non-malignant cells. Analysis of PCNA structure revealed that the L126-Y133 region forms part of a pocket suitable for binding by a small molecule. We designed and tested a series of small molecules that target this binding pocket and identified AOH1160, a potent PCNA inhibitor, which kills small cell lung cancer (SCLC) cells at high nanomolar concentrations, but causes no significant toxicity to a broad range of non-malignant cells up to a concentration of 10 μM. AOH1160 is orally available to animals and inhibits tumor growth without causing any observable side-effects, including weight loss, in mice. These studies demonstrated the feasibility of inhibiting the growth of SCLC cells by targeting a specific region of PCNA without causing unacceptable toxicity to normal tissues. Further development of AOH1160 may lead to a novel anti-cancer therapy.

Translational application of microRNA profiling for early detection of lung cancer: A comparison of sputum and blood

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Background: Lung cancer has the highest mortality rates of all the cancers in Canada with a 5 year survival rate of less than 15%. Asymptomatic in its early stages, methods to screen high risk individuals are in dire need to allow earlier diagnosis and curative intent treatment. MicroRNAs (miRNAs) are small, non-coding strands of RNA that are shown to lead to carcinogenesis when dysregulated. They are promising candidates for biomarkers as they are stable, detectable in small quantities and are expressed in a tissue specific manner. Through the use of a miRNA panel developed by our group that demonstrated good sensitivity and specificity using sputum as a medium to measure miRNA., we aimed to compare the efficacy of measuring miRNA in sputum and blood to develop a miRNA profile for non-small cell lung cancer (NSCLC).

Objective: To examine miRNA profiles of NSCLC cases versus healthy controls to compare the efficacy of sputum and blood for potential screening purposes using microarray analysis.

Methods: A case control study of stage I/II cancers, matched with controls having similar smoking history, age, and gender, was performed. Participants were recruited at the Royal Alexandra Hospital in Edmonton, Alberta, Canada. Both sputum and blood are collected and analyzed via Qiagen miRNA kits. 10 cases and 10 controls miRNA samples were submitted for microarray analysis. miRNAs were labelled, hybridized, and quantified using single-color experimental design. Specific miRNAs from past literature were then compared in cases and controls using Mann Whitney U test.

Results: Sputum does not have consistent levels of miRNA present when compared to blood, and principle component analysis (PCA) plots show more random patterns in sputum when compared to blood. By using heat maps and hierarchical clustering, no apparent clusters are seen when compared cases and controls in both sputum and blood. A type II error could be responsible for this finding due to the small sample size. In an independent analysis looking at specific miRNAs seen to be dysregulated in past literature, miR-147a is significantly different in sputum, and miR-126-5p is significantly different in blood.

Conclusions: Microarray analysis shows that sputum is less consistent when measuring miRNAs compared to blood overall. These findings have already been applied to the next phase of our research which will examine miRNA levels in high risk individuals as a means of establishing it as a robust screening test for lung cancer.

Targeting immunosuppressive mechanisms in KRAS mutant lung cancer

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Clinical trials with single agent immune checkpoint inhibitors, mainly the anti-PD-1 antibody, have achieved noteworthy benefit with an objective response rate in 17% of non-small cell lung cancer (NSCLC) patients. However, minimal or no response in a large proportion of patients suggest that additional immune suppression pathways need to be identified in the tumor microenvironment to define combination immune therapies for future therapeutic intervention. We have focused our studies on the KRAS mutant subset, as it accounts for $>$30% of NSCLC patients with high mortality rates due to a conspicuous lack of effective FDA approved targeted therapies.

To enable selection of appropriate immunotherapies, we have performed comprehensive analysis of immune microenvironments in a mouse model of

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.

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.

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. EFGR 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 SMARCB1 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).