# Relationship of hypoxia signature with variant subgroup of clear cell renal cell carcinoma (ccRCC) and its association with clinical activity on tivozanib hydrochloride

Murray Robinson,[1] Bin Feng,[1] Richard Nicoletti,[1] Anna Berkenblit,[1] Andrew Strahs,[1] Brooke Esteves,[1] Timothy Eisen,[2] Robert Motzer[3]

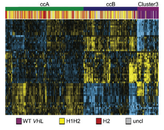
[1]AVEO Pharmaceuticals, Inc., Cambridge, MA, USA; [2]Cambridge University Health Partners, Cambridge, England; [3]Memorial Sloan-Kettering Cancer Center, New York, NY, USA

**Abstract/Poster No: 361**

## Introduction and Objective

- TIVO-1 (NCT01030783), a randomized Phase III trial of first-line targeted therapy in patients with clear cell renal cell carcinoma (ccRCC), demonstrated significant improvement in progression-free survival (PFS) in patients receiving tivozanib vs sorafenib [11.9 vs 9.1 months, *P*=0.042; 12.7 vs 9.1 months in treatment-naive, *P*=0.037][1]
- Recent work identified intrinsic molecular subtypes within ccRCC **(Figure 1)**[2]
- To further characterize the molecular ccRCC subtypes and assess relationships between subtypes and vascular endothelial growth factor (VEGF) tyrosine kinase inhibitor activity, we characterized available molecularly annotated data sets from TIVO-1
- Characterizing signatures associated with molecular subtypes can provide testable hypotheses to identify subtypes that are more likely to respond to tivozanib

**Figure 1. Using consensus clustering, ccRCC has been previously classified into three intrinsic subtypes: ccA, ccB and "Cluster 3".**




**Meta-analysis of Clear Cell Renal Cell Carcinoma Gene Expression Defines a Variant Subgroup and Identifies Gender Influences on Tumor Biology[2]**

*Brannon AR, et al. European Urology October 2012;61:258–268.*

## Methods

- The TIVO-1 Biomarker Statistical Analysis Plan (bSAP) pre-specified, as the primary analysis to investigate association between efficacy and hypoxia, a test for the hypoxia-subset factor (positive, negative) in a Cox regression model in which PFS depends on treatment arm and hypoxia subset
- The hypoxia index was computed as a function of the expression of nine genes from the hypoxia transcript cluster as assessed by quantitative PCR

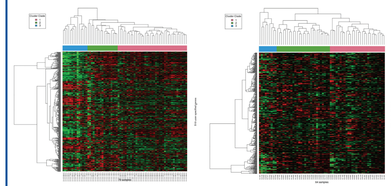
## Results

- Using large cancer microarray data sets from multiple tumor types, bioinformatics visualization tools and applied statistics, we have identified 51 non-overlapping functionally relevant groups of genes whose intragroup transcript level is coordinately regulated (ie, strongly correlated, or "coherent," across various microarray data sets)
  - We have designated these groups of genes Transcription Clusters 1-51 (TC1-TC51)
- For the primary analysis:
  - Hypoxia index (positive, negative) was associated with PFS in TIVO-1, hazard ratio (HR)=0.380 (95% CI: 0.155–0.935) and *P*=0.035 (low HR means better PFS for hypoxia positive disease)
  - In this model, treatment arm was also significant HR=0.457 (95% CI: 0.241—0.869) and *P*=0.017 (low HR means better PFS for tivozanib)
- For the subgroup analyses:
  - Continuous variable:
    - The hypoxia index as continuous variable was associated with PFS in the tivozanib arm, HR=0.510 (95% CI: 0.275–0.946) and *P*=0.033; n=33
    - The hypoxia index as continuous variable was not significantly associated with PFS in the sorafenib arm, HR=0.792 (95% CI: 0.519–1.207) and *P*=0.278; n=36
  - Dichotomous variable with pre-specified cutoff:
    - The hypoxia index (positive, negative) was associated with PFS in the tivozanib arm, HR=0.285 (95% CI: 0.078–1.043) and *P*=0.043; n=33 **(Figure 6C)**
    - The hypoxia index (positive, negative) was not significantly associated with PFS in the sorafenib arm, HR=0.503 (95% CI: 0.144–1.760) and *P*=0.273; n=36 **(Figure 6D)**

**Figure 2. Hierarchical clustering of two independent microarray data sets of ccRCC identify three intrinsic subtypes.**

Data set 1:
70 Agilent clear cell from TCGA

Data set 2:
64 clear cell from GeneLogic



These clustering results were consistent with those previously observed: "Cluster 3" in Brannon *et al*[2] corresponds to Cluster 3 (blue) in these plots.

