the combination of Pembrolizumab and HFRT. The secondary objective was the radiographic response of metastatic lesions outside the radiation field as measured by RECIST.

Results: A total of 59 patients aged 27-90 years (median 60) were enrolled from March 2015 to December 2018 (24 in the Safety Phase and 35 in Expansion Phase). 40 patients (67.7%) had treatment-related AEs, of which 4 were grade 3 and none were grade 4. One patient experienced hepatitis, classified as DLT. While most patients did not have a radiologic response, in patients with metastatic melanoma, 7 of 16 (43.8%, exact 95% CI 19.8-70.1%) had an objective response of non-irradiated lesions to HFRT + pembrolizumab, including 3 complete and 4 partial responses. Responses are durable with 3/3 complete responders alive with no progression, and 3/4 partial responders alive with 2 having no evidence of progression. Among melanoma patients, only 2 of 7 (29%) responders received ipilimumab prior to enrollment, compared to 8 of 9 (89%) non-responders ($P = 0.035$). An increase in Ki67+ PD-1+ non-naïve CD8 T-cells was observed in the blood 2 weeks after HFRT, but the magnitude did not correlate with likelihood of response. Responses were observed after either 17 Gy x 1 fraction or 8 Gy x 3 fractions, with no difference in response rate by fractionation.

Conclusion: This study suggests that HFRT administered with concurrent pembrolizumab is associated with acceptable toxicity and that in patients with metastatic melanoma progressing on anti-PD-1 therapy, this approach yields an ORR of 44%.

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Combined Stereotactic Ablative Radiotherapy and Fibroblast Activation Protein Based Whole Cell Tumor Vaccine Synergize to Suppress Tumor Growth and Metastasis

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Purpose/Objective(s): Cancer-associated fibroblasts (CAFs), which occupy a major portion of the TME, play an important role in tumor growth, immunosuppression, treatment resistance. Our studies found that fibroblast activation protein α (FAPα) based whole cell tumor vaccine (WCTV) can induce a cross-immune response to produce specific neutralizing antibodies and CTL effects to kill tumor cells and CAFs. However, the therapeutic efficacy of vaccination is limited. Recent studies have revealed that stereotactic ablation radiotherapy (SABR) also elicit systemic antitumor immunity, which makes it an ideal partner for immunotherapy. The goal of this study was to examine synergistic effect of SABR and FAP-based WCTV.

Materials/Methods: We produced FAPα-based WCTV by infecting tumor cells with lentiviral vector pCDH encoding FAPα, and then inactivating them with radiation (100Gy) for immunotherapy **in vivo**. To investigate the optimal protocol, we compared different SABR schemes and sequence of the combination of irradiation and FAPα- based WCTV in mouse LLC Lewis lung cancer model. Subsequently, we tested whether the combination protocol would result in enhanced anti-tumor activity and antifibrotic effect in mouse 4T1 breast cancer and CT26 colon cancer models. Furthermore, we analyzed the impact of treatment on cellular immunophenotypic characterization in TME via immunohistochemical staining and flow cytometry.

Results: We demonstrated that the therapeutic effect of SABR combined with FAPα-based WCTV is better than radiotherapy or vaccination alone. We identified the optimal dose scheme of SABR synergistically with FAPα- based WCTV is a single dose of 8Gy × 3 times, and the best treatment sequence is radiotherapy within one week after the first vaccination (three times in total). The optimal combination protocol also resulted in enhanced anti-tumor activity and antifibrotic effect against 4T1 breast cancer and CT26 colon cancer, and inhibited lung metastasis in breast cancer. No adverse effects were found based on gross measures of health. Importantly, collagen type I and FAPα expression in tumor tissue was significantly lower in SABR+ Vaccine treated mice than in control mice. SABR combined with FAPα-based WCTV reduced microvascular and lymphatic invasion, allowed polarization of tumor-associated macrophages to the M1-like phenotype. The relative percentage of M2 macrophages, MDSCs, and Tregs significantly decreased in the peripheral blood and lymph nodes. By contrast, CD8+ T lymphocytes especially activated cells increased.

Conclusion: Our findings indicate that combined FAPα-based WCTV and SABR synergize to suppress tumor growth and metastasis via activating the systemic anti-tumor immune response, attenuating CAFs-mediated immunosuppression, interstitial fibrosis and treatment resistance. This novel combination of radio-immunotherapy may add a new perspective to cancer treatments.

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Overcoming Resistance to Anti-PD-1 With Tumor Agnostic NBTXR3: From Bench to Bedside

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Purpose/Objective(s): Immune checkpoint inhibitors (ICI) can improve outcomes in patients who respond to treatment, however most patients exhibit resistance. Overcoming this resistance is the main challenge in immune-oncology and recent studies suggest radiotherapy (RT) may improve ICI response rates. NBTXR3, composed of functionalized hafnium oxide nanoparticles, is injected intratumorally and activated by RT. NBTXR3 increases RT energy deposit inside tumor cells and subsequent tumor cell death, without adding toxicity to healthy tissues. Here we present evidence that NBTXR3 activated by RT primes the immune system, producing an anti-tumor immune response, including activation of the cGAS-STING pathway, that overcomes anti-PD-1 resistance both in murine models and patients.

