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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

reduces total c-Met in various tumor cell lines and inhibits proliferation of tumor cells that depend on high c-Met expression. In this poster, we further explore LA480 mechanism of action. Our data demonstrate that LA480 can block HGF-induced p-Met, p-Akt and p-Erk in multiple tumor cell lines. In ligand independent MKN45 cells (a model of cancer cells with high c-Met amplification), LA480 treatment *in vitro* results in G1 cell cycle arrest, but not apoptosis. In mouse xenograft studies, LA480 significantly inhibits MKN45 tumor growth by 34% at day 15 and 91% at day 35. Consistent with cell culture data, the percentage of mitotic proliferating cells is decreased in the treated tumors by 71%. Tumors treated with LA480 are less hypoxic and have a decreased percent total apoptotic area. These findings suggest that LA480 may be a promising therapy for treatment of cancers driven by ligand-dependent and ligand-independent c-Met activation.

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Anti-IGF1R therapy with dalotuzumab is efficacious in a sub-set of KRAS mutant cetuximab refractory CRC models

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The monoclonal antibody targeting EGFR, cetuximab is currently used in the treatment of advanced colorectal cancer. Recent studies indicate that cetuximab is ineffective in the treatment of patients with colorectal cancers harboring activating mutants in KRAS or BRAF, components of the RAS-MAPK pathway. Cross-talk between EGFR and insulin like growth factor receptor (IGF1R) has been reported. MK-0646 (dalotuzumab), a monoclonal antibody targeting IGF1R is currently being developed for the treatment of various cancers. Here we have investigated activity of MK-0646 in KRAS or BRAF mutant, cetuximab refractory pre-clinical colon cancer models. A subset of cetuximab-refractory CRC cell lines (3/12) were responsive to MK-0646 treatment. Addition of cetuximab did not further potentiate MK-0646 mediated growth inhibition in KRAS/BRAF mutant CRC models. Strikingly, MK-0646-mediated inhibition of IGF1R signaling enhanced the sensitivity to irinotecan. In xenograft models that expressed high levels of IGF1R, MK-0646 significantly enhanced irinotecan-mediated growth inhibition. The combination of MK-0646 with irinotecan produced lasting tumor growth inhibition that persisted even after treatment withdrawal, indicating a durable response to this combination. In contrast, in xenograft tumors with low levels of IGF1R expression, the combination of MK-0646 and irinotecan failed to enhance irinotecan-mediated growth inhibition. In this study a molecular rationale for the combination benefit with MK-0646 and irinotecan was established. Irinotecan treatment resulted in the activation of IGF1R and PI3K signaling pathways, representing as a possible tumor survival mechanism. Combined treatment with MK-0646 and irinotecan prevented the activation of these survival signals, leading to increased anti-tumor activity. These studies suggest that MK-0646 in combination with irinotecan may have utility in the treatment of KRAS or BRAF mutant colorectal cancer patients. This hypothesis is currently being tested in the clinic. Preliminary data, from early Phase 1 clinical studies have shown activity of MK-0646 based therapy in KRAS mutant patients.

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Essential role of fibroblast growth factor receptor 2 (FGFR2) in tumorigenesis of human cancers harboring FGFR2 amplification demonstrated by a functional blocking antibody

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Fibroblast growth factors (FGFs) play important roles in regulating many fundamental biological processes including embryogenesis, tissue homeostasis, metabolism, angiogenesis, and wound healing. Dysregulated FGF signaling has been implicated in the pathogenesis of human cancers. We generated monoclonal antibodies (mAbs) against the extracellular ligand binding domain of fibroblast growth factor receptor 2 (FGFR2) to address the role of FGFR2 in tumorigenesis and to explore the potential of FGFR2 as a novel therapeutic target. Human gastric and breast cancer cell lines harboring *FGFR2* amplification predominantly express the IIIb-isoform of FGFR2. Therefore, we used an FGFR2-IIIb specific antibody, GP369, to investigate the importance of FGFR2 signaling in such cell lines *in vitro* and *in vivo*. GP369 specifically and potently suppressed ligand-induced phosphorylation of FGFR2-IIIb and downstream signaling *in vitro*. The administration of GP369 in mice significantly inhibited the growth of *FGFR2*-amplified human cancer xenografts. Our findings strongly support an essential role of FGFR2 in the initiation and/or maintenance of human cancers harboring *FGFR2* amplification. Cancer patients with activated/amplified FGFR2 signaling could potentially benefit from therapeutic intervention with FGFR2-targeting antibodies.

