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Exploring optimal sequencing of radiation and immunotherapy combinations.

Andrew J Gunderson
Earle A. Chiles Research Institute, Providence Portland Medical Center, Portland, Oregon

Kristina H Young
Earle A. Chiles Research Institute, Providence Portland Medical Center, Portland, Oregon

Critical Review

Exploring optimal sequencing of radiation and immunotherapy combinations

Andrew J. Gunderson PhD [a], Kristina H. Young MD, PhD [a,b,*]

[a]Earle A. Chiles Research Institute, Providence Portland Medical Center, Portland, Oregon
[b]Radiation Oncology Division, The Oregon Clinic, Portland, Oregon

Abstract

Purpose: The purpose of this article is to assemble, review, and provide a synopsis of the historical and current literature regarding optimal sequencing of radiation (RT) and immunotherapy combination treatments.

Materials and methods: A review of the literature was performed using PubMed with the query "radiation" and "Immunotherapy", "PD1", "PDL1", "CTLA4", "OX40", "checkpoint", "vaccine", "macrophage", "STING", and "TGFbeta". Studies that included sequencing of therapy were evaluated and the studies were included at the authors discretion.

Results: A paucity of primary literature exists examining the best order of radiation and immunotherapy, most of which was performed in the pre-clinical setting. The observations are that optimal sequencing of various radiation plus immune therapy combinations is dependent on the mechanism(s) of activation by the combination treatment. Immunosuppressive molecules tend to be better inhibited prior to RT while engagement of costimulatory genes is better activated concomitantly with RT.

Conclusions: These data should compel more basic research into both the direct investigation of sequencing efficacy and studies on the mechanisms of immune mediated cell death potentiated by radio-therapy.

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Purpose

The advent of immune therapies approved by the U.S. Food and Drug Administration for patients with late-stage cancer, which consist primarily of monoclonal antibodies (mAbs) that neutralize the inhibitory actions of T-cell checkpoint molecules, has stimulated a wave of research into novel immune-oncology (IO) combination treatment strategies. The central hypothesis of these strategies hinges on the idea that cell-mediated cytotoxicity can be harnessed and exploited to eradicate existing tumors as well as surveil undetectable malignancies. The evidence that necessitates this research is clear, but despite remarkable responses in melanoma,[1] responses to treatment are much more modest in other tumor types where the majority of patients do not respond. Among

patients who exhibit an objective response, many become therapeutic resistant while on immune checkpoint blockade (ICB) treatment. As more immunotherapeutic options become available, determining when to use them and how to combine them with standard-of-care cytotoxic therapy is critical.

Radiation is a common antineoplastic treatment modality that is primarily related to its efficacy as a focal cytotoxic agent. Additional benefits of radiation are its ability to expose tumor antigens, create a focal inflammatory response, enhance major histocompatibility complex upregulation, induce maturation of antigen-presenting cells, lead to danger-associated molecule pattern (DAMP)/pathogen-associated molecular pattern expression, and sensitize tumor cells to immune-mediated killing to create a potent in situ vaccine.[2-4] Primarily because of this, combination radiation and immunotherapy can enhance efficacy over either modality alone. Herein, we will discuss the data for the most effective combination of radiation and various immunotherapies to potentiate the vaccination effect (Table 1). Specifically, we will focus on the sequencing of treatments to maximize tumor killing both locally and in metastatic tissues.

Methods and Materials

A review of the literature was performed using PubMed with the query "radiation" and "Immunotherapy", "PD1", "PDL1", "CTLA4", "OX40", "checkpoint", "vaccine", "macrophage", "STING", and "TGFbeta". Studies that included sequencing of therapy were evaluated and the studies were included at the authors discretion.

