Adenoid Cystic Carcinoma (ACC) is a relatively rare cancer that typically arises in salivary tissues of the head and neck. There are currently no approved systemic agents for ACC and no data supporting the delivery of chemoradiation for ACC patients. The scarcity of validated model systems has hampered research efforts. We report the establishment and propagation of an ACC patient-derived xenograft (PDX), genomic evaluation of cancer-associated mutations, and in vivo response profiles to personalized radiosensitization agents based on next-generation sequencing.

**Materials/Methods:** An ACC PDX was established and maintained in preclinical systems. PDXs were treated with focal radiation or chemoradiation at 5 Gy x 8 fractions twice weekly for 4 weeks. A set of PDXs was implanted in nude mice and expanded in secondary and tertiary generations. The PDXs were then treated with targeted therapies based on next-generation sequencing.

**Results:** Twenty ACC tumors were categorized as either radiation sensitive (RS, N=9) or radiation resistant (RR, N=11). Six were early-stage tumors (RR: N=3 and RS: N=3). Basic demographic factors were balanced between the two groups. Among all 20 samples, we found increased somatic mutations in the NOTCH pathway in RR patients (NOTCH 2: 44% vs. 0%, P=0.04; NOTCH 3 44% vs. 11%, P=0.19). In the 6 early-stage ACC PDX patients, we found all 3 RR tumors to have mutations in the KEAP1/NFE2L2 pathway, while none of the RS tumors had mutations (P=0.014).

**Conclusion:** In RR patients, there was a higher somatic mutational burden involving the NOTCH family. Interestingly, all early-stage ACC patients with RR (N=3) had mutations in the KEAP1/NRF2 oxidative stress pathway. In ACC patients, downregulation of the NOTCH1/NOTCH2 pathway may result in RT resistance. Further validation in a larger pathway. In LSCC patients, we found all 3 RR tumors to have mutations in the KEAP1/NRF2 pathway, while none of the RS tumors had mutations (P=0.014).

**Purpose/Objective(s):** Cancer stem cells (CSC) can play an important role in cancer recurrence due to their resistance to therapy. We sought to identify and characterize the CSC population in head and neck cancer cell lines and to identify approaches to improve the therapeutic index in these difficult to treat cancers.

**Materials/Methods:** CSCs were identified by their ability to grow as tumorspheres on low-attachment plates. Flow cytometry of CSCs was used to confirm CSC-specific activities. CSCs were expanded by growing tumorspheres in suspension culture. Tumorigenicity was assessed by injecting 50 and 500 cells subcutaneously in nude mice. Expression of stem cell related genes (NANOG, OCT4, SOX2) was assessed by quantitative real-time PCR and in situ hybridization. Radiation and chemotherapy were evaluated by colony formation assay.

**Results:** Sphere frequency (spheres formed/cell plated) in head and neck cancer cell lines ranged from 0.3% to 5.0% and were seen in 17 of the 19 investigated cell lines. A subset of cell lines was selected for further analysis (2 non-sphere forming and 5 sphere forming). Sphere-forming cell lines had higher ALDH activity, and a greater proportion of side-population cells than non-sphere forming cell lines. Cells derived from tumorspheres displayed higher expression of stem cell marker genes Nanog, OCT4 and SOX2 compared to differentiated attached cells. SOX2 expression was assessed in a TMA from patients with known therapy response. A trend toward higher SOX2 expression was seen in patients with disease persistence compared to those with disease control (P=ns). Data extracted from the Recurrent and Metastatic Head and Neck Cancer dataset from The Cancer Genome Atlas revealed worse overall survival in patients with alteration in SOX2 (P=0.02). Subcutaneous flank tumors were established in nude mice when injected with as few as 50 (13/16) and 500 (15/16) spheroid derived cells whereas non-CSCs did not initiate any tumors under identical conditions. Sphere-initiating cells demonstrated resistance to radiation and conventional chemotherapies compared to differentiated attached cells. Cetuximab and cisplatin were shown to have minimal effects on sphere formation and sphere frequency (average surviving fraction [SF]: 1.01 and 0.913). Radiation had a moderate effect on sphere formation and frequency in UM-SCC47 (average SF: 0.679), but had minimal effect on UM-SCC6 (average SF: 0.962).

**Conclusion:** Cancer stem cells can be identified in existing head and neck cancer cell lines, are capable of forming tumors in nude mice, and are resistant to treatments typically used for the treatment of head and neck cancer. The presence of CSCs correlates with worse outcomes in head and neck cancer patients.

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Purpose/Objective(s): We have established a repository of head and neck cancer patient-derived xenografts (PDXs) to support the translational mission of the University’s Head and Neck Cancer SPORE. We have used mutational profiling of these tumors to identify actionable cancer associated mutations to match tumor to targeted therapy.

