Conclusions: Mexican NSCLC patients exhibit a specific mutation profile with alterations in several cancer-related genes mainly in EGFR, TP53, GNAQ, HNF1A, VHL and KRAS. The NGS technology showed greater sensitivity than PCR to detect relevant mutations in NSCLC. Several mutations found in high incidence in this study could become relevant targets for novel targeted therapies in NSCLC.

Detection of viral induced double-stranded RNA intermediates in archival paraffin blocks

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Double strand RNA (dsRNA) species were previously thought to be a 'junk DNA' equivalent are related to endogenous retroviral elements (ERV) inserted into the human genome. Genomic integration sites and blocking of these processes have been well documented in HIV and Hepatitis C related diseases. Viral integration into human genome has been shown in a range of solid tumors, but mechanisms less understood. The detection of dsRNA in the research setting has been largely limited to cell line models and controlled transfections. To better characterize function significance of ERV and interactions at the cellular level, we outline efforts to detect and quantify such viral genomic elements with a focus on archival clinical samples in the commonly stored paraffin block. Tissue blocks issues with clinical and biochemical documented viral infection and associated viral cytopathic changes were selected from the Department of Pathology and Biorepository and standard 4 micron sections were prepared. Two (2) commercially available antibodies raised to dsRNA fragments (J2 and K1 antibodies) were directly compared by chromogenic and immunofluorescent methods. In addition, downstream biomarkers as identified by literature searches include RIG-1 and IRF 7 as moderate probable success and MDA-5 and STAT 1 as higher level success. Correlative analysis of downstream pathway markers with labeling results of dsRNA antibodies suffers from lack of sensitive measures of dsRNA fragments as opposed to the downstream cellular pathway markers. This lack of concordance likely reflects the short nature of dsRNAs in decade old paraffin tissue and/or differences in abundance. Future work will focus on developing more sensitive probes or IF probe signal amplification.

Prediction of distant recurrence-free survival in resectable lung adenocarcinoma

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Background: Recurrence and lung cancer mortality in resected, early stage lung cancer is likely due to the presence of micro-metastatic disease not detected during surgery or the pre-surgical assessment. The likelihood of such latent disease increases with higher stages and thus treatment with adjuvant therapy is currently determined by pathological stage. However, significant recurrent disease and cancer-related mortality even in stage I patients suggest that pathological stage alone does not adequately identify resected patients that could benefit from further treatment. Tumor molecular features can improve stratification of patients for increased risk of distant recurrence.

Methods: Formalin-fixed surgical samples from 318 stage I and II lung adenocarcinoma patients, treated with surgery alone, were examined for the expression of 31 proliferation genes (CCP score) by multiplex, quantitative RT-PCR. A prognostic score (PS) was calculated from pathological stage and the CCP score. The added prognostic value of the CCP score for the prediction of five–year distant recurrence-free survival and five-year cancer-specific survival was tested in Cox proportional hazards regression models with adjustment for clinical parameters. Estimates of disease-free survival and cancer-specific survival were calculated by the Kaplan-Meier method.

Results: Among 289 samples with successful expression analysis, 85 had developed a distant recurrence at five years post-surgery. After adjustment for age, gender, tumor size and pleural invasion, pathological stage (HR 2.28, 95%CI 1.16-4.50 for IA vs IB, p=0.0015) and the CCP score (HR 1.62 per IQR, 95% CI 1.15-2.29, p=0.0055) were independent predictors of distant recurrence and as well as for cancer-specific survival [pathological stage (HR 1.72, 95%CI 0.93-3.17 for IA vs IB, p=0.024), CCP score (HR 1.40 per IQR, 95%CI 1.03-1.90, p=0.031)]. The prognostic score was a better predictor of distant recurrence than pathological stage alone (PS: HR 2.22 95%CI 1.11-4.44, p=0.023, stage: HR 1.70 95%CI 0.86-3.35

for IA vs IB, p=0.29). Patients with a high-risk PS (>27) had a three-fold increased risk of distant recurrence compared to patients with a low-risk PS score. In stage I patients, the estimated risk of distant recurrence for tumors with high-risk prognostic scores was significantly higher (36%, 95%CI 28%-46%) than in low-risk PS tumors (15%, 95%CI 8%-29%, p=0.0005).