Results

Combination radiation and immunotherapy rationale

Although initially approved for metastatic melanoma with objective response rates up to 40%, some cancers such as pancreatic adenocarcinoma are virtually unresponsive to ICB monotherapy.[1,5,6] A recently identified biomarker to predict which patients are susceptible to ICB efficacy is the categorization of tumors as those with an already established T-cell inflamed phenotype (ie, hot tumors) and those with a relative paucity of T-cell infiltrates (ie, cold tumors) before ICB treatment.[7,8] The influence of T-cell infiltrate on response to standard-of-care treatment (excluding immunotherapy) is the number 1 independent prognosticator of cancer-specific survival in colorectal cancer[9,10] and has led to a 6-year global effort to standardize the immunoscore as defined by the level of cluster of differentiation (CD)3$^+$/8$^+$ T-cell infiltration.

These biomarkers are strongly considered to enhance patient stratification and likely will be employed for the clinical practice of some cancer types, which is a remarkable example of progress and translation from research to clinical practice. Additional biomarkers for response to ICB include the sum total of somatic mutations acquired in an individual's tumor, which portends an enhanced immunogenic state because of the acquisition of neo-antigens presented by cancer cells that can stimulate endogenous immunity.[7,11] Indeed, a strong association between ICB-responsive patients with higher tumor mutational burdens has been demonstrated.[12]

Curiously, these molecular patterns do not explain the difference between T-cell rich and poor tumors that are naturally generated from treatment-naïve melanomas.[13] To convert the phenotype of cold tumors into T-cell rich microenvironments, conventional cytotoxic therapies using ionizing radiation have significant utility. Radiation increases tumor-infiltrating T cells, both naïve and antigen-specific CD8+ T cells.[14] Exceedingly rare case report observations have long ago shown that radiation of a particular malignant lesion can lead to partial or complete regression of an unirradiated lesion within the same patient (abscopal effect).[15] This indicates that radiation therapy (RT) can elicit local and systemic antitumor immunity and provides an interventional opportunity to increase the frequency and magnitude of this abscopal response for complete tumor rejection and immune memory.

Preclinical evidence has revealed that the mechanism that mediates these abscopal responses is dependent on CD8$^+$ cytotoxic T lymphocytes. However, differences in radiation dose, fractionation, and sequence can have significant alterations on leukocyte function,[16,17] which highlights the need to understand immune response mechanisms to RT-induced cell death and tissue stress responses.

Immune-system activation is carefully controlled within tissues and finely balanced between eliminating pathogenic insults and repairing damaged tissue while limiting collateral tissue destruction through the suppressive actions of various immune regulatory cell types (eg, Tregs, Bregs, and M2 macrophages, which are discussed in more detail elsewhere in this issue).[18,19] Progressing cancers escape immune recognition and rejection in large part by co-opting these immune regulatory cells and wound-repair phenotypes to assist with angiogenesis, matrix remodeling, and cytotoxic T-cell inhibition.[20] Ionizing radiation can elicit greater proportions of nearly all these immunoregulatory cell types attributed to the activation of apoptotic cell death pathways in these regulatory cells, which favors resolution of immune function over potentiating inflammation.[19] To capitalize on the window of immune stimulation after radiation, cell-death-associated immunosuppression must be counteracted to prolong T-cell activation and residency within tumors. To that

Table 1 Reference categorization.

