carcinogenesis. 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-kB 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 a cytokine which is highly expressed and produced in our K-ras 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 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**

**Changhoon Song,1 Beom-Ju Hong,2 Seoyeon Bok,2 Chan-Ju Lee,2 Young-Eun Kim,2 Sang-Rok Jeon,1 Yun-Sang Lee,1 Gi Jeong Cheon,1 Jin Chul Paeng,1 G-One Ahn,2 Hak Jae Kim1,1 Seoul National University College of Medicine, Seoul, Korea, 2Pohang University of Science and Technology, Pohang, Korea**

**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.