to assess the feasibility of automatic gross tumor volume (GTV) delineations in DW-MRI, in laryngeal cancer, and to validate them with pathology.

Materials/Methods: DW-MRI (EPI sequence, b-values = 0/150/800 s/mm2) and T2 weighted MRI scans were obtained from 14 patients before total laryngectomy. The DW-MRI scans were visually compared with the T2w MRI scans, to exclude the cases where deformations in the tumor region were larger than 3.5 mm. The GTV was delineated automatically in the DW-MRI b800 images (GTV_{DWI}), using a threshold based on the large gradient of the signal intensity around the tumor. After laryngectomy, the specimen was processed into 3 mm thick-slices and whole mount hematoxylin-eosin (H&E) sections were obtained. A pathologist delineated the tumor in the H&E sections. The specimen was then reconstructed in 3D, and registered with the preoperative imaging with accuracy within 3.5 mm. The coverage of the GTV_{PATH} with the GTV_{DWI} was calculated, according to the formula: GTVcoverage = Intersection ($GTV_{DWI,}$ GTV_{PATH})/Volume (GTV_{PATH}).

Results: 3 of the 14 DW-MRI scans did not present observable geometric distortions, and were selected for the analysis. For these patients, excellent agreement was observed between the tumor delineated in the DW-MRI images and the pathology.

Conclusions: DW-MRI based delineations and the tumor on pathology showed similar volumes, and the tumor coverage was 75%. Due to geometric distortions present on the DW-MRI images, the number of patients that could be included in this analysis was limited. Nevertheless, our results indicate that DW-MRI is a promising technique for GTV delineation in laryngeal cancer, when geometric distortions are corrected.

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3095 Translational Development of EPR Oximetry for Assessment of Tumor Hypoxia

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Purpose/Objective(s): Tumor oxygenation is one of the most important factors that affect the response to therapy. Oxygenation changes with disease progression and with therapy in a complex and unpredictable manner, so that direct measurements are needed to follow it under clinically applicable conditions. We aim to provide quantitative pO2 measurements to better enable physicians to characterize disease status and monitor the effects of therapeutic measures. With this information treatments could then be applied with optimal effectiveness by taking into account the oxygen-dependent aspects of the therapy.

Materials/Methods: We have developed an approach based on electron paramagnetic resonance (EPR) that makes it feasible to make measurements in the clinical setting under conditions compatible with clinical routines. These measurements are made using low frequency (1.2 GHz) EPR spectroscopy and surface loop resonators which enable measurements at superficial sites using India ink as an oxygen reporter. This technique can be used to provide direct and repeatable measurements of tissue pO2 with a non-invasive measurement procedure following one-time injection of India ink. Ongoing studies in human subjects include oximetry in tumors during courses of radiation and chemo-therapy, and measurement of subcutaneous pO2 in the feet of healthy volunteers to develop procedures that could be used in the treatment of peripheral vascular disease. In addition, we have developed implantable resonators in order to enable measurements of deeper tissues.

Results: Oximetry measurements have been performed in tumors of patients during courses of radiation and chemotherapy. Tumor types include melanoma, basal cell, soft tissue sarcoma, and lymphoma, and measurement sites have ranged from the feet to the scalp. Oximetry measurements of subcutaneous tissue on dorsal and plantar foot surfaces have been made in volunteers, with measurements ongoing for each and the longest set of measurements carried out successfully over the last 5 years. In vivo oximetry measurements with implantable resonators were performed in rodent and pig models at several clinically relevant sites, including the brain, to evaluate their performance characteristics and potential for clinical use.

Conclusions: These studies demonstrate the feasibility of EPR oximetry in a clinical setting and the potential for more widespread use in the treatment of these and other oxygen dependent diseases. Ongoing developments include the expansion of the technique to determine pO2 in operative fields after irradiation, in wound healing and in restorative oncological surgery, extension of the technique to measure at greater depths using implantable resonators, and the development of capabilities to make the measurements at the bedside and in the operating room.

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3096 Radiosensitization with the PARP Inhibitor ABT-888 Is Independent of PTEN or TP53 Status in Cultured Murine High-grade Astrocytes

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Purpose/Objective(s): The majority of high grade astrocytomas (HGA) are ultimately refractory to multimodality therapy. Radiosensitization with poly (ADP-ribose) polymerase (PARP) inhibitors represents a novel therapeutic approach. PARP inhibitors have demonstrated synthetic lethality in cells deficient in DNA repair, including those lacking functional PTEN. Because PTEN and TP53, a gene intimately involved in DNA damage response, are both frequently altered in HGA, we hypothesized that PARP inhibition with would selectively sensitize PTEN- or TP53-deficient HGA to XRT.