Reference #	Reference	Preclinical	Clinical Retrospective	Clinical Prospective
1	Wolchok, J. D. *et al.* Nivolumab plus ipilimumab in advanced melanoma. *N. Engl. J. Med.* **369,** 122–33 (2013).			x
2	Zhang, B. *et al.* Induced sensitization of tumor stroma leads to eradication of established cancer by T cells. *J. Exp. Med.* **204,** 49–55 (2007).	x		
3	Reits EA, Hodge JW, Herberts CA, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med.* 2006;**203**(5):1259-1271. https://doi.org/10.1084/jem.20052494	x		
4	Ganss, R., Ryschich, E., Klar, E., Arnold, B. & Hämmerling, G. J. Combination of T-cell therapy and trigger of inflammation induces remodeling of the vasculature and tumor eradication. *Cancer Res.* **62,** 1462–70 (2002).	x		
5	Brahmer, J. R. *et al.* Safety and Activity of Anti–PD-L1 Antibody in Patients with Advanced Cancer. *N. Engl. J. Med.* **366,** 2455 –2465 (2012).			x
6	Royal, R. E. *et al.* Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. *J. Immunother.* **33,** 828–33 (2010).			x
7	Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science.* 2015;**348**(6230):69-74. https://doi.org/10.1126/science.aaa4971		x	
8	Hellmann, M. D. *et al.* Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. *N. Engl. J. Med.* NEJMoa1801946 (2018). https://doi.org/10.1056/NEJMoa1801946			x
9	Pagès, F. *et al.* International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. *Lancet* 1–12 (2018). https://doi.org/10.1016/S0140-6736(18)30789-X		x	
10	Galon, J. *et al.* Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* **313,** 1960–4 (2006).		x	
11	Llosa, N. J. *et al.* The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov.* **5,** 43–51 (2015).		x	
12	Snyder, A. *et al.* Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma. *Nejm* **371,** 2189–2199 (2014).		x	
13	Spranger, S. *et al.* Density of immunogenic antigens does not explain the presence or absence of the T-cell-inflamed tumor microenvironment in melanoma. *Proc. Natl. Acad. Sci. U. S. A.* **113,** E7759–E7768 (2016).	x		
14	Lugade, A. A. *et al.* Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor. *J. Immunol.* **174,** 7516–23 (2005).	x		
15	Kaur, P. & Asea, A. Radiation-induced effects and the immune system in cancer. *Front. Oncol.* **2,** 191 (2012).	x		
16	Crittenden, M. R. *et al.* The peripheral myeloid expansion driven by murine cancer progression is reversed by radiation therapy of the tumor. *PLoS One* **8,** e69527 (2013).	x		
17	Shiao, S. L. *et al.* TH2-Polarized CD4(+) T Cells and Macrophages Limit Efficacy of Radiotherapy. *Cancer Immunol. Res.* **3,** 518 –25 (2015).	x		

Table 1 (*continued*)

