Tivozanib Activity in Combination With Capecitabine, 5-fluorouracil (5-FU) or Docetaxel, in Traditional or Engineered Subcutaneous Breast Tumor Models

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Abstract

Background: Taxanes and fluoropyrimidines are 2 major classes of chemotherapy drugs used to treat advanced breast cancer. Recent clinical data indicate that vascular endothelial growth factor (VEGF) signaling pathway inhibitors exhibit promising activity in combination with the 5-fluorouracil (5-FU) prodrug capecitabine. The antitumor activity of tivozanib, a potent and selective kinase inhibitor of VEGF receptors-1, -2, and -3, was evaluated alone and in combination with capecitabine in a human epidermal growth factor receptor 2 (Her2)-engineered murine tumor that exhibits tivozanib resistance. In addition, tivozanib was evaluated alone and in combination with representative taxane and fluoropyrimidine chemotherapy drugs in a xenograft model of breast cancer.

Materials and methods: Subcutaneous BH216 Her2-engineered murine or MX-1 human breast tumors were grown in female nude mice. Mice bearing Her2-engineered tumors were treated with oral tivozanib (5 mg/kg/day), capecitabine (300 mg/kg/day using a 14 days on, 7 days off schedule), or both drugs in combination. Mice bearing MX-1 tumors were treated with oral tivozanib (20 mg/kg/day), 5-FU (100 mg/kg weekly for 3 weeks) or docetaxel (10 mg/kg weekly for 3 weeks), or a combination of tivozanib and chemotherapy. Tumor growth inhibition (TGI) was calculated using standard criteria.

Results: In the Her2-engineered breast tumor model, treatment with tivozanib alone or capecitabine alone resulted in continued tumor growth with only modest TGI. However, the combination of tivozanib plus capecitabine led to complete growth inhibition. In the MX-1 xenograft model, tivozanib monotherapy exhibited robust TGI, and modest improvements in TGI were observed when tivozanib was combined with 5-FU or docetaxel relative to tivozanib monotherapy. Following discontinuation of tivozanib alone or in combination with 5-FU, tumor regrowth was observed, whereas mice treated with tivozanib plus docetaxel were tumor-free by Day 34, with no tumor recurrence at Day 63.

Conclusions: When examined in a tivozanib-resistant murine breast tumor model, tivozanib combined with capecitabine appeared to reverse tivozanib resistance. Tivozanib treatment also potently inhibited MX-1 breast tumor growth and enhanced the anti-cancer effects of 5-FU and docetaxel.

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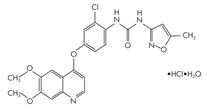
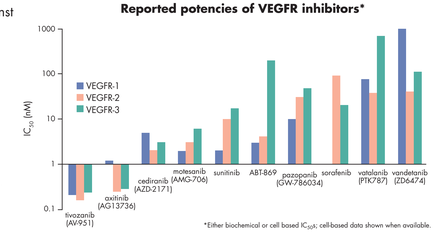
Combination treatment with tivozanib and capecitabine

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

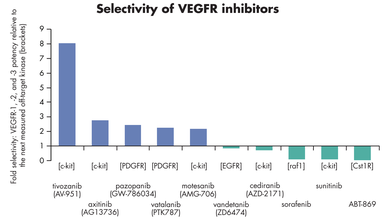


Reported potencies of VEGFR inhibitors*



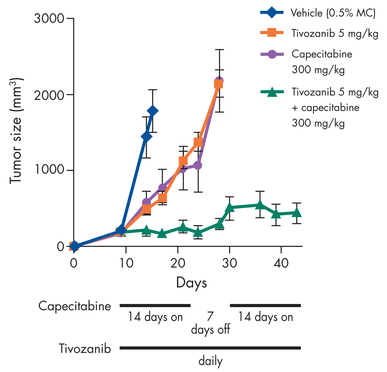
*Either biochemical or cell based IC_{50}; cell-based data shown when available.

Selectivity of VEGFR inhibitors



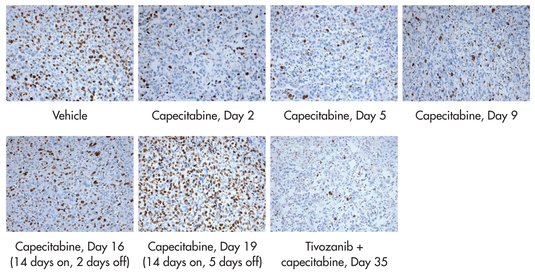
Antitumor activity of tivozanib + capecitabine in Her2-engineered murine breast tumors

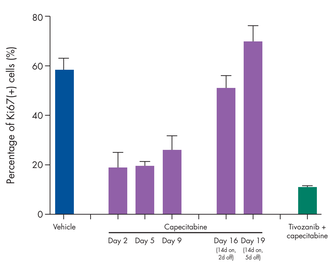
- Her2-engineered murine breast tumors were grown in female nude mice
- Tumor-bearing mice were treated with capecitabine 300 mg/kg with or without tivozanib 5 mg/kg once daily
- Both tivozanib alone and capecitabine alone moderately slowed tumor growth, but the combination induced tumor stasis
- The combination of tivozanib and capecitabine is required to achieve complete growth inhibition