Materials/Methods: Colony formation assays (CFA) in the presence of a PARP inhibitor ABT-888 (0 - 10 μM) and/or XRT (0 - 10 Gy) were performed on AdCre-infected primary cortical astrocytes harvested from conditional, genetically-engineered mice with inactivated RB and constitutively activated KRAS, with or without concomitant inactivation of PTEN or TP53. Data were fit to the linear quadratic equation (LQE) and radiosensitization was determined either by comparing best fit LQE variables or sensitizer enhancement ratios (SER) calculated at a fractional survival of 0.1.

Results: 5μM ABT-888 was sufficient in completely ablating PARP activity in these cells, as immunoblot analysis demonstrated a complete loss of a broad band centered at 150kD representing proteins modified by variable length poly (ADP-ribose) polymer. ABT-888 inhibited proliferation of PTEN-deficient cells in the presence (IC50 0.98 μM) and absence (IC50 6.4 μM) of 5 Gy XRT. Radiosensitization with ABT-888 was independent of dosing schedule if administered prior to XRT, whereas relative survival increased to 1.56 and 2.17 when added 30 minutes and 4 hours after XRT, respectively, a finding consistent with radiosensitization being dependent on DNA repair. The effect of PARP inhibition on both cellular proliferation and radiosensitization was independent of PTEN and TP53 status: PTEN-deficient, TP53-deficient, and PTEN/TP53 wild-type cells treated with 1μM ABT-888 alone showed surviving fractions relative to no drug of 0.94, 1.1, and 0.91 (ANOVA P = 0.31); while 1μM ABT-888 showed significant radiosensitization in all three genotypes (F test P<0.001), the SER of 1.30, 1.32, and 1.28, respectively, were not significantly different (ANOVA P = 0.40).

Conclusions: Inhibition of cellular proliferation and radiosensitization of murine HGA cells with inactivated RB and activated KRAS by the PARP inhibitor ABT-888 is independent of PTEN or TP53 status. These data suggest that the previously identified correlation between PTEN status and PARP inhibitor response may not generalize to all tumor types and may depend on the spectrum of co-occurring genetic abnormalities present within individual tumors.

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3097 Therapeutic Implications of Targeting of the Sonic Hedgehog Pathway in Non-small Cell Lung Cancer

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Purpose/Objective(s): About 25% of all human tumors require Sonic Hedgehog (SHH) signaling to maintain tumor cell viability and drive tumor growth and constitutive SHH signaling is frequent in non-small cell lung cancer (NSCLC). In the cancer stem cell model, SHH signaling is important for the maintenance of these putative cancer stem cells. SHH inhibition may deplete this tumorigenic population and may have significant radiosensitizing effects. In addition, an interaction between EGFR and SHH pathway in NSCLC is postulated. Our studies focus on NSCLC cells with wild type and mutant EGFR as well as a subpopulation of lung cancer cells with elevated aldehyde dehydrogenase (ALDH) activity to select cells with stem cell-like features. Our hypothesis is that inhibition of the SHH signaling pathway by IPI-926, a small molecule inhibitor of Smo, key factor in the SHH pathway will have significant radiosensitizing effects on the stem cell-like population and may overcome resistance to EGFR inhibition in select EGFR mutant cells.

Materials/Methods: An expression array of 249 human cancer cell lines showed differential expression of a Shh-Gli gene signature and increased expression of Shh and Gli was noted by immunocytochemistry in lung cancer cell arrays showing active HH signaling. SHH pathway activity and its modulation via IPI-926 in wild-type and EGFR mutant NSCLC were examined via western blotting and real-time RT-PCR. Several NSCLC cell lines treated with IPI-926 and radiation demonstrated decreased colony formation in soft agar as well as decreased colony formation in clonogenic assays. When evaluating CSC-like properties of ALDH+ cells in lung cancer cells, sorted ALDH+ cells demonstrated enhanced colony formation compared to ALDH- cells.

Results: Lung cancer cells treated with chemotherapeutic agents exhibited a 2 - 3 fold increase in the fraction of ALDH+ cells which showed enhanced radioresistance. Treatment with IPI-926 significantly decreased the selective enrichment of stem cell-like population among the tumor cells after radiation treatment and furthermore, ALDH+ cells exhibited increased radiosensitization.

Conclusions: The stem-cell like population in NSCLC is not directly targeted by standard chemotherapeutic and radiotherapeutic approaches however targeting of the SHH pathway via inhibition of Smo may be an effective treatment approach to improve patient survival.

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3098 WITHDRAWN

3099 Whole Brain Radiotherapy and Chloroquine in Patients with Brain Metastases: Outcomes and Response related to IDO2 Gene Single-Nucleotide Polymorphisms

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Purpose/Objective(s): Chloroquine (CQ), has been shown to enhance cellular sensitivity to radiotherapy. IDO2 (indoleamine 2,3-dioxygenase 2) is an immunomodulatory enzyme that has been implicated in cancer progression. Studies suggest that