Reference #	Reference	Preclinical	Clinical Retrospective	Clinical Prospective
18	Bergsbaken, T., Fink, S. L. & Cookson, B. T. Pyroptosis: host cell death and inflammation. *Nat. Rev. Microbiol.* **7,** 99−109 (2009).	x		
19	Kroemer, G., Galluzzi, L., Kepp, O. & Zitvogel, L. Immunogenic cell death in cancer therapy. *Annu. Rev. Immunol.* **31,** 51−72 (2013).	x		
20	Coussens, L. M., Zitvogel, L. & Palucka, A. K. Neutralizing tumor- promoting chronic inflammation: a magic bullet? *Science* **339,** 286−91 (2013).	x		
21	Cannarile, M. A. *et al.* Colony-stimulating factor 1 receptor (CSF1R) inhibitors in cancer therapy. 1−13 (2017). https://doi.org/10.1186/s40425-017-0257-y	x		
22	Leao, I. C., Ganesan, P., Armstrong, T. D. & Jaffee, E. M. Effective depletion of regulatory T cells allows the recruitment of mesothelin-specific CD8+T cells to the antitumor immune response against a mesothelin-expressing mouse pancreatic adenocarcinoma. *Clin. Transl. Sci.* **1,** 228−239 (2008).	x		
23	Muroyama, Y. *et al.* Stereotactic Radiotherapy Increases Functionally Suppressive Regulatory T Cells in the Tumor Microenvironment. *Cancer Immunol. Res.* **5,** 992−1004 (2017).	x		
24	Kaneda, M. M. *et al.* PI3Kγ is a molecular switch that controls immune suppression. *Nature* **539,** 437−442 (2016).	x		
25	Soliman, H. H. *et al.* A first in man phase I trial of the oral immunomodulator, indoximod, combined with docetaxel in patients with metastatic solid tumors. *Oncotarget* **5,** 8136−46 (2014).			x
26	Monjazeb, A. M. *et al.* Blocking Indolamine-2,3-Dioxygenase Rebound Immune Suppression Boosts Antitumor Effects of Radio-Immunotherapy in Murine Models and Spontaneous Canine Malignancies. *Clin. Cancer Res.* **22,** 4328−40 (2016).	x		
27	Morris, Z. S. *et al.* Tumor-Specific Inhibition of *In Situ* Vaccination by Distant Untreated Tumor Sites. *Cancer Immunol. Res.* 825−835 (2018). https://doi.org/10.1158/2326-6066.CIR-17-0353	x		
28	Lee, Y. *et al.* Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment. *Blood* **114,** 589−95 (2009).	x		
29	Tran, E. *et al.* T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer. *N. Engl. J. Med.* **375,** 2255−2262 (2016).			x
30	Tran E, Turcotte S, Gros A, et al. Cancer Immunotherapy Based on. *Science.* 2014;**9**(May):641-645. https://doi.org/10.1126/science.1251102			x
31	Ott, P. A. *et al.* An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* **170,** 1120−1125.e17 (2017).			x
32	Chamoto, K. *et al.* Combination immunotherapy with radiation and CpG-based tumor vaccination for the eradication of radio- and immuno-resistant lung carcinoma cells. *Cancer Sci.* **100,** 934−9 (2009).	x		
33	Hodge, J. W., Sharp, H. J. & Gameiro, S. R. Abscopal regression of antigen disparate tumors by antigen cascade after systemic tumor vaccination in combination with local tumor radiation. *Cancer Biother. Radiopharm.* **27,** 12−22 (2012).	x		
34	Crittenden, M. R. *et al.* Tumor cure by radiation therapy and checkpoint inhibitors depends on pre-existing immunity. *Sci. Rep.* **8,** 7012 (2018).	x		
35	Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: The next generation. *Cell* **144,** 646−674 (2011).	x		

Table 1 (*continued*)

Reference #	Reference	Preclinical	Clinical Retrospective	Clinical Prospective
36	Dovedi, S. J. *et al.* Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade. *Cancer Res.* **74,** 5458–68 (2014).	x		
37	Sade-Feldman, M. *et al.* Resistance to checkpoint blockade therapy through inactivation of antigen presentation. *Nat. Commun.* **8,** 1136 (2017).		x	
38	Morisada, M. *et al.* PD-1 blockade reverses adaptive immune resistance induced by high-dose hypofractionated but not low-dose daily fractionated radiation. *Oncoimmunology* **7,** e1395996 (2018).	x		
39	Antonia, S. J. *et al.* Durvalumab after Chemoradiotherapy in Stage III Non—Small-Cell Lung Cancer. *N. Engl. J. Med.* NEJMoa1709937 (2017). https://doi.org/10.1056/NEJMoa1709937			x
40	Young, K. H. *et al.* Optimizing Timing of Immunotherapy Improves Control of Tumors by Hypofractionated Radiation Therapy. *PLoS One* **11,** e0157164 (2016).	x		
41	Gough, M. J. *et al.* OX40 agonist therapy enhances CD8 infiltration and decreases immune suppression in the tumor. *Cancer Res.* **68,** 5206–15 (2008).	x		
42	Moran, A. E., Kovacsovics-Bankowski, M. & Weinberg, A. D. The TNFRs OX40, 4-1BB, and CD40 as targets for cancer immunotherapy. *Curr. Opin. Immunol.* **25,** 230–237 (2013).	x		
43	Arce Vargas, F. *et al.* Fc Effector Function Contributes to the Activity of Human Anti-CTLA-4 Antibodies. *Cancer Cell* **33,** 649–663.e4 (2018).	x	x	
44	Postow, M. A. *et al.* Immunologic correlates of the abscopal effect in a patient with melanoma. *N. Engl. J. Med.* **366,** 925–31 (2012).		x	
45	Hiniker, S. M. *et al.* Translational Oncology A Systemic Complete Response of Metastatic Melanoma to Local Radiation and Immunotherapy. **5,** 404–407 (2012).		x	
46	Twyman-Saint Victor, C. *et al.* Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature* **520,** 373–7 (2015).	x		x
47	Hiniker, S. M. *et al.* A Prospective Clinical Trial Combining Radiation Therapy With Systemic Immunotherapy in Metastatic Melanoma. *Int. J. Radiat. Oncol. Biol. Phys.* **96,** 578–588 (2016).			x
48	Demaria, S. *et al.* Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. *Clin. Cancer Res.* **11,** 728–34 (2005).	x		
49	Baird, J. R. *et al.* Radiotherapy combined with novel STING-targeting oligonucleotides results in regression of established tumors. *Cancer Res.* **76,** 50–61 (2016).	x		
50	Deng, L. *et al.* STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. *Immunity* **41,** 543–852 (2014).	x		
51	Woo, S. R. *et al.* STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. *Immunity* **41,** 830–842 (2014).	x		
52	Corrales, L. *et al.* Direct Activation of STING in the Tumor Microenvironment Leads to Potent and Systemic Tumor Regression and Immunity. *Cell Rep.* **11,** (2015).	x		

