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515H7, a novel anti-chemokine receptor 4 (CXCR4) antibody. Part II: in vivo efficacy on CXCR4-dependent tumor models

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Background: Chemokines are small, secreted peptides that control the migration of leukocytes along a chemical gradient of ligand, especially during immune and inflammatory reactions. They are divided into two major subfamilies, CC and CXC, based on the position of their NH_2-terminal cysteine residues, and bind to G protein coupled receptors, whose two major sub families are designated CCR and CXCR. More than 50 human chemokines and 18 chemokine receptors have been discovered so far. CXCR4 receptor is over-expressed in a large number of cancers: colon, breast, prostate, lung, ovary, pancreas, ... The ligand of CXCR4 receptor, Stromal-cell-Derived Factor-1 (SDF-1) is secreted in lymph node, bone marrow, liver and lung. CXCR4/SDF-1 axis plays a key role in cancer and is directly implicated in migration, invasion leading to metastases, cell proliferation and angiogenesis. Moreover, CXCR4 overexpression correlated with poor prognosis in many types of cancer.
A novel monoclonal antibody (Mab 515H7) was raised against the human CXCR4. It displayed efficacious antagonist properties for all major pathways associated with SDF-1-induced CXCR4 signaling (see companion poster, *in vitro* studies). Its antitumor activity was investigated *in vivo* using several human tumor models.
Materials and Methods: S.c. xenograft models. Cells [MDA-MB-231 (breast) and KARPAS299 (T-cell NHL)] in exponential phase of growth were harvested, pelleted and resuspended in sterile PBS without Matrigel. Cells (between 5 and 10.10^6 in 100 µl) were implanted s.c. into the right flank region of each mouse (Nod/SCID) and allowed to grow to the designated size before administration of antibodies. Antibody treatments were injected twice a week. The mice were followed twice a week for the observation of xenograft growth. Tumor volume was calculated using the formula: p/6 X length X width X height.
U937 survival model. U937 cells (AML) in exponential phase of growth were pelleted and resuspended in sterile PBS. Cells (10.10^6 in 200 µl) were implanted I.P. in female Nod/SCID mice. Antibody treatments were injected twice a week and the mice followed for survival.
Results: We demonstrated that 515H7 Mab was able to significantly inhibit *in vivo* growth of xenograft tumors in mice. In addition to its effect on tumor growth, 515H7 Mab was also able to significantly improve mice survival in the lethal U937 model.
Conclusion: Taken together *in vivo* data suggest that 515H7 Mab targeting CXCR4 is a promising candidate for the treatment of tumors.

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High in vivo anti-tumor activity of the immunoconjugate BT-062 against CD138 positive solid tumors

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CD138 (Syndecan-1) is highly upregulated on a variety of solid tumors and hematological malignancies and used as an identification marker for multiple myeloma (MM). Therefore, CD138 represents a promising target for therapeutic intervention in several malignancies. BT-062 is an immunoconjugate comprising a chimerized anti-CD138 antibody conjugated to maytansinoid (DM4), an inhibitor of tubulin polymerization. We have previously reported that BT-062 exerts highly selective *in vitro* and *in vivo* cytotoxic activity against CD138 positive multiple myeloma (MM) cells (Ikeda et al., 2009). Based on these results, a phase I/II clinical trial has been conducted in relapsed/refractory multiple myeloma patients, which demonstrated overall good tolerability up to 160 mg/m^2 after repeated single dosing, as well as first signs of efficacy in this heavily pretreated patient population (Khan et al., ASH 2009). Here, we investigated the potential of BT-062 for the treatment of solid tumor indications. In accordance with published data, immunohistochemistry studies showed high CD138 expression in a variety of patient-derived tumor tissues of the pancreas, bladder, breast, head & neck, cervix and lung. Treatment of CD138 positive cell lines derived from these solid tumor indications exhibited selective cytotoxic activity down to subnanomolar IC_{50} values. In order to assess *in vivo* efficacy, primary tumors (pancreatic, mammary, lung or transitional cell bladder carcinoma) were xenografted in SCID mice and treated with weekly intravenous injections of BT-062; very high anti-tumor activity was observed in all of these primary xenograft models. Complete remission in all treated animals was observed in the pancreatic carcinoma model after 6 weeks treatment with 23.85 mg/kg or 13.25 mg/kg BT-062 (T/C = 0%) without tumor re-growth during the treatment-free observation period. Even when lower doses were tested for 5 weeks (4 mg/kg), BT-062 treatment in the mammary carcinoma bearing animals resulted in complete tumor eradication (T/C = 0%) without any tumor re-growth. As the tumor displayed a triple negative phenotype (ER^-, PR^-, $HER2^-$), BT-062 may offer a future treatment option for this poor-prognosis patient population. In conclusion, the high *in vitro* and *in vivo* cytotoxic activity towards CD138 expressing solid tumors would support clinical evaluation of BT-062 in these indications in addition to multiple myeloma.