TCGA (Agilent) and commercially acquired (Affymetrix) RCC microarray expression data sets were grouped by hierarchical clustering using 510 genes. The top three clusters were used to annotate the samples in each data set.

**Table 1. Top TC Gene Sets Exhibiting Differential Expression in Cluster 3 by GSEA Analysis**

| | Name | Size | Nom *P*-val | FDR q-val | FWER *P*-val |
|---|---|---|---|---|---|
| TCGA Kidney RCC (predominantly clear cell) | | | | | |
| DOWN in Cluster 3 | TC39.Hypoxia_ responsive | 52 | 0.000 | 0.037 | 0.026 |
| | TC46.Endothelium_and_com-plement | 59 | 0.011 | 0.194 | 0.290 |
| | TC40.Endothelial_specific | 86 | 0.019 | 0.276 | 0.275 |
| | TC41.Extracellular_ matrix_cell_contact | 54 | 0.026 | 0.146 | 0.400 |
| | TC49.Hematopoietic _ dendritic_cell | 90 | 0.027 | 0.133 | 0.330 |
| | TC47.Hematopoietic_CD8_Tcell_ enriched | 89 | 0.030 | 0.159 | 0.317 |
| | TC45.Extracellular_ matrix_and_cell_ communication | 78 | 0.041 | 0.125 | 0.436 |
| UP in Cluster 3 | TC50.Myeloid | 119 | 0.052 | 0.139 | 0.426 |
| | TC32.Metabolism | 132 | 0.000 | 0.028 | 0.041 |
| | TC20 | 54 | 0.002 | 0.026 | 0.069 |
| | TC36 | 61 | 0.031 | 0.128 | 0.368 |
| GeneLogic Kidney (clear cell) | | | | | |
| DOWN in Cluster 3 | TC39.Hypoxia_ responsive | 57 | 0.000 | 0.211 | 0.487 |
| | TC46.Endothelium_and_com-plement | 66 | 0.004 | 0.246 | 0.318 |
| | TC40.Endothelial_specific | 89 | 0.004 | 0.137 | 0.567 |
| | TC45.Extracellular_ matrix_and_cell_ communication | 79 | 0.006 | 0.152 | 0.551 |
| | TC41.Extracellular_matrix_cell_ contact | 58 | 0.008 | 0.180 | 0.338 |
| | TC49.Hematopoietic _ dendrit-ic_cell | 98 | 0.024 | 0.188 | 0.517 |
| | TC44.Extracellular_matrix | 81 | 0.042 | 0.165 | 0.528 |
| UP in Cluster 3 | TC50.Myeloid | 133 | 0.037 | 0.160 | 0.639 |
| | TC32.METABOLISM | 140 | 0.012 | 0.088 | 0.070 |

Representative sets of genes (8–10) for each transcript cluster (TC) associated with the indicated biological annotation were plotted as an expression heat map. "Cluster 3" is referred to as "hypoxia low" and shows the differential gene expression of each of the representative genes in the "hypoxia low" cluster versus the nccA or nccB clusters. Clusters with low expression in the hypoxia low intrinsic subtype: hypoxia, endothelial content, extracellular matrix, hematopoietic dendritic cell; clusters with high expression: metabolism.

In each of the two indicated clear cell RCC data sets, Gene Set Enrichment Analysis (GSEA) was used to identify which gene lists representing 51 pathways exhibited differential expression (UP or DOWN). Significantly enriched pathways are displayed, using a cutoff of False Discovery Rate <0.25.