Table 1 (*continued*)

Reference #	Reference	Preclinical	Clinical Retrospective	Clinical Prospective
53	Vanpouille-Box, C. *et al.* DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. *Nat. Commun.* **8,** 15618 (2017).	x		
54	Dewan, M. Z. *et al.* Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clin. Cancer Res.* **15,** 5379–88 (2009).	x		
55	Spranger, S., Dai, D., Horton, B. & Gajewski, T. F. Tumor-Residing Batf3 Dendritic Cells Are Required for Effector T Cell Trafficking and Adoptive T Cell Therapy. *Cancer Cell* **31,** 711–723.e4 (2017).	x		
56	Noy, R. & Pollard, J. W. Tumor-Associated Macrophages: From Mechanisms to Therapy. *Immunity* **41,** 49–61 (2014).	x		
57	Xu, M. M. *et al.* Dendritic Cells but Not Macrophages Sense Tumor Mitochondrial DNA for Cross-priming through Signal Regulatory Protein α Signaling. *Immunity* **47,** (2017).	x		
58	Zhang, J. G. *et al.* The Dendritic Cell Receptor Clec9A Binds Damaged Cells via Exposed Actin Filaments. *Immunity* **36,** 646–657 (2012).	x		
59	Hashimoto, D., Miller, J. & Merad, M. Dendritic Cell and Macrophage Heterogeneity In Vivo. *Immunity* **35,** 323–335 (2011).	x		
60	Young, K. H. *et al.* TGFβ Inhibition Prior to Hypofractionated Radiation Enhances Efficacy in Preclinical Models. *Cancer Immunol. Res.* (2014). https://doi.org/10.1158/2326-6066.CIR-13-0207	x		
61	Vanpouille-Box, C. *et al.* TGFb is a master regulator of radiation therapy-induced antitumor immunity. *Cancer Res.* **75,** 2232–2242 (2015).	x		
62	Formenti, S. C. *et al.* Focal Irradiation and Systemic TGFβ Blockade in Metastatic Breast Cancer. *Clin. Cancer Res.* (2018). https://doi.org/10.1158/1078-0432.CCR-17-3322			x
63	Bouquet, F. *et al.* TGFβ1 inhibition increases the radiosensitivity of breast cancer cells in vitro and promotes tumor control by radiation in vivo. *Clin. Cancer Res.* **17,** 6754–6765 (2011).	x		
64	Lan, Y. *et al.* Enhanced preclinical antitumor activity of M7824, a bifunctional fusion protein simultaneously targeting PD-L1 and TGF-β. *Sci. Transl. Med.* **10,** eaan5488 (2018).	x		
65	Strauss, J. *et al.* Phase I Trial of M7824 (MSB0011359C), a Bifunctional Fusion Protein Targeting PD-L1 and TGFβ, in Advanced Solid Tumors. *Clin. Cancer Res.* **24,** 1287–1295 (2018).			x
66	DeNardo, D. G. *et al.* Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. *Cancer Discov.* **1,** 54–67 (2011).	x	x	
67	Crocenzi, T. *et al.* A hypofractionated radiation regimen avoids the lymphopenia associated with neoadjuvant chemoradiation therapy of borderline resectable and locally advanced pancreatic adenocarcinoma. *J. Immunother. Cancer* **4,** 1–13 (2016).			x
68	Mosely, S. I. S. *et al.* Rational Selection of Syngeneic Preclinical Tumor Models for Immunotherapeutic Drug Discovery. *Cancer Immunol. Res.* **5,** 29–41 (2017).	x		
69	Sivan A, Corrales L, Hubert N, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science.* 2015;**350**(6264):1084-1089. https://doi.org/10.1126/science.aac4255	x		

