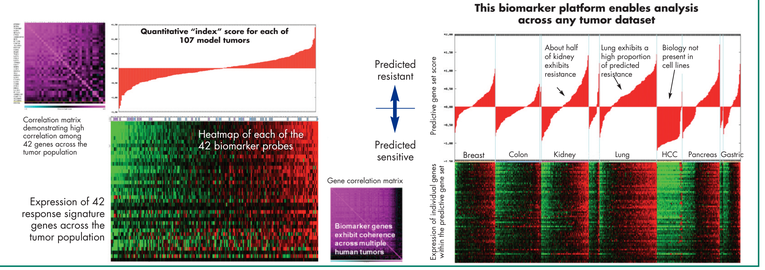
Correlated genes among the 200 genes were used as a seeding set to identify a 33-gene set of coherently expressed genes

42-gene biomarker retains correlated expression across many human tumor datasets



Standard statistical approaches to gene signatures typically perform poorly across tumor types

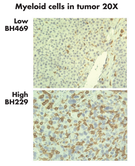
42-gene predictive gene set predicts tivozanib response in mouse tumors and is coherent in human tumors



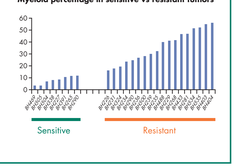
Myeloid cell density highly correlates with tivozanib resistance in the tumor population

- Measuring the degree of myeloid cell infiltration into the tumor revealed a correlation with tivozanib resistance
- Percent myeloid infiltration exhibits good correlation to multi-gene biomarker score (Pearson R = 0.62, *P* = 0.0004)

Myeloid cells in tumor 20X

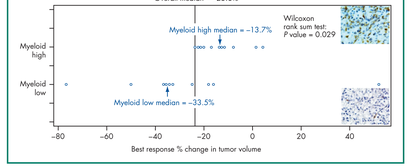


Myeloid percentage in sensitive vs resistant tumors



Retrospective analysis from tivozanib phase 2 trial: median tumor volume stratified by CD68 biomarker low vs high

- Of the 272 patients in the phase 2 trial, only 21 samples had data for tumor volume assessment, were not randomized to placebo, and had available formalin fixed paraffin embedded (FFPE) material of sufficient quality for analysis



POSTER PRESENTED AT THE EORTC-NCI-AACR INTERNATIONAL SYMPOSIUM ON MOLECULAR TARGETS AND CANCER THERAPEUTICS, 16-19 NOVEMBER 2010, BERLIN, GERMANY.

Tivozanib biomarker identifies tumor-infiltrating myeloid cells contributing to tivozanib resistance in both preclinical models and human renal cell carcinoma

Jie Lin, Xiaojian Sun, Bin Feng, Feng Jiang, Richard Nicoletti, Guangmu Li, M. Isabel Chiu, Brooke Esteves, Pankaj Bhargava, Murray O. Robinson

AVEO Pharmaceuticals, Inc., Cambridge, MA.

POSTER PRESENTED AT THE EORTC-NCI-AACR INTERNATIONAL SYMPOSIUM ON MOLECULAR TARGETS AND CANCER THERAPEUTICS, 16-19 NOVEMBER 2010, BERLIN, GERMANY.