**Abstract**

**Background:** To identify biomarkers associated with tivozanib response, a population-based, genetically engineered breast tumor model comprising 127 tumors was developed. The tumors tested included 59 Her2+ breast cancers, 29 triple negative breast tumors, and 49 normal breast tissues, using the VEGFR-1, -2, and -3 kinase inhibitors that have shown clinical activity in renal cell carcinoma (RCR) (AEO-2007, Abstract #5032).

**Results:** Twenty-five tumors from the archive were treated with tivozanib, revealing both responding and nonresponding tumors (40% responders, 60% resistant). Gene expression analysis of RNA from tumoral lesions revealed significant differences between the tumor populations. To identify genes contributing to resistance, we performed correlation analysis using RNA from 127 breast tumors identified as a set of 200 genes that were significantly associated with clinical efficacy using a VEGFR inhibitor (correlation heat maps were created to assess correlation among genes in any dataset). An extremely potent (~200 pM) 200-gene signature does not retain high correlation in other datasets (correlation heat maps were created to assess correlation among genes in any dataset). The 42-gene signature retains correlated expression in multiple human tumor datasets. The 42-gene signature signature comprising infiltrating myeloid cells as a primary chemo-sensitizing element.

**Tivozanib mechanism of action in preclinical tumors**

- Complete tumor growth inhibition
- Typical histology change for blockade of angiogenesis

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**Summary**

- We have generated a population-based, engineered mouse tumor model of Her2+ primary breast tumors to study mechanism of resistance to tivozanib
- Significant inter-tumor variations in biology, gene expression, and DNA copy number as well as resistance to tivozanib in individual preclinical breast tumors
- Twenty-five tumor lines were treated for sensitivity to the VEGFR kinase inhibitor tivozanib to determine the mechanisms of resistance and the predictive biomarkers
- A resistant biomarker, comprising infiltrating myeloid cells, was discovered
- Retrospective analysis of 31 tumor samples from a phase 2 clinical trial in RCC showed significant correlation between biomarker and resistance

**Acknowledgements**

The authors thank AVEO Pharmaceuticals, Inc., for support of this work.

**Background:** Her2+ breast cancers exhibit frequent amplification of the Her2 gene, leading to overexpression of Her2 protein and constitutive activation of the Her2 signaling pathway. This frequently results in resistance to anti-HER2 therapy. Therefore, a need exists for additional strategies to target Her2 signaling in resistant breast cancers.

**Materials and methods:** Tumor xenografts were generated from 25 tumor samples from the archive. These tumors were treated with tivozanib, a potent and selective VEGFR kinase inhibitor that has shown clinical activity in renal cell carcinoma (RCR) using the VEGFR-1, -2, and -3 kinase inhibitors that have shown clinical activity in renal cell carcinoma (RCR) (AEO-2007, Abstract #5032).

**Results:** Twenty-five tumors from the archive were treated with tivozanib, revealing both responding and nonresponding tumors (40% responders, 60% resistant). Gene expression analysis of RNA from tumoral lesions revealed significant differences between the tumor populations. To identify genes contributing to resistance, we performed correlation analysis using RNA from 127 breast tumors identified as a set of 200 genes that were significantly associated with clinical efficacy using a VEGFR inhibitor (correlation heat maps were created to assess correlation among genes in any dataset). An extremely potent (~200 pM) 200-gene signature does not retain high correlation in other datasets (correlation heat maps were created to assess correlation among genes in any dataset). The 42-gene signature retains correlated expression in multiple human tumor datasets. The 42-gene signature signature comprising infiltrating myeloid cells as a primary chemo-sensitizing element.

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

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