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RNAi-based identification of potential targets in colorectal cancers

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Background: Despite the implementation of sophisticated therapeutic strategies into clinical practice, colorectal cancer is still a major cause of cancer death in the Western world. Thus, understanding cancer progression and establishing novel therapeutic options remains of considerable clinical interest.
Materials and Methods: We have recently profiled a series of 90 primary colorectal cancers and 50 matched normal mucosa biopsies using gene expression microarrays and identified differentially expressed genes, among them up-regulated mRNA levels of *MYC* and *HMGA1*. Towards functional validation, mRNA expression levels of 28 differentially expressed genes were established in 25 colorectal cancer cell lines by semi-quantitative real-time PCR. Using RNAi analysis, we then systematically silenced a subset of these genes in SW480 cells, and screened for siRNA duplexes that reduced cellular viability. The siRNA-mediated reduction in mRNA levels was validated 48 hours after transfection using a branched-DNA/RNA assay.
Results: Screening our panel of 25 colorectal cell lines, we first confirmed that the majority of genes were similarly deregulated comparing colorectal cancers and matched mucosa samples and comparing the cell lines and a mucosa pool. Using RNAi analysis, we then silenced 14 highly up-regulated genes in SW480 cells using two siRNAs per gene, and could show that knockdown of a subset of these genes resulted in reduced cellular viability. This effect was independently confirmed for up to four different siRNA duplexes, and for HT-29 as well as DLD-1 cells.
Conclusions: Our experimental strategy led to the identification of novel genes critical for colorectal tumorigenesis, and we have now started to analyze the global transcriptomic changes that occurred as a consequence of gene silencing using gene expression microarrays. We surmise that some of these genes represent potential targets for therapeutic intervention.

Signal transduction modulators

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Targeting MET with XL184 to reverse EGFR tyrosine kinase inhibitor (TKI) resistance in NSCLC: impact of preclinical studies on clinical trial design

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Background: Most NSCLC patients (pts) who initially respond to EGFR TKIs develop drug-resistance. Recent studies have implicated the MET receptor tyrosine kinase in ~20% of EGFR TKI resistance. Thus, targeting MET may be a therapeutically viable strategy in the setting of EGFR TKI resistance in lung cancer. We tested the combination of a potent MET/VEGFR2/RET inhibitor, XL184, with gefitinib or erlotinib in vitro and in vivo using an EGFR TKI resistant NSCLC xenograft model harboring MET amplification. The outcome from these studies guided the initiation of a phase 1b/2 clinical trial of XL184 administered either alone or in combination with erlotinib in pts with NSCLC who progressed after prior benefit from erlotinib.
Methods: The EGFR mutant (E746_A750 del) HCC827 human NSCLC cell line was made resistant to gefitinib by culturing in increasing concentrations of gefitinib. The resulting clonally derived resistant cell line HCC827GR6 contains a focal amplification of MET, which causes resistance to gefitinib. The ability of XL184, gefitinib, or the combination of both to inhibit cell proliferation in vitro was determined using the MTS assay. The ability of XL184 to reverse erlotinib resistance was tested in vivo in a xenograft tumor growth experiment.
Results: Proliferation of gefitinib-sensitive HCC827 cells was potently inhibited in a dose-dependent manner by gefitinib (IC50 ~0.01 uM), less potently inhibited by XL184 (IC50 ~3 uM), and with no change in sensitivity to gefinitib by the combination of XL184 and gefitinib. In contrast, proliferation of gefitinib-resistant HCC827GR6 cells was not inhibited by gefitinib (IC50 > 10 uM), and only weakly by XL184 (IC50 ~3 uM). In this setting, equimolar concentrations of both agents were substantially more potent than either agent alone (>50% inhibition at 0.01 uM concentrations of both agents). In HCC827GR6 xenograft tumor growth inhibition (TGI) studies, erlotinib alone (100 mg/kg/day) resulted in weak TGI, while XL184 alone (10 mg/kg/day) had slightly better TGI. However, the combination of XL184 + erlotinib in this model caused tumor regression in all animals.
Conclusions: The preclinical rationale for the inhibition of MET in the setting of EGFR TKI resistance is encouraging and may have therapeutic potential for patients with NSCLC. A phase 1b/2 trial of XL184 with or without erlotinib in EGFR TKI resistant NSCLC is ongoing, and updated results will be presented.

