The antitumor immune response generated by fractionated radiation therapy may be limited by tumor cell adaptive resistance and can be circumvented by PD-L1 blockade

S J Dovedi* and T M Illidge
Targeted Therapy Group; Institute of Cancer Sciences; Manchester Cancer Research Center; University of Manchester; Manchester Academic Health Sciences Center; Manchester, United Kingdom

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Fractionated radiation therapy (RT) leads to adaptive changes in the tumor microenvironment that may limit the generation of an antitumor immune response. We demonstrated that fractionated RT led to increased tumor cell expression of programmed cell death ligand 1 (PD-L1) in response to CD8\textsuperscript{+} T cell production of interferon gamma. Our data reveal that the efficacy of fractionated RT can be significantly improved through the generation of durable systemic immune responses when combined with concurrent, but not sequential, blockade of the PD-1/PD-L1 pathway.

Radiation therapy (RT) is delivered to approximately 50–60% of all cancer patients and is a component of curative treatment in approximately 40% of cancer patients. Several studies demonstrate that, in addition to its direct cytoreductive effect, RT-induced cell death can be immunogenic. Treatment with RT leads to the release of damage-associated molecular patterns (DAMPs) by cancer cells that can facilitate the recruitment and activation of antigen presenting cells (APC), such as dendritic cells (DCs), and priming of tumor antigen-specific T cells (reviewed in ref. 1\textsuperscript{1}). The contribution of the anticancer immune response to the clinical efficacy of RT is under investigation; however, generation of systemic immunity and the “abscopal effect” are rare in clinical practice. Preclinical data demonstrate that whereas vaccination of mice with irradiated tumor cells can lead to potent T-cell priming, in situ vaccination of established experimental tumors with RT alone is rarely able to induce durable systemic antitumor immune responses.\textsuperscript{2,3} This lack of a durable immune response to RT in established tumors is thought to be a consequence of an immunosuppressive tumor microenvironment that may limit the magnitude and durability of the anticancer immune response.\textsuperscript{4} This immune failure may contribute to disease recurrence, which is the leading cause of death following RT.

To date, the majority of preclinical studies have investigated the effect of a large single dose of RT on the generation of systemic immune responses. Despite substantial improvements in image-guided stereotactic body radiation therapy (SBRT), which permits the delivery of larger doses of RT over a much shorter time period (hypofractionation), and in the administration of a single large ablative dose, the majority of RT in the clinic is delivered in multiple daily fractions of approximately 1.8–2 Gy. However, relatively few studies have addressed the immunogenicity of fractionated RT with this lower dose (reviewed in \textsuperscript{5}), therefore this was the focus of our study.

We conducted phenotypic analysis of the tumor microenvironment following treatment with fractionated RT to identify drivers of immunosuppression. Our data revealed that local fractionated RT to the tumor led to upregulation of programmed cell death ligand (PD-L)-1 on cancer cells in vivo. Through binding its cognate receptor PD-1 expressed on lymphocytes, PD-L1 is involved in the maintenance of peripheral tolerance and modulation of acute inflammatory responses through inhibition of T-cell receptor (TCR) signal transduction, leading to apoptosis of antigen-specific T-cells.\textsuperscript{6,7} Our studies revealed that PD-L1 expression was induced on tumor cells in vivo, but not in vitro, following RT. Given this disparity, we evaluated the contribution of the local tumor microenvironment to the expression of PD-L1 by tumor cells. We determined that RT led to CD8\textsuperscript{+} T-cell production of interferon.
gamma (IFNγ), which was responsible for the upregulation of tumor cell PD-L1 expression. These data reveal that tumor cells can adapt to the microenvironmental changes following RT to circumvent T-cell immunity, potentially contributing to treatment failure. Moreover, treatment with RT also led to increased expression of PD-L1 on cells with a myeloid-derived suppressor cell (MDSC) phenotype (CD11b+Gr1hi), further demonstrating the potential of blockade of the PD-1/PD-L1 axis to improve the effectiveness of RT. Using syngeneic models of melanoma, colorectal cancer, and triple negative breast cancer, we demonstrated that the efficacy of RT can be enhanced when delivered in combination with monoclonal antibody (mAb) targeted against PD-1 or PD-L1. The efficacy of combination therapy was dependent on the activity of CD8+ T cells, and long-term (>100 days) surviving mice were protected against disease recurrence by the generation of a tumor antigen-specific memory immune response. In a separate preclinical study, Deng and colleagues demonstrated that high-dose RT (12 or 20 Gy) led to increased expression of PD-L1 on the surface of both tumor cells and DCs, and that anti-PD-L1 treatment improved the efficacy of RT. Together, these studies rationalize blockade of the PD-1/PD-L1 axis to improve outcomes in patients receiving SBRT or low-dose fractionated RT (see Fig. 1).

A critical question to support translation of this therapeutic approach is which combination schedule should be adopted for maximal immunogenicity. To address this issue we tested 3 different regimens: (1) concurrent RT and αPD-L1 mAb starting at the beginning of the RT cycle, (2) concurrent RT and αPD-L1 mAb starting at the end of the RT cycle, and (3) sequential therapy with αPD-L1 mAb administered 7 d after the completion of RT. We showed that concurrent therapy was optimal for generating effective antitumor immunity and long-term tumor control. In contrast, the efficacy of sequential therapy was not significantly different to that of RT alone. Measurement of the kinetics of tumor cell PD-L1 expression revealed an acute increase following RT that persisted for at least 7 d after completion of the RT cycle. In addition, we showed that the expression of PD-1 on...
tumor-infiltrating T cells, which has been shown to identify the tumor antigen-specific T-cell repertoire, is elevated immediately after RT. Taken together, these data suggest that blockade of the PD-1/PD-L1 pathway is optimal when synchronized with the delivery of fractionated RT to prevent the induction of immunological anergy. These observations may be important for clinical translation as a sequential dosing schedule is often the de facto regimen adopted where there are uncertainties about the tolerability of a novel combination.

In summary, we have demonstrated that fractionated RT can prime antitumor CD8+ T-cell responses, which may be attenuated by signaling through the PD-1/PD-L1 axis. Inhibition of this pathway augments the generation of durable effective antitumor responses and improves survival. However, scheduling of the combination may be important for optimal outcome and our findings suggest that concurrent blockade of the PD-1/PD-L1 axis is required to improve the efficacy of fractionated RT. This study provides a strong rationale for clinical translation and has important implications for clinical trial design.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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