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

Chuck Caldwell, Raghuraman Kannan, Gerald Arthur, Dmitry Shin, Ilker Ersoy, Richard Hammer University of Missouri-Columbia, Columbia, MO

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 therapeutic 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

Alshehri Abdulrahman,\(^1\) Eunkiung An,\(^2\) Fabiola Cecchi,\(^2\) Adele Blackler,\(^2\) Shankar Sellappan,\(^2\) Matt Smolkin,\(^1\) Todd Hembrough,\(^2\) Manish Monga\(^1\) 1West Virginia University, Morgantown, WV, 2NantOmics, Rockville, MD

Lung cancer remains the leading cause of cancer mortality in United States and globally.
combined with platinum chemotherapy is specifically indicated for treatment of non-squamous non-small cell lung cancer (non-sq NSCLC). Pemetrexed is a folate-analog metabolic inhibitor that disrupts folate-dependent processes essential for cell replication. Pemetrexed inhibits thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycaminide ribonucleotide formyltransferase (GARFT), which are folate-dependent enzymes involved in the de novo biosynthesis of thymidine and purine nucleotides. Folate receptor alpha (FRalpha) is a folate/antifolate transporter protein that is overexpressed by a number of epithelial tumors. The purpose of this study is to identify proteomic biomarkers predictive of response to pemetrexed-based chemotherapy in non-sq NSCLC.

Methods: Patients with advanced non-sq NSCLC who received pemetrexed-based chemotherapy at West Virginia University from 2009 to 2014 were retrospectively identified. Formalin-fixed, paraffin-embedded tumor biopsies were laser microdissected, solubilized, enzymatically digested and subjected to quantitative proteomic analysis. A multiplexed, selected reaction monitoring (SRM) mass spectrometry (MS) assay was used to determine the absolute levels of 46 different candidate proteomic markers, including those in the folate receptor pathway. TS analysis was also performed by IHC. The Kaplan-Meier method and log-rank test were used in statistical analysis of overall survival (OS) and progression-free survival (PFS).

Results: The 74 patients included in the study had a median follow-up of 26 months, a median OS of 16.6 months (95%CI: 11.6 - 43.4), and a median PFS of 9.61 months (95%CI: 8.43, 12.98). There were 65 patients who received pemetrexed-based regimen as a first line therapy and 9 patients as subsequent salvage treatment. In a comparison between patients who survived >24 months and < 8 months, there were no significant differences between the two groups in terms of sex, age, ECOG performance status, TNM stage at diagnosis, and smoking history. Among the 37 patients with sufficient tumor specimens available for multiplexed proteomic analysis, 30 biomarkers were detected with varying levels of expression. Sixteen additional biomarkers were undetectable. TS protein expression was detected in by SRM in 2 patients and by IHC in 32 patients (tumor staining>1); however, TS IHC was not predictive of outcome (PFS-HR ratio = 1.06). Patients whose tumors expressed low levels of GARFT protein (≤900 amol/μg; n=7) had statistically significantly longer median PFS than those whose tumors expressed high levels of GARFT (>900 amol/μg; n=30) (40.6 vs. 11.4 months; p = 0.014). Patients with high FRalpha protein expression (>1510 amol/μg, n=9) had significantly longer median PFS than those with low FRalpha expression (≤1510 amol/μg; n=28) (>50 vs. 11.4 months; p = 0.021). Moreover, the 23 patients with both high GARFT expression (>900 amol/μg) and low FRalpha expression (≤1510 amol/μg) fared considerably worse than the remainder of patients (median PFS 10.1 vs. 40.6 months; p=0.0003).

Conclusion: Multiplexed mass spectrometry-based proteomics offers a feasible and promising approach for tumor biomarker profiling and quantification to predict therapeutic response. Of note, our results show that FRalpha and GARFT protein expression may be predictive of response to pemetrexed-based treatment in patients with non-sq NSCLC. Further investigation is needed to validate the utility of these biomarkers for guiding personalized treatment decisions in clinical practice.

Deregulated SOX2 drives dysplasia in a novel 3D organotypic model of bronchial dysplasia

Lúcia L. Correia, Trevor D. Littlewood, Gerard Evan, Frank McCaughan

University of Cambridge, Cambridge, United Kingdom

Introduction and objectives: Squamous lung cancer (SQC) is a devastating disease for which the currently available treatments are poorly effective. There are no licensed targeted therapeutic agents. Improving the early detection and chemoprevention of SQC, and identifying novel and tractable therapeutic targets are key challenges to improving outcomes.

The development of model systems that recapitulate the human disease will facilitate the development of new therapeutic/chemopreventive agents and new insights into the pathobiology of SQC.

SOX2 amplification is a frequent genetic alteration in squamous lung cancer. We have shown that amplification and overexpression of SOX2 is an early and consistent event in the pathogenesis of this disease.

Our hypothesis is that deregulated expression of SOX2 is a key event in the initiation of bronchial dysplasia/squamous carcinogenesis.

Our aims are:

1) To develop a novel 3D in vitro model of human bronchial dysplasia that recapitulates the molecular and phenotypic characteristics of the in vivo disease.