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AP24534: an orally active kinase inhibitor that targets multiple pro-angiogenic receptors and exhibits potent anti-tumor activity in vivo

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Background: AP24534 is a potent orally active inhibitor of the Bcr-Abl kinase and variants, including T315I, and a phase 1 clinical trial is underway that includes patients with CML and other hematologic malignancies. AP24534 also potently inhibits a discrete subset of additional kinases, suggesting the potential for activity against solid tumors.
Results: A broad kinase screen demonstrated that AP24534 potently inhibits a number of tyrosine kinases involved in tumor growth and angiogenesis, including members of the Src (0.2 to 6 nM IC50s), VEGFR (1.5 to 13 nM), FGFR (0.5 to 10 nM) and PDGFR (1.5 to 6 nM) families as well as Tie-2 (7 nM). Cellular activity against a subset of these receptors was examined by stimulating HUVEC or NHDF cells with cognate ligand. In these systems AP24534 inhibited phosphorylation of VEGFR2 (KDR), FGFR1, PDGFRβ and Tie2 with IC50s of approximately 3, 50, 25 and 8 nM, respectively. A functional consequence of receptor inhibition was demonstrated for KDR, FGFR1 and PDGFRβ whereby ligand-induced proliferation was inhibited with IC50s of approximately 4, 25 and 100 nM respectively. Potency against all 4 receptor families was a unique characteristic of AP24534 compared to other multi-targeted kinase inhibitors tested (e.g. sunitinib, sorafenib and dasatinib). In vivo activity was examined in Colo205 (colon) and A375 (melanoma) xenograft models. Statistically significant inhibition of tumor growth was demonstrated with daily oral doses as low as 5 mg/kg with nearly complete inhibition of growth observed at 50 mg/kg. An intermittent (2x/week) dosing schedule was also efficacious. The activity of AP24534 compared favorably to that of sunitinib in both models as reflected by a greater degree of tumor growth inhibition at equivalent doses. At efficacious doses AP24534 blood levels generally did not exceed the IC50 for inhibiting proliferation of Colo205 and A375 cells in vitro suggesting that tumor growth is inhibited primarily through an anti-angiogenic mechanism. Studies to test this hypothesis are underway.
Conclusions: AP24534 is an oral kinase inhibitor with potent activity against a variety of tumor growth and angiogenesis targets. Promising anti-tumor activity has been observed in vivo. The compound's unique spectrum of targets suggests promise in settings where VEGF blockade is ineffective due to activation of alternate angiogenic pathways. A phase 1 clinical trial in patients with solid tumors is planned.

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Pharmacokinetic (PK) and pharmacodynamic (PD) results of Phase I studies of IMC-A12, a fully human insulin like growth factor-I receptor IgG1 monoclonal antibody, in patients with advanced solid malignancies

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Background: IMC-A12 is a fully human IgG_1 monoclonal antibody directed against the human insulin like growth factor-I receptor. Two Phase I trials evaluated the safety and maximum tolerated dose of IMC-A12 in patients with advanced, treatment-refractory malignancies.
Methods: Patients (pts) received IMC-A12 weekly at doses of 3, 6, 10 or 15 mg/kg or every other week (q2w) at doses of 6, 10 or 15 mg/kg until progression. After Cycle 1, there was a 2-week observation period. Extensive PK sampling and noncompartmental analysis was performed around Cycle 1. PD samples were obtained in conjunction with each cycle.
Results: In patients receiving IMC-A12 at escalating doses of 3, 6, 10 or 15 mg/kg on a weekly schedule, mean $t_{1/2}$ was 166 h, 171 h, 205 h, and

