Commentary

Fractionated radiotherapy combined with PD-1 pathway blockade promotes CD8 T cell-mediated tumor clearance for the treatment of advanced malignancies

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Programmed death-1 (PD-1) is a T cell inhibitory receptor, expressed on recently activated and chronically stimulated CD4 and CD8 T cells (1,2). Through interacting with programmed death ligand-1 (PD-L1), PD-1 limits T cell receptor signaling, and maintains peripheral tolerance (1,2). PD-1 pathway blockade has the potential to restore effector function to exhausted T cells, thus boosting their antiviral and antitumor activity (2). This has prompted the development of PD-1/PD-L1 antibodies for treating cancer. Success in numerous preclinical studies (3-5) led to multicenter clinical trials, and FDA approval of anti-PD-1 agents (nivolumab or Opdivo® and pembrolizumab or Keytruda®) for the treatment of metastatic melanoma and non-small cell lung cancer (6-8). With as many as 31% of patients benefiting from treatment and median response duration lasting 2 years (8), it is not surprising that PD-1 pathway blockade, and interference with other T cell signaling checkpoints such as cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), continues to generate excitement for cancer immunotherapy. Given that PD-1 and CTLA-4 blockade exert distinct effects on tumor control, combined blockade has been successful in mouse models of melanoma and clinical trials with 53% response rate (9). In order to further increase efficacy, it is necessary to understand how immunotherapy could complement already approved treatment modalities, such as chemotherapy and radiation.

Radiation therapy (RT) is the most widely used non-surgical intervention for treating primary solid malignancies, and relieving cancer-associated pain stemming from bone metastases (10). Besides directly inducing tumor cell death, RT has an immunomodulatory effect. Dying tumor cells release danger-associated molecular patterns (DAMPs) such as deoxyribonucleic acid (DNA), high mobility group box 1 (HMGB-1) and adenosine triphosphate (ATP), as well as tumor-associated antigens. DAMP recognition induces a strong type I interferon (IFN) signature in dendritic cells, increases major histocompatibility complex (MHC) Class I and Class II expression, and helps prime tumor-reactive CD4 and CD8 T cells. Immune-mediated regression of the irradiated tumor and improved control of non-irradiated lesions (abscopal effect) sometimes arise in RT-treated hosts (10). However, more often than not, RT is not sufficient to override tumor-induced immunosuppression and escape (10). Several case reports pointed to a potential benefit of combining RT with immune checkpoint intervention, while a retrospective study and a phase I and II trial concluded combination therapy was safe (11-13). This has spurred extensive preclinical investigation into RT-checkpoint blockade combination therapies, with a focus on dosing, scheduling, and mechanisms underlying potential synergistic effects. RT delivered in smaller daily fractions over several days increases tumor immunogenicity compared to single-dose radiation (14). Previous work has shown that fractionated RT synergized with CTLA-4 blockade as well as other immunotherapies (e.g., Toll-like receptor 7 agonist treatment) to yield better survival than single-dose radiation, or single-dose radiation with immunotherapy (15,16).

Recently, Dovedi and colleagues published an exciting report in *Cancer Research* examining whether fractionated RT combined with PD-1 pathway inhibition could enhance

survival following tumor challenge (17). The authors first noted that fractionated RT (delivered in 5 daily fractions of 2 Gy) led to increased PD-L1 expression on CT26 colon carcinoma cells. Interestingly, this effect was noted *in vivo* and not *in vitro*, suggesting that tumor-associated stroma or infiltrating T cells mediated the increase in PD-L1 expression after RT. Through elegant depletion experiments, the authors demonstrated that CD8 T cells were required for the enhanced PD-L1 expression on the tumor cells. Since IFNγ can induce PD-L1 expression (18), the authors utilized anti-IFNγ neutralizing antibodies and IFNγR1 shRNA to determine whether the noted increase in PD-L1 expression was IFNγ-dependent. Blocking IFNγ signaling abrogated RT-induced PD-L1 expression in the presence of CD8 T cells, suggesting that CD8 T cell-mediated IFNγ secretion is responsible for enhanced PD-L1 expression on CT26 cells (17). The increased PD-L1 expression following RT could therefore explain how some tumors evade the endogenous immune response, and provides a rationale for combining checkpoint blockade with RT for enhanced tumor control. To test the functional significance of RT-induced PD-L1 increase, Dovedi *et al*. combined fractionated RT with PD-1 pathway blockade. Mice bearing established CT26, 4T1 (triple negative breast) or 4,434 (melanoma) tumors exhibited significantly improved tumor control (184.3±13.5 *vs*. 292.8±14.3 mm^2 for 4T1 at day 10 post treatment) and overall survival when treated with fractionated RT and either anti-PD-1 or anti-PD-L1, than the animals treated with either monotherapy (17). Specifically, 66–80% of treated animals survived past 100 days and were protected from a subsequent recall challenge at a distinct site (17).

