treatment. Therefore, DCE-MRI would be useful as a pharmacodynamic biomarker to predict tumor response to TAK-593.

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VEGFR2 targeted antibody and small molecule combinations in renal cell and hepatocellular cancer models

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Sunitinib and sorafenib are small molecule tyrosine kinase inhibitors (TKIs) whose targets include VEGFR2. Both TKIs are approved for the treatment of renal cell cancer (RCC), and sorafenib is also approved for the treatment of hepatocellular cancer (HCC). We examined the benefits of an antibody targeted to VEGFR2, alone or in combination with the small molecule inhibitors, in RCC and HCC models.
In the subcutaneous SKRC-29 RCC model established in nu/nu mice, a rat antibody to mouse VEGFR2, DC101 (40 mg/kg, IP, Mon-Wed-Fri), showed comparable effects to sunitinib (40 mg/kg, PO, daily) on tumor growth (T/C% =28 and 36, respectively) ($p = 0.36$). Combination of these two agents resulted in significantly increased efficacy compared to monotherapy (T/C% = 16, $p < 0.02$), with an increase in partial regression frequency to 64%, compared to <10% in the monotherapy groups. Utilizing thin and thick section histological analysis, the loss of MECA-32 +ve or CD31 +ve blood vessels was similar in all treatment groups, although slightly greater in the combination group ($p < 0.05$). Interestingly, DC101 caused a greater loss of LYVE-1 +ve tumor lymphatic vessels and alpha-smooth muscle actin (alphaSMA) positive vessel like structures than sunitinib ($p < 0.05$). In fact DC101 caused a dramatic loss of both CD31 and alphaSMA in the most common vessel type, leaving only type IV collagen sleeves, while sunitinib decreased only the CD31 component of these triple stained vessels.
Similar to DC101 + sunitinib, the combination of DC101 and sorafenib (100 mg/kg, PO, daily) in a subcutaneous SK-Hep1 HCC model resulted in significantly increased efficacy compared to monotherapy (T/C% = 25 versus 43 and 51 respectively; $p < 0.003$), with 100% partial tumor regression frequency, compared to 18% in the DC101 monotherapy group. At the dose used, sorafenib monotherapy was associated with 100% mortality by Day 23 of treatment, while in the combination group no mortality was observed through Day 30 of treatment. Thin and thick section histological analysis in the SK-Hep1 model are pending.
The above results support the potential utility of combining antibody and TKIs targeting the VEGFR2 pathway for the treatment of RCC and HCC. In addition, results suggest that small molecule VEGFR2 inhibitors, at the doses utilized, may not completely block VEGFR2 function in cancer models, given the benefits observed following the addition of an antibody specifically targeting VEGFR2.

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Combination treatment of VEGFR inhibitor AV-951 and rapamycin reveals distinct mechanisms of each agent's anti-tumor activity

