mesenchymal markers, vimentin and N-cadherin in STM cells. Enhanced invasion and migration capabilities were observed in STM cells consistent with a mesenchymal phenotype. Src has been shown to play a role in E-cadherin regulation and EMT. Thus, treatment of cells with dasatinib, a Src inhibitor, suppressed cell growth in STM cells, but not in ALK+/TKI sensitive cell lines. The addition of a low dose of dasatinib sensitized STM cells to the anti-proliferative effects of crizotinib. STM cells were subjected to genetic analysis to identify new genetic anomalies that could be driving resistance. Top hits are being evaluated. To our knowledge, this is the first report that demonstrates EMT occurring in an ALK+ crizotinib resistant clinical sample. Collectively these data support EMT as a mechanism of resistance to crizotinib and identifies dasatinib as a potential therapeutic for treatment of crizotinib resistance associated with EMT.

Immune profiling of malignant pleural mesothelioma by flow cytometry identifies distinct T-cell activation and exhaustion phenotypes in PD-L1 positive versus PD-L1 negative tumors

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Although PD-L1 immunohistochemical staining appears to be a partially predictive biomarker of response to PD-1 inhibitors in some cancers, many PD-L1 positive tumors do not respond to these agents. Possible explanations for this observation are that some PD-L1 positive cancers may have a paucity of infiltrating lymphocytes and/or T cells within tumors may express multiple exhaustion markers. We have developed a method for comprehensive immune cell phenotyping using flow cytometry on solid tumors that have been dissociated into single cell suspensions. Applying this technique to 33 resected malignant pleural mesothelioma samples, here we show that compared to PD-L1 negative tumors, PD-L1 positive tumors have significantly more infiltrating CD45+ immune cells, a significantly higher proportion of infiltrating CD3+ T cells, a significant increase in proliferating CD4+ and CD8+ T cells, and a significantly higher percentage of CD3+ cells displaying the activation antigens HLA-DR+/CD38+. PD-L1 positive tumors also have a significantly higher proportion of FOXP3+/CD4+ regulatory T cells. We found that CD4+ and CD8+ T cells in PD-L1 positive samples are significantly more likely to express the T-cell exhaustion markers PD-1 and TIM-3 compared to PD-L1 negative samples. Flow cytometric analysis also identified two immunologically distinct PD-L1 positive mesothelioma samples: PD-1 positive, TIM-3 negative “single positive” tumors, and PD-1 positive, TIM-3 positive “double positive” tumors. Successful incorporation of comprehensive immune profiling by flow cytometry into prospective clinical trials should hopefully refine our ability to predict which patients will respond to immune checkpoint blockade and lead to rationally designed combination immunotherapy trials.

Novel epidermal growth factor receptor inhibitor accumulates in the brain and inhibits the growth of brain metastatic non-small cell lung cancer

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Deaths from solid tumors are often not due to the primary lesion but to metastatic disease at distal sites such as the lung, liver, and brain. Patients with non-small cell lung cancer (NSCLC) experience brain metastases, a poor prognostic marker, at an incidence rate of 30-50%. A significant proportion of the metastatic tumors express activating mutations of the EGFR, including exon 19 deletions, which confer increased sensitivity to EGFR inhibitors such as erlotinib and gefitinib. Despite successful use of small molecule kinase inhibitors for the treatment of EGFR+ primary lung tumors, current therapeutics poorly inhibit the growth of NSCLC brain metastases due to difficulty crossing the blood-brain barrier. For this reason, NSCLC patients with brain metastases are often excluded from clinical trials with novel therapies and thus have access to very few emerging treatment options.