Materials/Methods: Abscopal assays were conducted in immunocompetent mice. Anti-PD-1 sensitive or resistant lung tumor cell lines were injected in both flanks. Intratumoral injection of NBTXR3 (or vehicle) followed by RT was performed in right flank (primary) tumors only. Some mice also received anti-PD-1 injections. Tumor growth was monitored, and tumor immune cell infiltrates analyzed by immunohistochemistry (IHC). Separately, in the phase II/III randomized Act.in.Sarc [NCT02379845] trial patients with locally advanced soft tissue sarcoma (STS) received either NBTXR3+RT or RT alone followed by tumor resection. Pre- and post-treatment tumor samples from patients in both groups were analyzed by IHC and Digital Pathology for immune biomarkers. The safety and efficacy of NBTXR3 plus stereotactic body radiotherapy (SBRT) in combination with anti-PD-1 is being evaluated in three cohorts of patients with advanced cancers in the Phase I 1100 [NCT03589339] trial.
Results: Pre-clinical studies demonstrated that NBTXR3+RT induces an immune response not observed with RT alone and enhances systemic control. IHC showed significant increase of CD8+ T-cell infiltrates in both NBTXR3+RT treated and untreated tumors compared to RT alone. Increased CD8+ T-cell and decreased FOXP3+ Treg density (pre- vs post-treatment) was also observed in tumors from STS patients treated with NBTXR3+RT. Furthermore, NBTXR3+RT in combination with anti-PD-1 improved local and systemic control in mice bearing anti-PD-1 resistant lung tumors, produced long-term memory, and reduced spontaneous lung metastases. Preliminary efficacy data from the 1100 trial showed tumor regression in 8/9 patients. Of note, tumor regression was observed in 6/7 patients who had progressed on prior anti-PD-1.
Conclusion: The clinical efficacy of NBTXR3+RT has been demonstrated as a single agent in STS. Here we demonstrate that it overcomes resistance to anti-PD-1 treatment mechanisms in mice and led to tumor regression in patients having progressed on anti-PD-1 therapy. These results highlight the potential of NBTXR3+RT to positively impact the immuno-oncology field.

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Activation of Sting in Response to Partial-Tumor Radiation Exposure

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Purpose/Objective(s): It has been shown that ionizing radiation can mediate antitumor immunity **via** the activation of the cytosolic DNA sensor cGAS/STING pathway. Therefore, the purpose of this study, was to determine whether STING activation is involved in partial volume radiation therapy (RT).

Materials/Methods: We investigated 67NR murine orthotopic breast tumors in Balb/c mice and LLC cells injected in the flank of $C_{57}Bl/_6$, cGAS or STING KO mice. RT was delivered to 50% or 100% of the tumor volume using a 2×2 cm collimator on a microirradiator allowing precise irradiation. Tumors were collected at different time points post-RT and assessed for different measurements.
Results: We previously showed that a single dose of radiation delivered to half of the tumor (50% RT) activated an anti-tumor immune response comparable to the response in a fully-irradiated tumor in the immunogenic 67NR murine breast carcinoma tumor model and in the less immunogenic and more radioresistant Lewis lung carcinoma (LLC) tumor model. We have also demonstrated that this immune response was due to the infiltration of $CD8^+$ T cells along with an increased expression of ICAM adhesion molecules. Treatment with either anti-CD8 or anti-ICAM antibodies abrogated the hemi-RT response. Furthermore, a significant abscopal effect was observed after partial irradiation with a single dose of 10Gy in a bilateral 67NR tumors model. In this study we tested whether the hemi-irradiation-mediated immune response involves the cGAS/STING canonical pathway, or a non-canonical activation of STING, in the 67NR or LLC tumor models. It has been reported that STING can be activated, independently of cGAS, **via** non-canonical activation of STING, involving ATM and TRAF6, among other factors. We found a significant activation of the cGAS/STING pathway in the hemi-irradiated tumors as compared to control and to 100% exposed 67NR tumors. Interestingly, the increased expression of the cGAS/STING pathway was found in the hemi-irradiated tumor but, also in the non-irradiated part of this hemi-irradiated tumor. In the LLC model, a non-canonical activation of STING was involved. Using both cGAS and STING KO mice, we demonstrated that the partial exposure RT-mediated immune response is dependent on STING activation in the host while cGAS is dispensable.
Conclusion: Upstream pathways responsible for STING activation are tumor type specific. Identifying the upstream pathways responsible for STING activation in the partial RT-mediated immune response in different tumor types would improve this therapy and its potential combination with immune checkpoint blockade.

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Inhibiting ATR Modulates Response to Radiation and Stimulates Immune Responses

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Purpose/Objective(s): Radiotherapy (RT) has been shown, albeit rarely, to stimulate anti-tumor immune responses. Recent evidence suggests this can, in part, be attributed to the accumulation of cytoplasmic DNA in the form of micronuclei (MN) following RT, which can stimulate IRF3 and NF-κB immune signaling that recruits various immune components to the tumor microenvironment. We hypothesized this effect is increased by combining RT with AZD6738, an inhibitor of ATR, which is a key protein in DNA repair and cell cycle regulation. Furthermore, we propose that the more clustered DNA damage caused by protons results in a higher level of MN than photons when these radiation types are combined with AZD6738.
Materials/Methods: H1299 and PANC-1 cells were treated with 6 MV x-rays and 9.9 keV/mm (dose-weighted LET in water) protons alone or with AZD6738 (1 μM) to assess DNA repair, cell cycle distribution, MN and c-