submandibular triangle (p < 0.0001). In 69% of patients (n = 11), the dose to the transferred submandibular gland was below the QUANTEC dose constraint of mean < 35 Gy. Four of the remaining patients had pathologic involvement of the contralateral level I nodes and could not have the transferred gland spared, while one patient had a large pT4 lip lesion and coverage of the tumor bed resulted in a dose of 36.0 Gy to the transferred gland.

**Conclusions:** The mSGT technique significantly reduced the dose to the submandibular gland from a median dose very likely to produce xerostomia to a dose below accepted dose constraints. Adoption of this technique may reduce the rate of xerostomia and improve quality of life in patients with oral cavity cancer undergoing adjuvant RT.

**25 CARO FELLOWSHIP**

**THE BIOLOGICAL ROLE AND CLINICAL SIGNIFICANCE OF LONG NON-CODING RNA UROTHelial CARCINoma ASSOCIATED 1 (UCA1) IN PROSTATE CANCER (PCA)**


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**Purpose:** Urothelial carcinoma-associated 1 (UCA1) is a recently identified long non-coding RNA which plays an oncologic role in several cancers and enhances cellular proliferation, invasion, and tumour growth. It is upregulated in prostate tumors, but its involvement in therapy response has not been investigated.

**Methods and Materials:** To simulate the clinical scenario of ionizing-radiation resistance (IRR), we created IRR PCA cell lines by treatment with mock irradiation (parental cells) or IR (conventional fractionation (CF): 2 Gy daily X 59 for DU145-CF-IRR cells). We performed gene array profiling to discover dysregulated genes in DU145-CF-IRR cells and identified UCA1. We investigated the mechanism of UCA1 on aggression and response to radiation and chemotherapy by performing miRNA comparative profiling (NanoString platform), Proteome Profiler Array, proliferation assays, soft agar colony formation, invasion assay, y-H2AX assay, cell cycle profiling, MTS assay and western blotting. We also studied the clinical significance of UCA1 expression in two cohorts of PCA patients; CPC-GENE (n = 210; patients with intermediate-risk PCA) and MSKCC (n = 131; PCA patients treated with radical prostatectomy).

**Results:** DU145-CF-IRR cells were radioresistant and acquired an aggressive phenotype. We found that UCA1 expression was significantly higher in DU145-CF-IRR compared to parental cells using gene array profiling and confirmatory qRT-PCR analysis (170-fold). Interestingly, UCA1 siRNA knockdown reversed the aggressive phenotype and significantly increased sensitivity to IR and docetaxel. We demonstrated that UCA1 depletion inhibited cell cycle arrest at the G2/M transition and decreased activation of the pro-survival Akt pathway. Furthermore, we found that UCA1 overexpression was associated with a trend toward lower biochemical recurrence-free survival in CPC-GENE patients (HR = 1.4, p = 0.28) and its aberrant expression was significantly associated with decreased five-year disease-free survival in publically available MSKCC database (84.5% versus 52%; HR = 2.19, log-rank test p = 0.005).

**Conclusions:** We showed for the first time that UCA1 can influence cancer aggression, radiation and chemotherapy response in PCA, which may occur through altered Akt signaling. Our results also suggest that UCA1 can have prognostic value in PCA. Future work will investigate UCA1 as a therapeutic target and prognostic biomarker for PCA.

**26 URINARY CYTOKINES/CHEMOKINES PATTERN AFTER MAGNETIC RESONANCE- GUIDED HIGH INTENSITY FOCUSED ULTRASOUND FOR PALLIATIVE TREATMENT OF PAINFUL BONE METASTASES**


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**Purpose:** Pain is experienced by 50%-75% of patients with bone metastases, representing a major source of morbidity amongst cancer patients. Magnetic Resonance- Guided High Intensity Focused Ultrasound (MRgHIFU) is a new, non-invasive, outpatient treatment modality for painful bone metastases. The aim of this study is to analyze urinary cytokines/chemokines pattern after MRgHIFU for palliative treatment of painful bone metastases. The findings will be compared to the cytokines/chemokines pattern post single 8 Gy fraction radiation from our previous study.

**Methods and Materials:** Urine samples were collected from patients with painful bone metastases three days before and two days after treatment with MRgHIFU. Each urine sample was tested for pro-inflammatory cytokines and anti-inflammatory cytokines. Patients received teaching on how to collect urine samples on their own. The Millipore Milliplex 42-Plex Cytokine/Chemokine Kit™ was used to measure urinary levels of a panel of cytokines/chemokines.

**Results:** Ten patients were enrolled for the study and provided urine samples three days before and two days after treatment with MRgHIFU. The following fifteen cytokines were above the level of detection (LOD) in at least 50% of patients at both pre-MRGHIFU and post-MRGHIFU: EGF, Eotaxin, Fit-3 Ligand, Fractalkine, G-CSF, GRO, IFN-alpha2, IL-1ra, IL-8, IP-10, MCP-1, PDGF-AA, RANTES, sIL-2Ra, and VEGF. Nine urinary cytokines significantly decreased post MRgHIFU, namely, Eotaxin, GRO, IL-8, IL-13, IP-10, MCP-1, MIP-1beta, RANTES, and sIL-2Ra. In addition, there were significant differences between post MRgHIFU and post-8 Gy fraction radiation in most urinary cytokines.

**Conclusions:** Nine urinary cytokines significantly decreased post MRgHIFU which correlated with pain response in patients with painful bone metastases.

**27 UTILIZING METABOLIC ALTERATIONS INDUCED BY ADIPOSE DERIVED STROMAL CELLS TO IDENTIFY NOVEL DRUGS FOR THE TREATMENT OF RADIATION FIBROSIS**

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**Purpose:** Radiation fibrosis (RF) is a side effect of radiotherapy characterized by irreversible scarring of normal tissue resulting in functional morbidity and decreased quality of life. Adipose-derived stromal cells (ADSCs) have been shown to regulate the metabolic profile of target tissue through the secretion of cytokines and growth factors. We hypothesize that ADSCs may be therapeutically effective for RF through metabolic reprogramming and that these ADSC-mediated metabolic alterations can be utilized to identify novel drugs for the treatment of RF.

**Method:** An RF model was created by irradiating the hind limb of C3H mice. This model showed a dose dependent leg contracture and histological findings of fibrosis. Primary cultured ADSCs derived from C3H mice were identified based on immunophenotyping and their differentiation potential down the mesenchymal lineage.

**Results:** ADSC transplantation was shown to be therapeutically effective for RF based on an improvement in leg contracture (p < 0.05) and reduced collagen deposition (p < 0.01). Whole transcriptome over-representation and gene set enrichment pathway analysis revealed that lipid metabolism and glycolysis were two pathways strongly dysregulated in RF and were reversed with ADSC treatment. To identify novel drugs to be repurposed for the treatment of RF, ADSC-mediated transcriptome alterations were compared with drug-mediated transcriptome alterations in CMAP, a pharmacogenomics
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 TGFB 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 analysis characterizing the types and frequencies of mutations in anal cancers from patients treated with radical chemoradiotherapy (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. McF 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 100x5x. 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 using Ion Read Inspector's Integrative Genomics 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.

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 subject numbers. 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.