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The approval of an increasing number of molecularly targeted therapies in cancer creates an opportunity to contemplate combinations of targeted agents. This is particularly important because single agent activity of targeted therapies typically exhibit only modest activity in the clinic. A current challenge is to identify appropriate rational combinations that combine mechanisms to elicit maximal anti-tumor activity and to avoid antagonistic drug combinations.
AV-951 (formerly KRN951) is a small molecule VEGFR inhibitor currently in phase 2 clinical trials, as well as in phase 1B combination studies with the rapamycin analogue temsirolimus. AV-951 inhibits VEGFR 1, 2 and 3 activity at picomolar concentrations (IC50 of 0.21, 0.16 and 0.24 nM respectively), while it inhibits c-Kit and PDGFR at 10-times higher concentrations (IC50 of 1.63 and 1.72 nM respectively). Rapamycin targets mTOR, a protein involved in integrating nutrient availability with cellular functions, including proliferation. mTOR is also known to promote hypoxia inducible factor (HIF1) activity, which in turn drives angiogenesis, such that in some settings, rapamycin is thought to elicit anti-tumor activity through an anti-angiogenic mechanism. We utilized a genetically engineered breast HER2 driven adenocarcinoma model to explore the activity of AV-951 and rapamycin as single agents and in combination. Treatment of these Breast HER2 tumors with rapamycin resulted in complete tumor growth inhibition persisting for at least 6 weeks. Histological analysis of representative tumors after 5 or 42 days of treatment using the proliferation marker KI67 revealed significant reduction in tumor cell proliferation across the tumor mass. In contrast, no change was observed in tumor vessel density or morphology in rapamycin treated tumors. Treatment of tumors with single agent AV-951 also resulted in complete growth inhibition, however the tumor histological phenotype was dramatically different, with alteration in vessel morphology, significant central necrosis and KI67 positive proliferating tumor cells at the tumor margin. Treatment of these Breast HER2 tumors with both Rapamycin and AV-951 resulted in partial regression, and histological evidence of both mechanisms in effect, with central necrosis as well as significant reduction of KI67 positive cells at the tumor margin. At 42 days of treatment, the emergence of regions of KI67 positive tumor cells was observed in tumors treated with either rapamycin or AV-951 single agents, whereas no evidence of emerging drug resistant tumor regions was observed with the AV-951/rapamycin combination.
The distinct mechanisms of action of these two agents, along with the apparent suppression of tumor resistance suggests that AV-951/mTOR inhibitor may represent an attractive rational combination treatment for solid malignancies.

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Common usage of the GEP100-Arf6-AMAP1 pathway in tumor invasion, angiogenesis and vascular permeability

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Background: We have shown that Arf6 and its downstream effector AMAP1 are highly overexpressed in invasive breast cancer cells and constitute a robust signaling pathway for their invasion and metastasis. This Arf6 pathway in invasion is activated by a guanine nucleotide exchanger, GEP100. Arf6 expression is also known to be highly augmented in endothelial cells upon vascular endothelial growth factor (VEGF) stimulation, and its activity has been implicated in angiogenesis. Here, we show that the GEP100-Arf6-AMAP1 pathway is also involved in angiogenesis and vascular permeability.
Material and Methods: We examined the effects of siRNA treatment of GEP100, Arf6 and AMAP1 on VEGF-induced tubular formation in vitro and angiogenesis in vivo. Permeability across endothelial cell monolayers was measured by use of FITC-conjugated dextran. VE-cadherin internalization were assessed by use of VE-cadherin antibody.
Results: We first found that AMAP1 is expressed at high levels in HUVECs, and that Arf6 is activated in these cells upon VEGF simulation. Arf6 silencing blocked the VEGF-induced tubular formation in vitro. We also found that GEP100 is primarily responsible for Arf6 activation for VEGF-induced tubular formation in vitro and VEGF- and tumor cell-induced angiogenesis in vivo. Blocking AMAP1 function also inhibited tubular formation in vitro and angiogenesis in vivo. Moreover, we found that the GEP100-Arf6-AMAP1 pathway is important for normal recycling of VE-cadherin and regulation of endothelial permeability.
Conclusions: The GEP100-Arf6-AMAP1 pathway plays integral parts in VEGF-induced angiogenesis and endothelial permeability, the latter of which appears to be mediated at least partly through regulation of recycling of VE-cadherin by this pathway.

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Combination therapy of an anti-PDGFRβ antibody with an anti-VEGFR2 antibody leads to enhanced antitumor activity

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Platelet-derived growth factor β receptor (PDGFRβ) is up-regulated in most of solid tumors. It is expressed by pericytes/vascular smooth muscle cells, fibroblasts, macrophages and some tumor cells. It has been implicated that PDGFRβ signaling is not only essential in stabilization and maturation of tumor vessels, but also plays a regulatory role in the tumor microenvironment. Previously, we demonstrated that 2C5, an antibody that reacts with both human and mouse PDGFRβ, has only modest efficacy as monotherapy in a number of tumor xenograft models. In this study, we studied the antitumor activity of 2C5 in combination with DC101, an antibody directed against mouse vascular endothelial growth factor receptor 2, in several xenograft models. Combination of 2C5 and DC101 resulted in enhanced antitumor activity in BxPC-3 (pancreatic)