CD8 T cells were critical for this tumor control, as CD8 T cell depletion prior to therapy abrogated the protective effect of combined treatment (17). NK cell depletion impacted initial tumor growth, but not overall survival (17). Collectively, these findings suggest that CD8 T, but not NK cells, are necessary and sufficient for tumor control after RT and PD-1 pathway blockade. Dovedi and colleagues also depleted CD4 T cells prior to RT + anti-PD-L1 treatment, and noticed improved tumor control, albeit without a significant increase in survival (17). These data indicate that regulatory T cells (T_{regs}) might play a role in restraining antitumor immunity after combined therapy. To test this, future studies could incorporate targeted T_{reg} depletion (e.g., using Foxp3-DTR mice) or anti-CTLA-4 treatment.

The authors noted that the combination therapy was well tolerated, since body weight was not impacted (17). However, with the use of checkpoint blockade, emergence of autoimmune-like events or overt autoimmunity is a major concern. Specifically, 9–14% of patients treated with anti-PD-1 or anti-PD-L1 developed immune-mediated grade 3 or 4 adverse events, which affected the skin, gastrointestinal tract, or thyroid (6,7). In addition, several patients developed type 1 diabetes as a result of PD-1 pathway inhibition (6,19). Future studies could evaluate serum autoantibody levels, intestinal pathology, and kidney and liver toxicity as direct readouts of immune-related adverse events following combination therapy.

Translating combination therapy into the clinic requires optimizing treatment schedule for maximizing clinical benefit, while minimizing side effects. In their report, Dovedi *et al*. investigated whether the order in which RT and PD-1 pathway blockade were administered affected treatment efficacy. Starting anti-PD-L1 treatment on the first or the last day of fractionated radiotherapy cured 57–60% of treated animals (17). However, anti-PD-L1 administered 7 days after the last dose of radiotherapy had no additive effect compared to radiation alone, and yielded no long-term survivors (17). These findings suggest that checkpoint blockade is most effective during, but not following radiation, and warrants further investigation.

In the year since Dovedi *et al*. published their findings, a phase I clinical trial examined the benefit of fractionated radiotherapy and CTLA-4 blockade in 22 patients with stage IV melanoma (20). Patients received fractionated RT, followed by four cycles of ipilimumab (anti-CTLA-4) treatment and were monitored for response with computed tomography (CT). Partial response (at least a 30% decrease in lesion diameter) was noted in 18% of patients (20). Another 18% had stable disease, while 64% of treated patients experienced progressive disease, suggesting that the majority of patients did not respond (20). Twyman-Saint Victor *et al*. then applied this treatment regimen to mice bearing B16-F10 melanoma, and similarly to Dovedi and colleagues, noted that concurrent checkpoint blockade synergized with RT, in a CD8 T cell-dependent manner. However, only 17% of animals responded to treatment. Even though combined treatment decreased the number of T_{regs} in the tumor, the number of effector CD8 T cells failed to increase (20). Importantly, transcriptional analyses of resistant tumors revealed that PD-L1 was in the top 0.2% of up-regulated genes that make up the gene signature of tumors refractory to combination therapy (20). Genetic deletion of PD-L1 by CRISPR rendered a resistant tumor cell line highly responsive to RT + anti-CTLA-4 therapy.

This prompted the authors to treat B16-F10 tumor bearing mice with anti-PD-1/PD-L1 in addition to anti-CTLA-4 and fractionated RT. In this case, 80% of animals were long-term survivors, with protective immunity against subsequent challenge (20). The authors showed that the three treatment modalities evoked non-redundant immune mechanisms. Radiotherapy led to increased CD8 T cell diversity in the tumor, CTLA-4 blockade decreased the number of tumor-infiltrating T_{regs}, while PD-L1 blockade allowed reinvigoration of exhausted intratumoral CD8 T cells (20).

Checkpoint blockade has revolutionized cancer therapy, and given hope to patient populations suffering from standard treatment-refractory tumors. Further understanding the ways in which checkpoint inhibitors complement each other and synergize with other therapies is necessary for increasing objective responses, minimizing relapse and side effects. Preclinical studies have already begun to explore optimal treatment schedules, and understand pathways driving resistance to combination therapy (17,20). Future work should focus on identifying biomarkers to predict treatment efficacy, as well as autoimmune risk screening (e.g., HLA typing, autoantibodies) to identify patients likely to develop immune-related adverse events.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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