Size = number of genes in gene list.

Nom *P*-val = nominal *P*-value of enrichment score.

FDR q-val = false discovery rate.

FWER *P*-val = familywise error rate.

**Figure 3. 510 genes from 51 signatures capture most expression variation in cancer.**

The non-overlapping transcription clusters can be represented by 51 sets of 10 genes, where each individual set exhibited correlated expression and defined the expression "index" of the group.

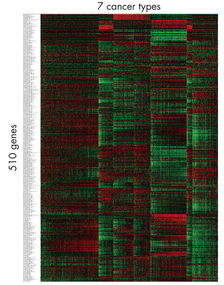



**Table 2. Baseline Characteristics: Overall TIVO-1 Population vs Biomarker Analysis Subset (Bx)**

| Characteristic | Tivozanib | Sorafenib | Bx Tivozanib | Bx Sorafenib |
|---|---|---|---|---|
| No. of patients | 260 | 257 | 33 | 36 |
| Median age, years (range) | 59 (23–83) | 59 (23–85) | 60 (25–74) | 56 (34–73) |
| Gender, male (%) | 71 | 74 | 79 | 81 |
| ECOG score, % | | | | |
| 0 | 45 | 54 | 27 | 31 |
| 1 | 55 | 46 | 73 | 69 |
| Number of organs involved,[a] % | | | | |
| 1 | 29 | 34 | 36 | 33 |
| ≥2 | 71 | 66 | 64 | 67 |
| Revised MSKCC | | | | |
| Good | 27 | 34 | 15 | 22 |
| Intermediate | 67 | 62 | 82 | 75 |
| Poor | 7 | 4 | 3 | 3 |
| Median time since initial diagnosis, months (range) | 14.7 (0.5–169) | 16.6 (1.0–264) | 7.1 (0.5–75) | 11.3 (1.4–209) |

[a]Based on retrospective independent radiological review.

**Figure 4. Expression heat maps of pathways identified by GSEA.**

Hypoxia

Metabolism

Endothelial Content

Extracellular Matrix

Hematopoietic Dendritic Cell

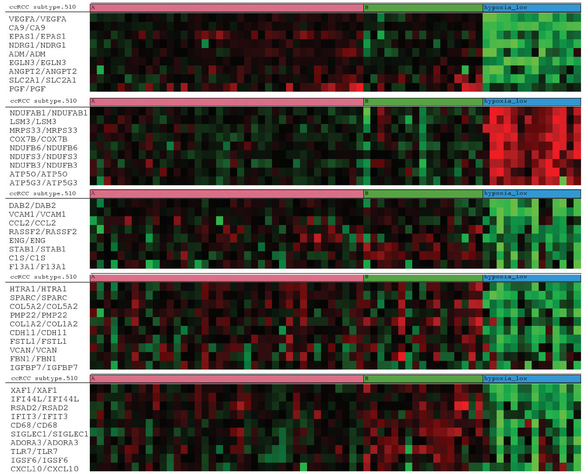
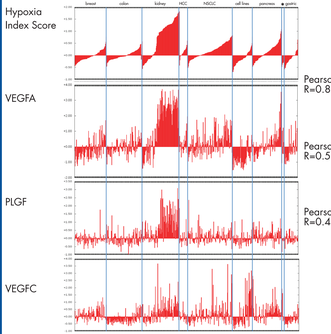



**Figure 5A. Hypoxia dysregulation represents a tumor state in which key VEGF pathway ligands, VEGFA, PLGF and VEGFC are activated.**

Nine representative genes from the "hypoxia" transcript cluster were summed using the following algorithm and plotted as an "index score"

$$PI = \frac{1}{m} * \sum_{i=1}^{m} Ei$$



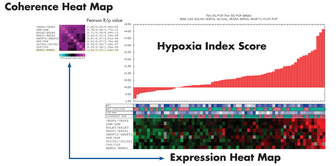

*Corresponds to prostate samples.

