for IA vs IB, p=0.29). Patients with a high-risk PS (>27) had a three-fold increased risk of distant recurrence compared to patients with a low-risk PS score. In stage I patients, the estimated risk of distant recurrence for tumors with high-risk prognostic scores was significantly higher (36%, 95%CI 28%-46%) than in low-risk PS tumors (15%, 95%CI 8%-29%, p=0.0005).

**Conclusions:** The prediction of risk of distant recurrence in resected lung adenocarcinoma patients can be improved over that obtained by the current standard of pathological stage by incorporating tumor expression of proliferation markers (CCP score). Improved risk stratification can help identify patients in need of additional treatment and prioritize patients for trials of emerging new therapies.

**Strong pharmacological activity of locally administered next-generation antisense oligonucleotides (ASOs) in orthotopic lung cancer mouse models**

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Lung cancer is the second most common cancer type and the leading cause of cancer-related death in both men and women in the United States. Although recent advances in genomic expression profiling of lung cancer has provided more individualized treatment based on target specific genetic alteration(s) in patients, there is a still high unmet medical need as many important driver mutations remain undruggable by conventional therapeutic approaches. Antisense oligonucleotide (ASO) technology offers a novel therapeutic modality to selectively target currently undruggable pathways. We have recently demonstrated that systemic delivery of unformulated next generation (Gen 2.5) ASO targeting human STAT3 mRNA (STAT3-Rx/AZD9150) leads to robust pharmacological activity in various preclinical animal models of lung cancer. Importantly, STAT3-Rx/AZD9150 displayed strong anti-tumor activity in patients with advanced treatment-refractory lymphoma as well as non-small cell lung cancer (NSCLC) in clinical trials*.

Although local delivery of ASOs has been proposed as a means to treat various pulmonary diseases, ASO pharmacology in lung tumors has not been previously evaluated. In this study, we evaluated the activity of an antisense inhibitor (targeting MALAT1 gene) given by different administration routes (by either a local (intratracheally=IT) or a systemic (subcutaneously=SC) delivery) in orthotopic lung cancer xenograft models. For this, human NSCLC A549 or H460 cells were injected to nude mice via tail vein and 2 weeks later, the tumor-bearing animals were treated with Gen 2.5 MALAT1 ASO either IT (at 10 to 20 mg/kg, 3 times per week) or SC (at 25 to 50 mg/kg, 5 times per week) for 3 weeks. Reductions in MALAT1 RNA levels in human tumor cells and mouse lung were greater with local delivery at lower doses compared to systemic delivery as assessed by qRT-PCR or in-situ hybridization using species-specific primers/probes (73% reduction at 20 mg/kg by IT dosing vs 50.5% reduction at 50 mg/kg by SC dosing in tumors; 72.5% vs 70% reduction in mouse lung at the same dosing regimen). Moreover, local administration of ASOs led to no notable increase in liver transaminase levels compared to systemic administration. Pharmacokinetic property-wise, the local administration showed greater and more even accumulation of drug in both tumor and lung tissues compared to the systemic route. Taken together, these findings suggest that local administration of Gen2.5 ASOs to lung malignancies may be a promising alternative approach to systemic delivery with greater potency and improved safety profile, especially in inhibiting lung cancer targets which cannot be tolerated when depleted systemically.


