551 POSTER

RNAi-based identification of potential targets in colorectal cancers

M. Grade[2], A.B. Hummon[1], J. Camps[1], G. Emons[2], M. Spitzner[2], J. Gaedcke[2], M.J. Difilippantonio[1], B.M. Ghadimi[2], N.J. Caplen[1], T. Ried[1]. [1]*National Cancer Institute, Genetics Branch, Bethesda, USA;* [2]*Georg-August University Göttingen, General and Visceral Surgery, Goettingen, Germany*

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

552 POSTER

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

P.A. Janne[1], M. Wax[2], J. Leach[3], J. Engelman[4]. [1]*Dana Farber Cancer Institute/Brigham and Women's Hospital, Lowe Center for Thoracic Oncology, Boston, MA, USA;* [2]*Summit Medical Group, Oncology/Hematology, Berkeley Heights, NJ, USA;* [3]*Park Nicollet Clinic, Oncology, St. Louis Park, MN, USA;* [4]*Massachusetts General Hospital, Cancer Center, Boston, MA, USA*

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.

553 POSTER

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

V.M. Rivera[1], R. DiRenzo[1], L. Berk[1], S. Wardwell[1], Y. Ning[1], N.I. Narasimhan[1], Q. Xu[1], W.C. Shakespeare[1], F. Wang[1], T. Clackson[1]. [1]*ARIAD Pharmaceuticals, Research Department, Cambridge, USA*

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.

554 POSTER

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

M.L. Rothenberg[1], E. Poplin[2], P. LoRusso[3], E. Yu[4], J. Schwartz[5], F. Fox[5], J. Mehnert[2], A.B. Sandler[1], E. Rowinsky[5], C.S. Higano[4]. [1]*Vanderbilt-Ingram Cancer Center, Hematology/Oncology, Nashville, TN, USA;* [2]*Cancer Institute of New Jersey, Hematology/Oncology, New Brunswick, NJ, USA;* [3]*Karnanos Cancer Institute, Hematology/Oncology, Detroit, MI, USA;* [4]*University of Washington, Hematology/Oncology, Seattle, WA, USA;* [5]*ImClone Systems Inc., Hematology/Oncology, Branchburg, NJ, USA*

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