**Figure 5B. Nine gene hypoxia signature converted to FFPE RTPCR assay and implemented in TIVO-1 clinical trial.**

Index score calculated as sum of RT-PCR expression levels of each gene:

$$PI = \frac{1}{m} * \sum_{i=1}^{m} Ei$$

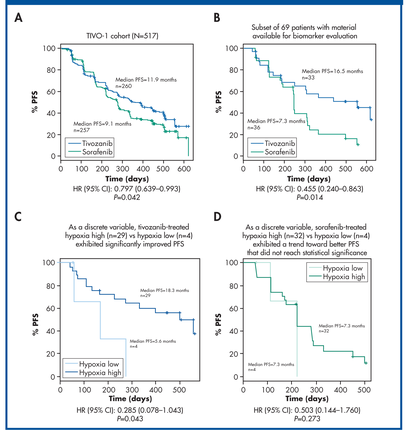
**Coherence Heat Map**




Hypoxia signature genes exhibited a minimum intergene Pearson correlation value of 0.44 ($P \leq 1.4 \times 10^{-4}$) in Formalin Fixed Paraffin Embedded (FFPE) RCC samples from available material collected from the TIVO-1 study. Coherent expression of the nine genes among the clinical samples is shown in the heat map (green: low expression; red: high expression). One to three 5uM sections of available FFPE material from each patient was processed to extract RNA, and Reverse Transcription PCR (RT-PCR) was conducted (Asuragen, Inc.) to establish the Ct expression value for each gene from each sample.

A five gene set of normalizer genes was used to control for variation in quality and quantity of RNA.

**Figure 6. Kaplan-Meier PFS estimates for overall TIVO-1 cohort compared to biomarker analysis subset (n=69).**




## Discussion and Conclusions

- Hierarchical clustering of microarray expression data from ccRCC samples demonstrated the presence of at least three intrinsic subtypes of ccRCC, consistent with previously published results
- Using a novel set of 51 TCs, representing 51 groups of correlated (coherently expressed) genes that exhibit coherence across multiple tumor types, Cluster 3 exhibited lower expression of genes associated with hypoxia, endothelial content, extracellular matrix and myeloid hematopoietic infiltration, but showed higher expression of genes associated with metabolism
- A nine gene signature representing hypoxia deregulation was developed as an RT-PCR based assay of FFPE material, and prospectively tested in available material from the Phase 3 TIVO-1 study (tivozanib vs sorafenib in ccRCC)
  - Given the small number of patients with available material, particularly for the low hypoxia subgroup, these data should be interpreted with caution
- Tivozanib patients exhibiting a high hypoxia biomarker signature exhibited statistically significantly improved PFS outcome, whereas sorafenib patients exhibited a trend toward better outcome that did not reach statistical significance
- These observations support a hypoxia biomarker hypothesis to be prospectively tested in an upcoming study of tivozanib in RCC
- This biomarker signature was seen in subsets of multiple tumor types including breast cancer, providing a testable hypothesis for tivozanib clinical activity outside of RCC

**References**

1. Motzer RJ, Eisen T, Bondarenko IN *et al.* Tivozanib versus sorafenib as initial targeted therapy for patients with advanced renal cell carcinoma: results from a phase III randomized, open label, multicenter trial. *J Clin Oncol* 2012;30(suppl):Abstract 4501.
2. Brannon AR, Haake SM, Hacker KE *et al.* Meta-analysis of clear cell carcinoma gene expression defines a variant subgroup and identifies gender influences on tumor biology. *Eur Urol* 2012;61:258–268.

**Acknowledgements**

This study was sponsored by AVEO Oncology and Astellas. AVEO Oncology and Astellas are parties to a collaboration agreement for the co-development of tivozanib. Editorial assistance was provided by Raffy M. Dakessian, PhD, Scientific Connexions, and was funded by AVEO Oncology and Astellas.

**POSTER PRESENTED AT THE AMERICAN SOCIETY OF CLINICAL ONCOLOGY GENITOURINARY CANCERS SYMPOSIUM; FEBRUARY 14-16, 2013; ORLANDO, FL**

*Corresponding author: Murray Robinson, mrobinson@aveooncology.com. Poster will be available on-line at http://investor.aveooncology.com*