database. Twenty-two FDA-approved candidate drugs shifted the transcriptome similarly to ADSC treatment; and are thereby promising for RF treatment. Drug screening revealed that candidates which upregulate lipid metabolism or gluconeogenesis decreased collagen production and/or secretion by TGF-B stimulated fibroblasts.

Conclusions: ADSC transplantation may be an effective treatment for the reversal of RF via metabolic reprogramming. Through pharmacogenomics analysis, we identified FDA approved drugs with potential to be repurposed for the treatment of RF based on their potential to induce metabolic alterations similar to ADSCs. Our data highlights the importance of metabolic dysregulation in the pathogenesis of RF and the importance of targeting these pathways in reversing RF.

28 MUTATIONAL SPECTRUM OF ANAL CANCERS FROM PATIENTS TREATED WITH RADICAL CHEMORADIOTHERAPY

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Purpose: The mutational landscape of anal cancers has not been well studied. The purpose of this study was to perform the first mutational analysis characterizing the types and frequencies of mutations in anal cancers from patients treated with radical chemoradiation therapy (CRT) using comprehensive next-generation sequencing (NGS).

Methods and Materials: Pre-treatment formalin-fixed, paraffin-embedded anal cancer specimens from 30 patients treated with radical CRT for anal cancer at a single tertiary institution were evaluated. Ninety percent of cases were squamous cell cancers. M:F ratio was 1:2.3; median patient age was 56 years (range: 34-80): 47% (n = 14) had T2 disease. Tumour DNA was extracted and assayed for 50 oncogenes and tumour suppressor genes using the Ampliseq Cancer Hot Spot Panel (CHPv2) on the Ion PGM using a 316v2 chip. Mean depth of target coverage was 100X5. Bioinformatic analysis was performed using Torrent Suite Software version 5.0.3. Variants from reference hg19 were called using variant Caller plugin 5.0.3.5 and annotated with Ion Reporter Software 5.0. All variants were manually reviewed usingstrand Integrative Genomic Bioinformatic analysis was performed using Torrent Suite Software version 5.0.3. Variants from reference hg19 were called using variant Caller plugin 5.0.3.5 and annotated with Ion Reporter Software 5.0. All variants were manually reviewed usingstrand Integrative Genomic Viewer. Mutational status was determined and associated with HPV status.

Results: Twenty-five of 30 cases (83%) were evaluable for full mutational analysis. The most common mutation identified was PIK3CA (4/25 of cases, 16%); 75% (3/4) were in exon 9. Overall, PI3K/akt/mTOR pathway activating mutations were seen in 24% (6/25 of cases). Other mutations were very rare: FBXW7 (n = 1, 4%), p53 (n = 1, 4%), IDH1 (n = 1, 4%). One tumour had NRAS mutation; notably all other MAPK pathway genes were wild-type. Twenty-one of 25 cases were HPV sub-typed: 90% (19/21) were positive for high-risk HPV. Only p53 mutation was associated with HPV negative status.

Conclusions: PI3K/akt/mTOR activating mutations were the most frequently observed in patients with anal cancer treated with CRT. Anal cancers have targetable mutations, making them amenable for consideration of therapeutic agents such as PI3K and EGFR inhibitors. Validation with a larger data set will be undertaken to confirm these findings, and to determine their association with clinical outcome parameters.

29 COMPUTER-ASSISTED IMAGE ANALYSIS OF AN ORAL CAVITY SQUAMOUS CELL CARCINOMA TISSUE MICROARRAY

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Purpose: The immune microenvironment within tumours is critical to oncogenesis, cancer progression, and radiotherapy (RT) efficacy. Immunohistochemistry (IHC) is a convenient and inexpensive method by which to characterize the immune infiltrates in pathology samples. However, manually reading multiple IHC stains on tissue microarrays (TMA) is labor intensive and subject to bias. Our objective is to apply computer image analysis tools to localize and quantify immune markers in oral cavity squamous cell carcinoma (OCSCC) samples and determine their prognostic implications.

Methods and Materials: A 91-patient OCSCC TMA was stained for the markers: CD3, CD4, CD8, FOXP3, IDO, and PD-L1. Tissue Studio (Definiens AG, Munich, Germany) was used to enumerate the number of marker-positive cells and to quantify the staining intensity for IDO and PD-L1. Cell populations were assigned to stromal or epithelial (tumour) compartments according to a mask derived from a pan-cytokeratin stain using a custom Matlab script. Automated methods were validated against manual tissue segmentation, cell count and stain intensity quantification. Univariate associations of cell counts and stain intensities with smoking status, TNM stage, overall survival (OS), and disease-free survival (DFS) were determined.

Results: 80.6% (737/910) of TMA cores were suitable for analysis, 39.8% (35/88) of patients had a known never-smoker history, and 34.5% (31/91) of patients were treated with CRT. Comparison of automated to manual tissue segmentation showed good agreement (Kappa coefficient range: 0.61 - 0.75). Automated and manual cell counts and stain intensities were highly correlated (Pearson correlation coefficient range: 0.46 - 0.91, p < 0.001 for all). Individual cell counts and stain intensities within the stromal, epithelial, or combined compartments did not display significant association with stage, OS or DFS in the set of all patients and in the subset of patients who received RT (p ≥ 0.05). Compared to never-smokers, current and ex-smokers had an increased density of FOXP3 cells in the epithelial compartment (OR 12.49, p = 0.06), and stronger PD-L1 stain intensity in both epithelial and stromal compartments (OR 4.54, p = 0.08; OR 6.58, p = 0.05); these results were confirmed by manual scores.

Conclusions: Computer-assisted image analysis can be used for robust quantification of cellular populations by IHC. Our automated methods show that current and ex-smokers have higher density of FOXP3 cells in the epithelial compartment and have more intense PD-L1 staining in both epithelial and stromal compartments. This result is validated with manual IHC scoring. This proof-of-principle study demonstrates the utility of computer-assisted image analysis for high-throughput assessment of multiple IHC markers on TMAs, with potential implications for studies on prognostic and predictive biomarkers.

30 EGFR MUTATIONS AND METABOLIC UPTAKE IN ADVANCED NON-SMALL CELL LUNG CANCER

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Purpose: Early studies have suggested a correlation between fluorodeoxyglucose-positron emission tomography (FDG-PET) uptake and epidermal growth factor receptor (EGFR) mutation status in patients with non-small cell lung cancer (NSCLC). Results from these few studies are conflicting and limited by small sample subject numbers and variances. The purpose of this study was to determine if such a correlation exists in a large population using standardized diagnostic protocols.

Methods and Materials: A retrospective review was conducted of patients with metastatic non-squamous, non-neuroendocrine, NSCLC who had EGFR mutation testing and FDG-PET imaging between March 2010 and March 2012. All patients had FDG-PET imaging at a central facility using the same scanning protocol. Data was collected on the maximum standardized uptake value (SUVmax) of the primary lung tumour. EGFR mutation testing was done at a central lab using a rapid polymerase chain reaction-based detection technique. Patients were divided into EGFR mutation positive (EGFR+) and EGFR wild type (WT) cohorts.