and be effectively combined with immunotherapeutic agents across oncology. More tissue samples are under evaluation to reinforce these findings.

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Anti-PD-1 Immunotherapy Potentiates the Radiation-Induced Lung Injury

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Purpose/Objective(s): Combination of thoracic radiotherapy and anti-PD1 immunotherapy has been investigated both in the lab and in the clinic. Pneumonitis is a rare but potentially fatal toxicity of ant-programmed death-1 (PD-1) monoclonal antibody (mAb). The purpose of this study is to address whether concurrent anti-PD-1 mAb during thoracic radiation leads to an increase in lung toxicity and mortality using a murine model using Small Animal Radiation Research Platform (SARRP) for lung-targeting irradiation (LIR) and the underlying mechanisms.

Materials/Methods: Both lungs of male C57bl/6 mice were targeted for 20Gy using the SARRP. Mice were stratified into 4 treatment groups receiving IgG, anti-PD1, LIR + IgG, or LIR + anti-PD1. IgG or anti-PD-1 mAb was administrated via i.p. injection, with a dosage of 10mg/kg, twice per week for five doses. The long-term survival was observed. Acute and late lung injuries were assessed at several time points by H&E staining; Masson's Trichrome staining and hydroxyproline for lung fibrosis, micro CT scan and lung physiological testing. The infiltration of lymphocytes and the expression of cytokines in irradiated lung tissues were measured by immunohistochemistry staining and RT-PCR, respectively. Furthermore, we examined the roles of CD4, CD8, macrophages and TGF-β1 by individual depletion using neutralizing antibody.

Results: LIR+anti-PD-1 led to worse survival with a median survival time of 18 weeks , compared with that of 31 weeks in the LIR+ IgG group ($p<0.05$). In the acute phase (4 weeks following LIR), LIR+anti-PD-1 treated mice showed more severe inflammation, abnormal alveoli and increased pulmonary resistance compared with LIR+ IgG. In the late phase (22～24 weeks following LIR), LIR+anti-PD-1 treated mice showed more severe fibrosis, increased lung density and resistance, as well as decreased compliance of the lung. LIR+anti-PD-1 mice had increased numbers of $CD8^+$ T cells and macrophages that are strongly positive for TGF-β1 in the lung tissues. Furthermore, depletion of CD8 lymphocytes (median survival 21 weeks), macrophages (median survival 23 weeks) or TGF-β1 (median survival 28 weeks) attenuated the mortality of the LIR+anti-PD-1 treated mice (median survival 17 weeks).

Conclusion: Concurrent anti-PD-1 mAb during thoracic radiation leads to an increase in lung toxicity and the consequent mortality, accompanied by increased $CD8^+$ T lymphocytes and macrophages with strong positivity for TGF-β1. Blocking TGF-β1 significantly attenuates mortality from LIR+anti-PD-1. Lung toxicities should be closely monitored in ongoing clinical trials of concurrent combination of thoracic RT and anti-PD-1 therapy.

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Combination of Radiation Therapy and Intratumoral IL-12/GM-CSF Leads to Systemic Toxicity in Pet Dog Subjects with Refractory Solid Tumors

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Purpose/Objective(s): We recently developed a potent cancer immunotherapy protocol that involves tumor-directed irradiation combined with intratumoral injection of adenoviruses encoding human IL-12 and GM-CSF. The combination protocol was safe and effective in multiple murine models. In this research, we conducted a phase I trial to determine its safety profile in pet dogs.

Materials/Methods: This study was approved by institutional animal care and use committee of National Taiwan University. The protocol followed traditional "3 + 3" dose escalation design to enroll 3 canine subjects with refractory solid tumors per dose level of adenovirus cocktail. The initial dose was set to 0.5×10^9 cfu for both IL-12 and GM-CSF, which is the total dose used in murine experiments. For combination therapy, 10 gray of radiation was delivered via a 6 MV beam with an linear accelerator. After irradiation, adenoviruses were immediately injected into the treated tumor. Clinical manifestation, blood samples, and CT scans were obtained along the course. The primary endpoints were safety profiles.

Results: A total of 3 pet dog subjects were enrolled. These subjects have metastatic orbital fibrosarcoma, recurrent malignant peripheral nerve sheath sarcoma of the forelimb, and metastatic buccal melanoma respectively. After treatment, thrombocytopenia was found in all subjects. 2 out of 3 subjects developed severe adverse effects, including 2 grade 5 hematological toxicities and 1 grade 3 cardiac toxicity. Immunophenotyping excluded pathogenic roles of adenovirally encoded human cytokines and suggested IL-6 is a major cytokine induced by the combination therapy. Furthermore, cytokine release syndrome induced by the combination therapy was successfully controlled with tocilizumab. In one subject with malignant peripheral nerve sheath sarcoma, significant partial response was noted 6 months after the procedure. The protocol was prematurely terminated due to high mortality rate.

Conclusion: We found significant immune-related systemic toxicity in a locally delivered radiation-immunotherapy for refractory solid tumors in pet dogs. The discordant safety profiles between murine models and canine subjects highlight the risk of translating potent radiation-immunotherapy combination into clinical settings. Further research is warranted to develop early toxicity biomarkers in species of interest that guide timely immunosuppression in translational immuno-oncology platforms.

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