Inhibition of tumor cell proliferation following treatment with capecitabine versus capecitabine + tivozanib

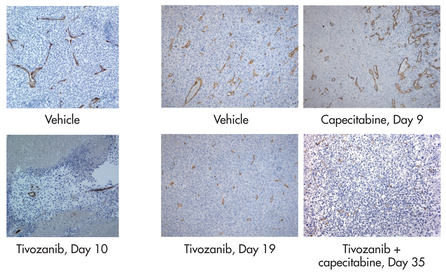
- Tumor cell proliferation (Ki67 IHC)
- Although capecitabine alone inhibited proliferation, the greatest inhibition was observed with tivozanib + capecitabine
- Capecitabine dramatically reduced tumor cell proliferation
- Between capecitabine cycles, tumor cell proliferation rebound was observed
- The combination of capecitabine and tivozanib achieved a near complete blockade of tumor cell proliferation





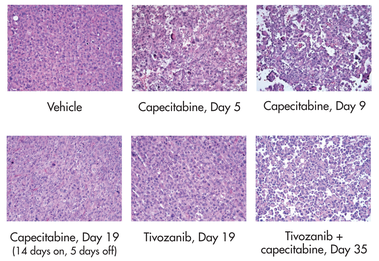
Tumor microvasculature

- Tumor sections from tumor-bearing mice were evaluated for microvessel density (CD31 IHC)
- Change of tumor vasculature in a breast Her2 tumor that was sensitive to tivozanib
- Tivozanib, but not capecitabine, inhibited tumor angiogenesis



Apoptotic/necrotic tumor cells

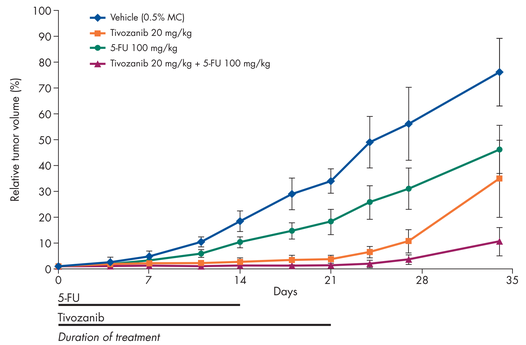
- Hematoxylin and eosin (H&E) staining was used to identify apoptotic and/or necrotic cells present in tumors from treated mice
- Capecitabine was found to induce rapid cell death in tumors



Combination treatment with tivozanib and 5-FU

Antitumor activity of tivozanib + 5-FU in MX-1 human breast tumors

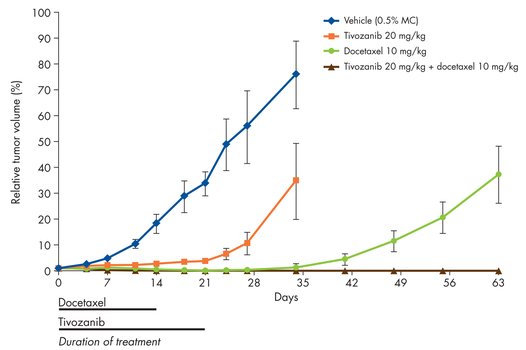
- MX-1 human breast tumors were grown subcutaneously in female nude mice
- Tumor-bearing mice were treated with tivozanib 20 mg/kg daily, 5-FU 10 mg/kg once weekly, or the combination of tivozanib + 5-FU
- Monotherapy with tivozanib or 5-FU resulted in modest tumor growth
- Further improvement in tumor growth inhibition was observed with the combination of tivozanib + 5-FU compared with either given as monotherapy



Combination treatment with tivozanib and docetaxel

Antitumor activity of tivozanib + docetaxel in MX-1 human breast tumors

- MX-1 human breast tumors were grown subcutaneously in female nude mice
- Tumor-bearing mice were treated with tivozanib 20 mg/kg daily, docetaxel 10 mg/kg once weekly, or the combination of tivozanib + docetaxel
- Tumor inhibition was observed with tivozanib or docetaxel monotherapy. However, treated tumors resumed progression after termination of the treatments
- Combination treatment resulted in robust antitumor activity, with all mice tumor free by Day 34 and no tumor recurrence through Day 63



Summary

- **Tumor models that exhibit resistance to tivozanib and chemotherapies were selected for this study**
- **In an engineered murine breast tumor model, combination of tivozanib and capecitabine resulted in complete tumor inhibition by compensatory mechanisms (ie, inhibition of angiogenesis and cell proliferation, respectively)**
- **Tivozanib greatly enhanced antitumor effects of 5-FU and docetaxel in the human breast tumor model MX-1**

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