Table 1 (*continued*)

Reference #	Reference	Preclinical	Clinical Retrospective	Clinical Prospective
70	Druzd, D. *et al.* Lymphocyte Circadian Clocks Control Lymph Node Trafficking and Adaptive Immune Responses Article Lymphocyte Circadian Clocks Control Lymph Node Trafficking. *Immunity* 1–13 (2017). https://doi.org/10.1016/j.immuni.2016.12.011	x		
71	Messenheimer, D. J. *et al.* Timing of PD-1 blockade is critical to effective combination immunotherapy with anti-OX40. *Clin. Cancer Res.* **23**, 6165–6177 (2017).	x		
72	Crittenden, M. R. *et al.* Mertk on tumor macrophages is a therapeutic target to prevent tumor recurrence following radiation therapy. *Oncotarget* **7**, (2014).	x		

end, performing radiation with therapies that target this suppression is appealing. Currently, macrophage inhibitors,[21] Treg depletion,[22,23] PI3Kγ antagonism,[24] and indoleamine 2, 3-dioxygenase 1 blockade[25,26] are promising immunoregulatory targets explored in clinical trials in a variety of indications. Preclinical evidence for enhanced systemic immune responses after radiation and immunotherapy when Tregs are depleted has laid the groundwork to move this research forward to the clinic.[27]

Altering tumor-infiltrating T cells before radiation

When formulating the correct sequencing of radiation and immunotherapies, understanding how the pre-existing immune microenvironment influences responses to RT is beneficial. Whole exome sequencing is becoming a standard laboratory practice for many cancer centers, primarily to identify molecular targets that can be treated with small molecule inhibitors of dominant oncogenic pathways. However, this offers an additional opportunity to characterize the immunological landscape for research and therapeutic exploitation, including the identification of tumor antigens and neoantigens, immune infiltrate and polarization, and cytokine profile. For those with cold tumors with potentially hidden or cryptic immune epitopes, radiation before whole exome sequencing–directed vaccination might be counterproductive.

Because response to radiation is partially dependent on CD8^{+} T cells,[28] strategies to increase T-cell infiltrate may improve radiation efficacy (Fig 1). Targeting tumor-cell–specific neoantigens in the forms of personalized vaccine and antigen-specific T-cell transfer can mediate tumor regression in patients with treatment-resistant tumors.[39–41] Preclinical studies that combine radiation and tumor-antigen vaccines have resulted in enhanced responses over either modality alone,[42] but genetic and cellular heterogeneity between patients likely precludes a one-size-fits-all approach to this particular combination therapy.