We have synthesized a novel class of compounds that inhibit the epidermal growth factor receptor (EGFR) in the nanomolar range in vitro, and demonstrate a high degree of selectivity for EGFR family members. The parent compound, LL001, from Capella Therapeutics, Inc, inhibited EGFR-mediated autophosphorylation and
phosphorylation of downstream targets Akt and ERK MAP kinase at a concentration of approximately 100nM and blocked the kinase activity of an EGFR mutant, T790M, which confers resistance to front-line EGFR inhibitors such as Tarceva. Compound LL001 induced full remission of subcutaneous tumors using the human NSCLC cell line H1975 (T790M/L858R+). Most notably, pharmacokinetic studies showed that LL001, when administered at a concentration of 150mg/kg in mice, showed no signs of toxicity and accumulated in the brain at concentrations ranging from 12-171 times higher than the GI50 for inhibition of EGFR+. NSCLC cell line proliferation in vitro.

In order to determine whether LL001 could effectively treat NSCLC brain metastases, we developed an intracranial injection orthotopic xenograft model for the study of NSCLC brain lesions. We found that LL001 could induce complete remission of brain tumors in 67% of mice injected with the human NSCLC cell line HCC827-luciferase, which expresses an exon 19 deletion mutant of EGFR. Compound LL001 (75mg/kg/day) reduced tumor burden in the brain to undetectable levels by in vitro imaging (IVIS) and performed significantly better than Tarceva, a reference compound. We conclude that compound LL001 crosses the blood brain barrier at levels sufficient to block the growth of EGFR+ NSCLC brain metastases and therefore may be therapeutically useful for a critically underserved patient population.

Gold nanorod immunoassay to quantify C-Met expression in NSCLC patients selected for anti-EGFR therapy

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Non-small cell lung cancer (NSCLC) is a subtype of lung cancer that is the leading cause of cancer-related mortality. The average 5-year relative survival rate for NSCLC patients is estimated to be 15.7% depending on the stage of diagnosis. 224,210 new cases of lung cancer, and 159,260 deaths were reported in the year 2014. Out of these reported numbers, NSCLC makes up almost 80% of cases. More importantly the patients are often found in late stages due to ineffective diagnosis and relative insensitivity to chemotherapy. NSCLC is a complex disease due to presence of large number of genomic alterations, such as EGFR overexpression that has been reported to be overexpressed anywhere from 43-89% depending on factors such as stage, ethnicity, and diagnostic method used. EGFR-expressing lung cancers are treated with anti-EGFR drugs such as monoclonal antibodies or small molecule tyrosine kinase inhibitors. Upon treatment with tyrosine kinase inhibitors (TKIs), NSCLC tumors have shown to acquire resistance to TKIs through mutations that circumvent the EGFR pathways targeted. One marker that has been shown to arise in tumors treated with anti-EGFR therapies is the c-Met (HGF) receptor. Once a patient has undergone several months of EGFR targeted therapy, often times a subsequent biopsy will show that the tumor now expresses a large amount of c-Met protein instead of the previously diagnosed EGFR expression. We will thus explore the notion that for a substantial amount of EGFR-expressing NSCLC tissues, there will be a population of cells which overexpress the c-Met receptor at the stage of initial diagnosis which are allowed to continue growth since EGFR is the only therapeautic target utilized for the patient treatment. As a result, a better recommendation for patient treatment would include therapies targeted both at c-Met and EGFR. For this study, we have acquired NSCLC tissues from patients who have been treated with anti-EGFR therapy. To assess levels of EGFR and c-Met in the tissues, we use our platform IHC nanotechnology consisting of gold nanorods (GNR) targeted to either receptor by means of surface modification. By adding a modified peptide specific for EGFR or c-Met to the surface of the gold nanorod, we assess levels of both EGFR and c-Met in the tissues through imaging. GNR stained tissue images can be analyzed quantitatively through specific image processing algorithms, allowing for a precise, accurate method of diagnosis compared to conventional IHC. Tissue levels of c-Met present and the patient response to anti-EGFR treatment will then be compared to determine if the patient would likely benefit from initial treatment targeted at both c-Met and EGFR.

Quantitative mass spectrometry-based proteomics identifies FRalpha and GARFT as predictive biomarkers in tissues of non-squamous NSCLC patients treated with pemetrexed

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Lung cancer remains the leading cause of cancer mortality in United States and globally. Pemetrexed