ND, respectively; mean C_{max} and AUC_{inf} increased in a greater-than-dose-proportional manner, suggesting nonlinear IMC-A12 pharmacokinetics (C_{max} = 333, 306, 788 and 443 μg/mL; AUC_{inf}=55744, 57613, 156460 and 669562 hr*μg/mL, respectively); and mean clearance decreased (0.07–0.02 mL/hr/kg), suggesting the approach of IMC-A12 saturation. In patients receiving IMC-A12 at escalating doses of 6, 10 or 15 mg/kg on a q2w schedule, mean $t_{1/2}$ was 149 h, 139 h, and 211 h, respectively; mean C_{max} and AUC_{inf} increased in a greater-than-dose-proportional manner, suggesting nonlinear IMC-A12 pharmacokinetics (C_{max} = 554, 734, and 1193 μg/mL; AUC_{inf}=69679, 134067, and 191176 hr*μg/mL, respectively); and mean clearance was relatively constant (0.141, 0.122, and 0.078 mL/hr/kg), suggesting IMC-A12 saturation. In the weekly and q2w groups, increases in serum levels of IGF-I and IGFBP3 were observed in all dose groups suggesting that IMC-A12 was blocking IGF-IR binding to its receptor. Serum trough concentrations observed in both weekly and q2w studies exceed target concentrations associated with anti-cancer activity in preclinical models (target trough concentration 60 to 158 μg/mL), especially at the doses recommended for further evaluation.
Conclusions: At clinically tolerable doses, IMC-A12 can achieve and maintain plasma concentrations that are sufficient to inhibit ligand binding to the IGF-IR. IMC-A12 will be further evaluated in prostate, breast, and other cancers at a dose of 10 mg/kg q2w.

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The PARP inhibitor, ABT-888 overcomes resistance in temozolomide refractory breast and prostate xenograft tumors implanted in metastatic sites in vivo

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PARP's role in DNA damage recognition/repair makes PARP inhibition an attractive cancer therapeutic target. ABT-888 is a potent PARP inhibitor with excellent oral bioavailability that readily crosses the blood brain barrier and has entered Phase 1 clinical trials. We have shown ABT-888's robust ability to potentiate temozolomide (TMZ) in tumors from different histological types having differential TMZ sensitivity. In this study we explored the ability of ABT-888 to potentiate TMZ efficacy in the human breast carcinoma, MDA-231-LN-luc implanted brain and human prostate carcinoma, PC3M-luc both in orthotopic and intra-tibial models. Bioluminescent cells were injected into the brain striatum of female SCID mice (MDA-231-LN-luc) and into the proximal epiphysis of the tibia in male SCID mice (PC3M-luc osteolytic cells). Zoledronic acid (ZA, 0.25 mg/kg/d, bi-weekly), a biphosphonate shown to inhibit bone resorption was also used in the intra-tibial model, where the area of decreased decalcification was quantitated in x-rays. Mice were staged based on the tumor burden evaluated in vivo with bioluminescent images (BLI) and treated with monotherapy and combinations of TMZ (50 mg/kg/d, q.d.x5) +/− ABT-888 (25 mg/kg/d, b.i.d.x5) for two cycles (PC3M-luc with 22 days rest) or three cycles (MDA-231-LN-luc with 11 days rest). There were no significant health concerns observed in the prostate study, but weight loss was observed in the breast study after the 2nd and 3rd cycles. In the prostate model, all groups with ABT-888/TMZ combination showed profound efficacy compared to groups with TMZ alone (>77% tumor growth inhibition, TGI) after the 1st cycle. However, the TMZ sensitivity was lost and tumors became refractory to TMZ during a 2nd cycle of TMZ+ZA but not with ABT-888/TMZ combination (>81% TGI). While ZA significantly improved bone integrity, no reduction in tumor burden was observed in groups without the addition of ABT-888. In the breast model, ABT-888 showed significant potentiation of TMZ activity after the 1st cycle (66% increase in tumor from size-match compared to 54% regression in the TMZ+ABT-888 group). After a 2nd cycle, tumors in the TMZ group were refractory to TMZ compared to the combination group where regression was maintained until the end of study (>40 days). We show a profound potentiation of TMZ activity by ABT-888 in two metastases models. While tumors can become refractory to TMZ treatment, ABT-888 is able to sustain sensitivity at the 2nd or 3rd cycles of combination treatment. More importantly, TMZ resistance can be prevented by ABT-888 combination therapy in crossover treatments indicating that in these studies, resistance may be overcome by PARP inhibition. Altogether, these suggest that tumors refractory to TMZ do not preclude sensitivity to ABT-888 combination therapy. The underlying mechanisms are not completely understood but may involve mechanisms independent of MGMT.