However, the combination of radiation and specific tumor-antigen vaccines can lead to the regression of tumors with disparate antigens because of antigen cascade.[37] Furthermore, T-cell priming to tumor antigens in implantable mouse models has recently been shown to occur in direct response to tumor cell injection, precluding the therapeutic generation of de novo immunity.[43] This provides a unique animal model to study immune-boosting scenarios to custom antigens, but is clearly an experimental artifact contrary to the 10-year average of human carcinogenesis.[44]

Despite these caveats, the majority of patients with cancer likely have sufficiently primed T cells at the time of diagnosis, and thus strategies to boost pre-existing immunity in these patients using local radiation would likely focus on enhancing cross-presentation and promoting T_H1 type inflammation. Clearly, more direct research on the mechanisms of radiation as an in situ vaccine is necessary to clarify these outstanding questions, as discussed in this special issue.

Radiation and immune checkpoint blockade

A robust T-cell infiltrate may be rendered ineffective via upregulation of immune checkpoint molecule expression. The combination of radiation and checkpoint blockade aims to enhance T-cell infiltrate into the tumor and prevent T-cell functional suppression. When evaluating the ideal timing of radiation and anti-programmed cell death protein 1 (PD-1) therapy, concurrent treatment was superior to sequential treatment, which suggests that an upregulation of anti-PD-1/programmed death-ligand 1 (PD-L1) occurs rapidly after radiation (Fig 1).[31] This timing is consistent with radiation-increasing interferon that leads to the upregulation of immune checkpoints.
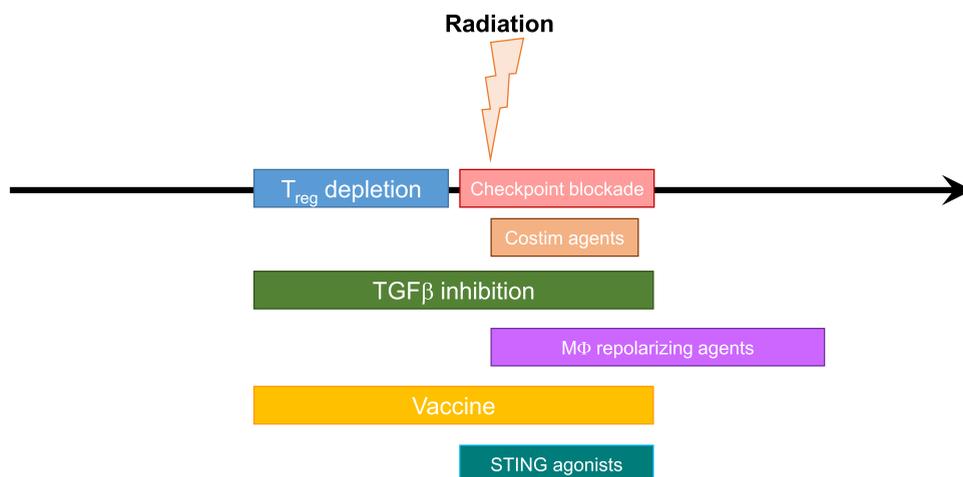
Radiation

Figure 1 Ideal efficacy of immune therapies around radiation. Studies demonstrate regulatory T-cell depletion or inhibition before radiation have enhanced efficacy.[29,30] Nondepleting anticytotoxic T-lymphocyte associated protein 4 and antiprogrammed cell death protein 1 therapy are effective when delivered concurrently with radiation.[29,31–33] Agonist anti-OX40 antibodies are most effective within 24 hours of delivering radiation. Transforming growth factor beta inhibition before and concurrently with radiation has demonstrated increased efficacy.[34,35] Macrophage repolarization after radiation improves radio-response.[36] Vaccination before and after radiation can lead to antigen spreading and systemic tumor responses, including tumors with disparate antigens.[37] Stimulator of interferon gene agonists delivered concurrently and after radiation demonstrate improved efficacy.[38]

Furthermore, resistance to ICB alone can develop via downregulation of antigen presentation machinery,[45] which hypofractionated radiation may be able to salvage.[46] Recent data also demonstrate that tumor cures from radiation and immune checkpoint blockade require pre-existing immunity, and that treatment does not generate a sufficient de novo immune response.[43]