**Impact of interleukin-22 on K-ras mutant lung cancer promotion and stemness properties**

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Activating mutations of K-ras, which are found in approximately 30% of non-small cell lung cancer (NSCLC), are the most common genetic alterations associated with tobacco exposure in lung cancer. Unfortunately, pharmacologic attempts directly targeting K-ras have thus far failed; clearly stating the need for new strategies to bring clinical benefits to patients displaying such a molecular profile. Tumor-promoting inflammation is a cancer hallmark, and it is now apparent that the cytokines and growth factors released during inflammation influence
cancerogenesis. Using a conditional K-ras induced lung cancer mouse model, CC-LR (CCSPCre/LSL-K-rasG12D), we previously showed that K-ras mutant lung tumors have intrinsic inflammatory characteristics. This was associated with activation of NF-κB pathway, release of inflammatory cytokines IL-6, and IL-17A, and activation of the IL-6 responsive transcription factor STAT3. We have further shown that IL-6/STAT3 pathway, and IL-17 producing CD4 helper T cells (Th17 cells) through their main cytokine, IL-17A, play critical roles in promotion of lung cancer in this model. Interleukin-22 (IL-22) is another effector molecule secreted by Th17 cells which is highly expressed and produced in our K-ras mutant mouse model. IL-22 is a unique cytokine in the IL-10 family which seems to act exclusively on nonhematopoietic cells, with basal IL-22R expression in the epithelial cells and fibroblast, and mostly signals through STAT3 pathway. We have found that genetic ablation of IL-22 in CC-LR mice (CC-LR/IL22-KO mice) results in a significant reduction in lung surface tumor numbers by 54% (2.1-fold) compared to age and sex matched control CC-LR mice. Histopathological analysis of H&E stained lung sections also confirmed reduction in number and size of tumors and less adenomatous lesions in CC-LR/IL22-KO mice compared to CC-LR mice. Immunohistochemical staining of lung tissues with specific markers, Ki-67, CD-31 and pSTAT3 demonstrated significantly lower tumor cell proliferation, angiogenesis and STAT3 activation in CC-LR/IL22-KO mice. IL-22 ablation also reduced the numbers of inflammatory cells in bronchoalveolar lavage fluid, decreased the expression of pro-tumor inflammatory cytokines such as IL-6, IL-17 and TNFα, and increased expression of anti-tumor inflammatory cytokines such as IFNγ. Recent studies have shown an association between IL-22 and stem-cell like properties in colon cancer. In lung cancer, populations expressing NANOG, SOX2, Oct4 and/or aldehyde dehydrogenase activity are enriched with stemness properties. Interestingly, in CC-LR/IL22-KO mice we found significant reduction in expression of NANOG, SOX2 and Oct4. Thus, we conclude that IL-22 promotes K-ras mutant lung tumorigenesis by inducing a pro-tumor inflammatory microenvironment with proliferative and angiogenic properties as well as protecting stemness characteristic in epithelial/tumor cells. Therefore, we propose pharmacological targeting of IL-22 as a potential therapeutic strategy in combination with conventional cytotoxic therapy, immune check point blockade, or other targeted therapies (e.g. MEK inhibition) for lung cancer patients with K-ras mutation.

Determination of real-time tumor oxygenation changes following high-dose radiotherapy in orthotopic and subcutaneous lung cancers in mice

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Purpose: To investigate serial changes of tumor hypoxia in response to ablative radiation treatment by using various clinical and pre-clinical methods in order to propose an optimal fractionation schedule for stereotactic ablative radiotherapy (SABR).

Methods and Materials: Syngeneic Lewis lung carcinomas were grown either orthotopically or subcutaneously in C57BL/6 mice and were irradiated with a single dose of 15 Gy to mimic SABR used in the clinic. Serial [18F]-misonidazole (F-MISO) positron emission tomography (PET) imaging, pimonidazole FACS analyses, hypoxia-responsive element (HRE)-driven bioluminescence, and Hoechst 33342 perfusion were performed before irradiation (d-1), at 6 hours (d0), 2 (d2), and 6 days (d6) after irradiation for both subcutaneous and orthotopic lung tumors. For F-MISO, the tumor-to-background activity ratio (TBR) was calculated.

Results: We observed that hypoxic signals were too low to quantitate for orthotopic tumors by F-MISO PET and HRE-driven bioluminescence imaging. In subcutaneous tumors we observed that TBR values were 2.87 ± 0.483 at d-1, 1.67 ± 0.116 at d0, 2.92 ± 0.334 at d2, and 2.13 ± 0.385 at d6, indicating that tumor hypoxia decreased after irradiation and returned to the pretreatment levels and then slightly decreased by 2 and 6 days post-radiation, respectively. Pimonidazole analysis also revealed similar patterns. By using Hoechst 33342 vascular perfusion dye and CD31 co-immunostaining, we found that there was a rapid and transient vascular collapse, which may have resulted in poor intratumoral perfusion of F-MISO PET tracer and pimonidazole at d0 hence leading to the decreased hypoxic signals.

Conclusions: We found tumor hypoxia levels to be returned to the pretreatment levels by 2 days after irradiation, hence supporting the use of current fractionation intervals of SABR being given at least 2 days. Our results also indicate that SABR may produce a rapid and transient vascular collapse in tumors.