Materials/Methods: PDXs are established with fresh tissue obtained through the University’s Translational Sciences Biocore BioBank. Tumor is implanted into nodu-SCID gamma (NSG) mice, passaged, and cryopreserved. Tumor identity is confirmed by short tandem repeat testing of patient tumor and PDX. Passage-to-passage stability is assessed by histologic and immunohistochemical analysis. Common cancer-associated mutations are assessed using an amplicon sequencing approach. Tumor size is measured over time and comparisons between treatment groups are made using the extra-sum-of-squares f test.

Results: We have established 40 head and neck cancer PDXs. These derive from a diverse group of patients reflecting heterogeneity in primary tumor site, nodal disease, and overall AJCC staging. Most (38/40) are squamous cell carcinomas, however one adenoid cystic carcinoma, and one NUT midline carcinoma have been established. HfO2-PDX comprise the majority although we have been successful at establishing 4 HPV+ PDXs. Mutational analysis has identified expected alterations based on previously reported large scale sequencing approach. Tumor size is measured over time and comparisons between treatment groups are made using the extra-sum-of-squares f test.

Conclusion: This growing repository of PDXs have been established from a broad spectrum of head and neck cancers. They represent a powerful experimental model system for investigating potential cancer therapies including personalized medicine.


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Hafnium Oxide Nanoparticles as a Promising Emergent Treatment for Head And Neck Cancer

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Purpose/Objective(s): In the treatment of head and neck cancer, tumor response has been improved by combining radiation therapy (RT) with chemical agents, radiosensitizers and monoclonal antibodies. However, these associations come with challenging limitations in terms of pharmacology, local control, clinical outcome benefits or patient quality of life. In addition, high doses of radiation may result in several undesired reactions which underline the need for new therapeutic approaches. A new class of material with high electron density, hafnium oxide, was designed at the nanoscale in the form of crystalline 50-nm particles (HfO2-NP) to efficiently absorb ionizing radiation and increase the radiation dose deposited—“hot spots” of energy deposit—from within the tumor cells to more focused and efficient cell killing.

Materials/Methods: Preclinical studies have demonstrated increase of cancer cell deaths in vitro and marked antitumor efficacy in vivo in the presence of these nanoparticles (HfO2-NP) exposed to RT, when compared to RT alone. Hafnium oxide nanoparticles efficacy was assessed in cancer epithelial and mesenchymal tumor models and on patient-derived tumor xenografts in nude mice, showing superior antitumor effects over RT alone in terms of complete response and overall survival.

Results: HfO2-NP (NBTXR3), administered as a single intratumoral injection and activated by RT, is currently evaluated in a phase 1 clinical trial for head and neck cancer [NCT01946867]. So far, patients treated in phase 1 showed good local and systemic tolerance to the product up to the highest dose level and received RT as planned, confirming a very good local safety profile. Regarding the patients, the durability of response so far is superior to 13 months, with some patients at 16 and 22 months follow-up without recurrence.

Conclusion: NBTXR3 nanoparticles constitute a rising hope for head and neck cancer patients as it could lead to a decrease in the long-term adverse effects of RT and an improvement in quality of life, associated with strong locoregional control. Besides, NBTXR3 + RT is also evaluated in clinical trials for soft tissue sarcoma, prostate, liver and rectum cancer and is showing promising results in terms of benefit-risk ratio assessment and efficacy, leading us to believe in its bright prospects for the treatment of head and neck cancer.


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The Effect of MATE1 Polymorphisms on Cisplatin Efficacy in the Treatment of Head and Neck Cancer

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Purpose/Objective(s): MATE1 (multidrug and toxin extrusion protein 1/SLC47A1) has an important role in the renal and biliary excretion of endogenous and exogenous organic cations including a number of therapeutic drugs. We recently reported that homozygosity for a single nucleotide polymorphism in MATE1 (rs2289669) (A/A) was independently protective for cisplatin-related ototoxicity in patients with head and neck squamous cell carcinoma (HNSCC) receiving cisplatin-based chemoradiation. To evaluate whether MATE1 A/A status had any effect on treatment efficacy we examined cancer outcomes in a subset of our patients expected to have a poorer prognosis.

Materials/Methods: Patients were identified from a prospective, single-center, observational cohort study of 200 HNSCC patients treated with curative intent cisplatin-based chemoradiation. Patients with HPV-related oropharyngeal and primary unknown cancers were excluded. Germline allelic variants of MATE1 were identified using TaqMan allelic discrimination assays as previously described. The disease specific survival and overall survival of patients with the ototoxic protective MATE1 homozygous A/A variant were compared to those MATE1 wild type (GG) and heterozygous (GA) using the log-rank test.

Results: A total of 10 non-HPV-related HNSCC patients were identified and included in the analysis. Median follow-up was 33 months. Twenty-eight (25.7%) patients had disease progression or recurrence and 30 (27.5%)