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Efficacy of VEGFR2 targeted mAb therapy in preclinical cancer models resistant to antiangiogenic therapy

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Several antiangiogenic agents are approved for the treatment of cancer, including the anti-VEGF mAb bevacizumab (metastatic breast and colorectal cancers) and the multi-targeted kinase inhibitor sorafenib (advanced hepatocellular carcinoma). These therapies do not confer tumor control in some patients, and the majority of tumors develop resistance. Identification of alternative treatment options that enable disease control in the setting of resistance is essential for improved patient outcomes. A monoclonal antibody targeting VEGFR2, IMC-1121B, has been associated with preliminary efficacy in TKI-refractory renal cancer and is currently being evaluated in bevacizumab and sorafenib resistant colorectal and hepatocellular cancers, respectively. We evaluated an antibody specific to murine VEGFR2, DC101, as monotherapy or in combination with chemotherapy in preclinical models of cancer resistant to sorafenib or anti-VEGF therapies.

In HuH-7 hepatocellular carcinoma xenografts, tumors that had grown on average by 100% over 8 days of sorafenib therapy (30 mg/kg, PO, daily), grew 20% over 15 days of therapy with the anti-VEGFR2 mAb DC101 (40 mg/kg, IP, 3×/week; $p < 0.0001$ versus rat IgG).

To mimic bevacizumab activity and resistance in preclinical cancer models, we developed a human antibody, S12, that specifically targets both human and mouse VEGF-A. S12 (40 mg/kg, IP, 3×/week) was combined with paclitaxel (10 mg/kg, IP, q7d) to develop refractory or non-responsive breast cancer models. In MDA-MB-231LP breast carcinoma xenografts, tumors that had grown on average by 100% over 8 days of paclitaxel + S12 therapy, grew 11% over 17 days of therapy with DC101 + 5-FU/LV (125/62 mg/kg) ($p < 0.0001$ versus saline). Antitumor benefits with the combination were significant compared to DC101 ($p = 0.0005$) or 5-FU/LV ($p = 0.0009$) alone. Similarly, DU4475 tumors that had grown on average by 100% over 17 days of paclitaxel + S12 therapy, regressed on average by 22% over 10 days of therapy with the DC101 + 5-FU/LV combination ($p < 0.0001$ versus saline), although the benefits did not reach statistical significance compared to 5-FU/LV.

These results support the conclusion that VEGFR2 targeted antibody therapy may be efficacious in breast and hepatocellular cancers that are refractory to anti-VEGF antibody based therapy or anti-VEGFR2 targeted TKIs.

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Unique molecular recognition of CD20 by the type II CD20 antibody GA101

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Background: CD20 is a specific cell surface marker found on normal and malignant B cells. Therapeutic anti-CD20 antibodies can be classified as type I and type II CD20 antibodies differing significantly in their mode of action. Rituximab is a type I CD20 antibody that has had a major impact on the treatment of malignant lymphomas. GA101 is a novel type II glycoengineered CD20 antibody. The molecular basis of the type I and type II classification of CD20 antibodies is incompletely understood.

Material and Methods: We used data from epitope mapping, point mutagenesis, co-crystallization, and protein tomography to precisely map the epitopes and characterize the molecular interactions of different anti-CD20 antibodies.

Results: The binding site of the monoclonal antibody GA101 on CD20 was found on the cyclic loop formed by the amino acids Cys167-Cys183 of human CD20. The CD20 epitope of GA101 and different type I and