Pharmacological Inhibition of PD-1 Exacerbates Radiation-Induced Cardiac Toxicity Through Cytotoxic T Cell–Mediated Myocarditis

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Purpose/Objective(s): It has been demonstrated that anti-PD-1 immunotherapy may have potentially life-threatening side effect (i.e., autoimmune myocarditis with consequent cardiac failure). The purpose of this work was to determine the incidence and severity of radiation-induced cardiac mortality and morbidity in a murine model when cardiac irradiation is delivered concurrently with an anti-PD-1 antibody.

Materials/Methods: Single fraction (20 Gy) was delivered specifically to the heart of female C57b1/6 mice with an image-guided SARRP, which allows on-board micro-CT imaging and real-time treatment planning. Mice were stratified into 4 treatment groups: (1) control IgG (n = 10); (2) anti-PD1 antibody (n = 10); (3) RT+ control IgG (n = 20); and (4) RT + anti-PD1 antibody (n = 20). Anti-CD4 and anti-CD8 antibodies were combined with RT and anti-PD1 antibody treatment to deplete subsets of T cells. Antibodies were intraperitoneally administered 24 hours before RT, and then every other day. Overall survival was determined. Echocardiographic assessment on all mice was performed to measure the cardiac contraction and output when clinical morbidity was encountered. The sick mice were then sacrificed for tissue collections, which were characterized by immunofluorescence for immune cells infiltration.

Results: Decreased survival was observed when anti-PD1 was combined with RT: 35% (7/20) mortality vs. 0% mortality in other three groups (P < 0.05). The median survival following RT in the RT + Anti-PD1 group was day 8 (day 5 to 19). Ejection fraction (EF) and fractional shortening (FS) were used to measure cardiac function. Combined therapy group exhibited significantly decline in both EF and FS, especially compared with mice treated with RT + IgG control: the EFs of group 1 to 4: 62.43% ± 7.54%, 60.19% ± 5.81%, 50.85% ± 5.06%, and 38.84% ± 6.06%, P < 0.01; the FSs: 33.48% ± 5.48%, 31.66% ± 4.09%, 25.50% ± 3.13%, and 18.58% ± 3.28%, P < 0.01. These data indicates that 20 Gy induced cardiac dysfunction, which was exacerbates by anti-PD1 treatment. Myocarditis was observed in the dying mice after RT. Further immunologic data on heart tissue changed showed increased tumor infiltration by CD45, CD3, CD4, and CD8. Consistent with worse cardiac dysfunction, considerably more immune cells were observed in heart tissues of the dying mice from RT+ anti-PD1 compared with those from RT plus control IgG. To test which subset of T cells contributes to the myocarditis, neutralizing antibodies against CD4 and CD8 were combined with RT and anti-PD1 treatment. Consistently, depletion of CD8 T cells prevented mortality from RT plus anti-PD1 whereas depletion of CD4 did not, indicating that CD8+ lymphocytes mediates cardiac injury from RT + anti-PD1.

Conclusion: The combination of PD-1 blockade increases the cardiac toxicity from irradiation through cytotoxic T cell-mediated myocarditis. Our study provides strong preclinical evidence that immune-related adverse events should be considered when PD-1 inhibitor is combined with RT.

Does Prophylactic Nodal Irradiation Inhibit Potential Synergy Between Radiation Therapy and Immunotherapy?

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Purpose/Objective(s): Prophylactic nodal irradiation (PNI) is a strategy used to treat early stage cancers to enhance local control and prevent metastatic spread. It remains unclear whether irradiation of tumor-associated draining lymph nodes (DLN) influences anti-tumor adaptive immune responses. Our aim is to investigate the in vivo effects of nodal irradiation on phenotype/function of tumor infiltrating lymphocytes and the impact of PNI on the anti-tumor effects of immunotherapy.

Materials/Methods: The Small Animal Radiation Research Platform (SARRP) delivered imaging-guided stereotactic radiation to tumor (T-only), DLN or both (T + LN). Syngeneic tumors (MC38 colon, B16 melanoma) were implanted in C57B1/6 mice and irradiated, 12 Gy x1. After radiation, the tumor microenvironment (TME) was assayed by flow cytometry. Adoptive transfer experiments were performed using OVA-specific CD8+ T-cells from Rag-/- OT-1 mice. Tumor lysate was collected for chemokine analysis.

Results: Inclusion of the DLN (T + LN) did not significantly impact tumor control compared with T-only radiation (P = 0.3). Despite similar tumor growth delay between T-only and T + LN, a significant increase (P < 0.01) in the proportion and absolute number of CD8+ effector T-cells in the TME was observed with T-only radiation. Conversely, T-only radiation increased TME infiltration of immunosuppressive regulatory T-cells (Tregs) and myeloid-derived suppressor cells. Ultimately, intra-tumoral CD8 effector: Treg ratio was similarly favorable between T-only (7.3 +/- 0.1) and T+LN (6.0 +/- 0.2) relative to untreated tumors (2.9 +/- 0.1); however, the absolute number of infiltrating CD8 effectors and Tregs was ~4-fold higher in the T-only group. To evaluate the impact of the radiation target on CD8 effector function, naïve OVA-specific T-cells were adoptively transferred into MC38-OVA tumor-bearing mice. The proportion of INFγ+ and TNFα+ tumor-antigen specific CD8 T-cells was significantly higher (P < 0.001) among mice treated with T + LN compared with T-only. Taken together, these data suggest that PNI (T + LN) promotes effector function of tumor-antigen specific CD8 T-cells while T-only numerically enhances immune infiltration of the TME. Mechanistically, we observed that the radiation target significantly modulated TME chemokine expression, with T-cell chemotactics CCL3/4/5 and CXCL10, all significantly increased (P < 0.05) in T-only irradiated samples.

Conclusion: We have successfully developed a SARRP-based early stage cancer model with the ability to target or spare the tumor-associated DLN. Results to date demonstrate significant immunological differences that are contingent upon inclusion/exclusion of the DLN. Future survival experiments combining αPD-1 and αCTLA-4 with T-only and T + LN will elucidate which radiation target will optimally synergize with immune checkpoint blockade. This preclinical model will promote rational design of future clinical studies that combine immunotherapy and radiotherapy.