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MetMAb significantly enhances anti-tumor activity of anti-VEGF and/or erlotinib in several animal tumor models

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Background: Crosstalk between the Met receptor tyrosine kinase (RTK) and other RTKs has been implicated in tumor cell growth and survival. For example, Met amplification leads to acquired resistance to epidermal growth factor receptor (EGFR) inhibitors in EGFR mutant NSCLC cell lines and primary tumors. We have shown that Met inhibition, via shRNA-knockdown or the anti-Met monovalent antibody, MetMAb, sensitizes EGFR wild-type NSCLC tumors to erlotinib (Tarceva®), possibly by modulation of HER3 levels. Here we further explore how Met interacts with the VEGF pathway and explore combination efficacy in xenograft tumor models.
Materials and Methods: HGF-induced human umbilical vein endothelial cell (HUVEC) sprouting assays were performed to investigate the impact of Met in endothelial cell function. To examine possible indirect effects on angiogenesis, mRNA and protein expression were analyzed in several Met-driven tumor cell lines and xenograft tumors after Met activation (HGF-treatment) or Met inactivation (shRNA or MetMAb). Anti-tumor efficacy studies were performed in multiple xenograft models, including Met amplified lines, HGF/Met autocrine lines, and paracrine models utilizing a human-HGF transgenic SCID model (hu-HGF-Tg-SCIDs). Met was targeted via shRNA against Met or treatment with MetMAb, in combination with anti-VEGF antibodies (B20−4.1).
Results: We show how Met can play both direct and indirect roles in modulating tumor angiogenesis and that combinations of MetMAb and anti-VEGF antibodies provide improved anti-tumor efficacy. First, MetMAb effectively blocked HGF induces HUVEC cell sprouting, highlighting how Met may directly modulate tumor endothelial cell organization and migration. Second, Met modulated angiogenesis in an indirect fashion by regulating tumor cell production of angiogenic factors, such as vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8). Third, targeting Met, via shRNA or MetMAb, significantly enhanced efficacy of anti-VEGF antibodies in multiple Met-driven human xenograft tumor models. Anti-VEGF had similar efficacy to MetMAb in hu-HGF-Tg-SCID mice. MetMab plus anti-VEGF treatment resulted in additive effects that surpassed MetMab plus erlotinib. In contrast, erlotinib plus anti-VEGF antibodies were equivalent to anti-VEGF alone, highlighting the importance of Met in this model. Triple combination of MetMAb, anti-VEGF, and erlotinib showed much better activity than any two agents alone, with prolonged anti-tumor activity and 9/10 partial responses and 1/10 complete response.
Conclusions: These data indicate how Met can be a direct and indirect driver of tumor angiogenesis and highlight the potential therapeutic value of combining inhibitors of Met (such as MetMab) with those for VEGF (such as bevacizumab (Avastin®)) and/or EGFR (such as erlotinib (Tarceva®)).

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Pre-clinical activity of the PARP inhibitor AZD2281 in homologous recombination repair deficient triple negative breast cancer

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Background: Recently, the novel PARP inhibitor AZD2281 has been shown to have clinical activity in tumours from patients with hereditary BRCA mutations (Yap et al., 2007), supporting the concept of synthetic lethality previously described in pre-clinical models (Farmer et al. 2005; McCabe et al., 2005; Evers et al., 2008). The BRCA genes, which are associated with an increased incidence of breast and ovarian cancer, play key roles in the homologous recombination (HR) repair of DNA double strand breaks. Sensitivity to PARP inhibition has also been demonstrated in cells deficient in non-BRCA components of the HR repair pathway (McCabe et al., 2006), suggesting the broader clinical potential of PARP inhibitors in tumours that are HR deficient (HRD). Triple negative (ER−, PR−, HER2−) breast cancers have been associated with HRD, however, the extent and make-up of deficiency in this tumour type is currently not well defined. We have undertaken pre-clinical studies to assess both the sensitivity of triple negative (TN) breast cancers to AZD2281 and the nature of any HRD associated with response.
Materials and Methods: In vitro sensitivity to AZD2281 in a panel of TN cancer cell lines (including a number with defined BRCA1 mutations)