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REGN421/SAR153192, a fully human anti-Dll4 monoclonal antibody, blocks tumor growth by reducing tumor perfusion

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Delta-like ligand 4 (Dll4) is an emerging new anticancer target given its predominant tumor vasculature expression and its role in regulating angiogenic sprouting. We have previously demonstrated that pharmacological blockade of the Dll4-Notch axis results in an excessive production of aberrant non-functional tumor vessels, and these changes were associated with reduced tumor growth. Using VelocImmune® mice, we identified a fully human IgG1 monoclonal antibody, termed REGN421/SAR153192, which binds human Dll4 and potently neutralizes Notch signaling with picomolar affinity. We further characterized this lead antibody for its therapeutic potential in recombinant immunodeficient mice engineered to express human Dll4. In mice bearing established HT1080 human sarcoma xenografts, twice weekly administration of REGN421 caused potent and dose-dependent inhibition of tumor growth. Maximal (⩾100%) tumor growth inhibition was observed at twice weekly doses of ⩾1 mg/kg which correlated with drug C_{max} values of approximately 10 mg/ml and C_{min} values of approximately 2 mg/ml. Tumor growth inhibition was associated with a pronounced increase of tumor endothelial cell density with enhanced sprouting and branching in highly vascularized HT1080 tumors at 7 days following REGN421 treatment. We further evaluated the acute effects of anti-Dll4 antibody treatment on tumor perfusion in HT1080 xenografts using contrast-enhanced micro-ultrasound imaging. Single-agent administration of Dll4 antibody (10 mg/kg) resulted in a 38% decrease in tumor perfusion as early as 24 hr post-treatment. Combination treatment with anti-Dll4 antibody plus the potent VEGF blocker, aflibercept (25 mg/kg), resulted in an even greater (64%) decrease in HT1080 tumor perfusion at 24 hr, along with enhanced anti-tumor activity. The combined effects of REGN421 treatment with standard chemotherapeutics were also evaluated in human colon tumor xenograft models. The combination of REGN421 with irinotecan or 5-FU resulted in augmented growth inhibition of HCT116 colon tumors compared to either single agent treatments alone. These and other studies lend further support for the therapeutic targeting of Dll4 as a promising new angiogenesis-based anticancer strategy. REGN421 is currently under investigation in a Phase 1 study in patients with advanced solid tumor malignancies.

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LA480, a bivalent humanized monoclonal c-Met antibody, inhibits tumor growth through an anti-proliferative mechanism

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The signaling pathway of c-Met and its ligand hepatocyte growth factor (HGF) has been linked to cancer progression and invasion. Inappropriate activation of c-Met can be induced by ligand-independent mechanisms such as gene amplification, specific genetic mutations, transcriptional up regulation, or by ligand-dependent autocrine or paracrine mechanisms. c-Met pathway activation leads to increased cell proliferation, motility, invasion, angiogenesis, and anti-apoptosis. Given the critical roles of the c-Met/HGF pathway in tumor growth and development, c-Met represents an attractive therapeutic target and is currently under intensive investigation. Past efforts to develop therapeutic anti-c-Met antibodies that inhibit both ligand-dependent and ligand-independent activation were largely unsuccessful because the antibodies tended to have agonistic rather than antagonistic properties. We reported that LA480, a humanized bivalent anti-c-Met antibody, inhibits HGF-dependent and HGF-independent c-Met pathway activation and tumor growth without stimulatory activities. LA480 treatment results in cell surface c-Met internalization and significantly