Conclusions: The prediction of risk of distant recurrence in resected lung adenocarcinoma patients can be improved over that obtained by the current standard of pathological stage by incorporating tumor expression of proliferation markers (CCP score). Improved risk stratification can help identify patients in need of additional treatment and prioritize patients for trials of emerging new therapies.

Strong pharmacological activity of locally administered next-generation antisense oligonucleotides (ASOs) in orthotopic lung cancer mouse models

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Lung cancer is the second most common cancer type and the leading cause of cancer-related death in both men and women in the United States. Although recent advances in genomic expression profiling of lung cancer has provided more individualized treatment based on target specific genetic alteration(s) in patients, there is a still high unmet medical need as many important driver mutations remain undruggable by conventional therapeutic approaches. Antisense oligonucleotide (ASO) technology offers a novel therapeutic modality to selectively target currently undruggable pathways. We have recently demonstrated that systemic delivery of unformulated next generation (Gen 2.5) ASO targeting human STAT3 mRNA (STAT3-Rx/AZD9150) leads to robust pharmacological activity in various preclinical animal models of lung cancer. Importantly, STAT3-Rx/ AZD9150 displayed strong anti-tumor activity in patients with advanced treatment-refractory lymphoma as well as non-small cell lung cancer (NSCLC) in clinical trials*.

Although local delivery of ASOs has been proposed as a means to treat various pulmonary diseases, ASO pharmacology in lung tumors has not been previously evaluated. In this study, we evaluated the activity of an antisense inhibitor (targeting MALAT1 gene) given by different administration routes {by either a local (intra-tracheally=IT) or a systemic (subcutaneously=SC) delivery} in orthotopic lung cancer xenograft models. For this, human NSCLC A549 or H460 cells were injected to nude mice via tail vein and 2 weeks later, the tumor-bearing animals were treated with Gen 2.5 MALAT1 ASO either IT (at 10 to 20 mg/kg, 3 times per week) or SC (at 25 to 50 mg/kg, 5 times per week) for 3 weeks. Reductions in MALAT1 RNA levels in human tumor cells and mouse lung were greater with local delivery at lower doses compared to systemic delivery as assessed by qRT-PCR or in-situ hybridization using species-specific primers/probes (73% reduction at 20 mg/kg by IT dosing vs 50.5% reduction at 50 mg/kg by SC dosing in tumors; 72.5% vs 70% reduction in mouse lung at the same dosing regimen). Moreover, local administration of ASOs led to no notable increase in liver transaminase levels compared to systemic administration. Pharmacokinetic property-wise, the local administration showed greater and more even accumulation of drug in both tumor and lung tissues compared to the systemic route. Taken together, these findings suggest that local administration of Gen2.5 ASOs to lung malignancies may be a promising alternative approach to systemic delivery with greater potency and improved safety profile, especially in inhibiting lung cancer targets which cannot be tolerated when depleted systemically.

*Hong D et al. AZD9150, a Next-Generation Antisense Oligonucleotide Inhibitor of STAT3 with Early Evidence of Clinical Activity in Lymphoma and Lung Cancer. Sci. Transl. Med. 2015 (in press).

Impact of interleukin-22 on K-ras mutant lung cancer promotion and stemness properties

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Activating mutations of K-ras, which are found in approximately 30% of non-small cell lung cancer (NSCLC), are the most common genetic alterations associated with tobacco exposure in lung cancer. Unfortunately, pharmacologic attempts directly targeting K-ras have thus far failed; clearly stating the need for new strategies to bring clinical benefits to patients displaying such a molecular profile. Tumor-promoting inflammation is a cancer hallmark, and it is now apparent that the cytokines and growth factors released during inflammation influence