KRAS driven NSCLC, to identify various potential mechanisms that may lead to effector T cell suppression. Validation of key findings in human KRAS adenocarcinomas identified several dominant immunosuppressive mechanisms employed by KRAS tumors. These mechanisms include spatiotemporal organization of immune cells (T cell exclusion/inclusion, MDSCs, Macrophages), expression of specific co-inhibitory checkpoints (PD1-PD-L1, LAG-3, TIM-3, TIGIT), and additional components of T cell suppression (IDO, Arginase, Tregs). Identification of concurrent dominant immune suppressive mechanisms employed by KRAS tumors has allowed us to employ rationally guided effective combination therapeutics, which are being tested in exploratory trials in preclinical models, together with immunomodulatory action of conventional standard of care chemotherapy and radiation therapy. Our approach is in line with recent success of combination immunotherapies in melanoma and colon cancer, and could impact the selection of immunotherapies for treating mutant KRAS NSCLC patients in the clinic.

**Methods:** Overall 31 tumor biopsies of Mexican patients with NSCLC were analyzed. DNA was extracted from biopsies using the Wizard Genomic DNA kit. DNA concentration was determined by Qubit. Quality control was performed with the FFPE QC kit for formalin-fixed, paraffin-embedded samples. Genomic libraries were constructed using the TruSeq Cancer Panel comprising 212 amplicons of 48 genes. Sequences were obtained in a MiSeq sequencer. EGFR mutations were analyzed alternatively by using real time PCR using the Rotor-Gene Q and the Scorpions and ARMS technologies.

**Results:** We found mutations in 19 cancer-related genes specific for the Mexican population. In 37% of cases we found mutations in the EGFR gene, in exon 19 (17%), exon 21 (13%) and two rare mutations in exons 2 and 3. We found a correlation for the diagnosis of mutations in the EGFR gene by PCR and NGS in seven patients. EGFR mutations were detected in four patients by NGS that were not detected by PCR. Additional mutations were found mainly in TP53 in 12 patients (40%), followed by mutations in the genes GNAQ (33%), HNF1A (16%), VHL (13%) and KRAS (10%). Several other mutations were detected in minor frequencies i.e. 6% for CTNNB1, FGFR2, MET and SMARC1 and 3% of the cases for APC, Braf, CDH1, ERBB2, FGFR3, GNAS, PTEN and RB1. The clinicopathological characteristics of the patients were a median age of 65 years in a range of 37-82 years, 93% of patients had adenocarcinoma with 34% of mixed subtype, 23% acinar, 17% poorly differentiated, 13% solid, 10% lepidic and 3% papilar. Metastatic NSCLC was detected in 53% of the patients, mainly in bones and brain (33%). Overall 70% were female, 55% were exposed to wood-smoke and 41% were smokers. The performance status of the patients was predominantly ECOG1 with 78%. Most patients (76%) were diagnosed at a disease stage IV while 24% presented stage IIIb. Treatment was established according the mutational status of the patients, 60% was treated with chemotherapy: Cisplatin-Paclitaxel (30%), Pemetrexed-Carboplatin (17%), Gemcitabine-Carboplatin (9%) and Vinorelbine-Cisplatin (4%). Tyrosine kinase inhibitor (TKI) treatment consisted of Erlotinib (17%), Gefitinib (13%) and Afatinib (10%). Concerning the relationship with clinical characteristics, 91% of the EGFR mutations appeared in patients over 60 years of age (p 0.02). Patients with EGFR mutations, particularly exon 19 deletions (83%) responded to TKIs either as partial response (50%) or stable disease (33%) (p 0.024). The presence of EGFR exon 19 deletions were associated with metastatic NSCLC, with bone and brain metastases as the most common secondary localization of disease (p 0.044).

**Mutational profile of non-small cell lung cancer by targeted next-generation sequencing in the Mexican population**

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**Background:** The mutation profile of many cancer-related genes in the Mexican population of patients with non-small cell lung cancer (NSCLC) remains largely unexplored and also their relationship to many clinical features of the patients. Next Generation Sequencing (NGS) allows multiplexing for sequencing several genes with higher sensitivity than other techniques and it is increasingly applied in clinical research. Hypothesis: The population of Mexican NSCLC patients presents a specific mutation profile in cancer-related genes that can be better characterized by targeted NGS than by PCR and may be related to clinical characteristics.

**Objectives:** To detect the presence of somatic mutations by targeted NGS and PCR in Mexican patients with NSCLC and find its association with clinicopathological features.
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