Recently, data from the landmark PACIFIC trial for patients with stage III locally advanced and unresectable non-small cell lung cancer revealed that the addition of consolidation durvalumab (anti-PD-L1 mAb) significantly improved progression-free survival over placebo plus chemoradiation alone.[47] Although not directly assessing the difference between radiation and immune-therapy sequencing, there was an important trend of improved responses for patients who received anti-PD-L1 mAb closer to the chemoradiation administration. This potentially indicates that concomitant combination therapy may be more optimal than significantly delaying immune checkpoint blockade.

Of even greater importance may be the responses of PD-L1 low/negative patients and nonsmokers to durvalumab therapy, which suggests a potential conversion of immunogenic-poor patients to T-cell rich environments by standard cytotoxic therapies. Other clinical trial results in breast cancer that combine chemotherapy with ICB mirror the treatment kinetics observed here (NCT02513472, clinicaltrials.gov).

Converse to the ideal timing of anti-PD-1 mAb around radiation, a preclinical study evaluating the timing of radiation and checkpoint blockade with anticytotoxic T-lymphocyte associated protein 4 (CTLA4) determined that the combination was the most effective when given 7 days preceding a single 20-Gy dose of radiation as opposed to concurrently or after RT (Fig 1).[29] Interestingly, this timing did not confer efficacy when using an agonist anti-OX40 antibody in the same model where 1 day after RT was the most effective course of combination treatment (Fig 1). This likely reflects the differing mechanisms of anti-CTLA4 versus anti-OX40 on T-cell biology. CTLA4 is known to raise the threshold for T-cell priming by blocking co-stimulation and is constitutively expressed after T-cell priming, but OX40 is transiently upregulated after antigen exposure and can serve as a powerful co-stimulatory molecule that is secondary to others, such as CD28.[48,49]

In addition, CTLA4 expression is the highest on regulatory T cells (Tregs), but OX40 expression is higher on other T-cell subsets, which indicates preferential binding to different cell populations. Furthermore, certain antibody isotypes are known to enhance antibody-dependent cell cytotoxicity of target cells and in this study, Treg depletion by anti-CTLA4 mAb was hypothesized to be partially responsible for the enhanced radiation efficacy. This approach has since been confirmed in independent studies to specifically deplete tumor Tregs.[27,30] Retrospective clinical reports have found that the delivery of anti-CTLA4 before radiation correlated with improved survival.[50,51] Consistent with this observation, 2 phase 1 trials have published their results combing RT with ipilumimab (Ipi) in patients with metastatic melanoma. The initial trial delivered stereotactic body RT followed by 4 cycles of Ipi, and the second trial delivered Ipi first, followed by RT 5 days later, then continued Ipi for a total of 4 cycles. Interestingly, stereotactic body RT followed by Ipi

resulted in an 18% partial response as the best response[52] compared with a 50% complete + partial response rate in the trial where Ipi preceded radiation, which also demonstrated a 27% complete response rate.[53]

In addition, the results of a recently completed phase 1 trial that attempted to limit the toxicity of Ipi by directly injecting tumors with TLR9-agonist SD-101 and Ipi followed by radiation a day later demonstrated only 1 partial response, with a median time to progression of 3 months (NCT02254772, clinicaltrials.gov). These results suggest less efficacy that is either related to the route of administration or the timing of radiation after injection. Of note, in preclinical models, the sequencing of anti-CTLA4 concurrently or after radiation still improved survival relative to radiation alone, presumably through the effects on the checkpoint blockade of CD8+ T cells.[29]

Consistent with these findings, anti-CTLA4 and radiation efficacy with concurrent therapy was dependent on CD8 T cells and correlated with decreased metastases.[32] Patients who demonstrated a response to RT and Ipi had higher levels of central memory CD8+ T cells and enhanced cytokine production by CD8+ T cells compared with patients who did not respond.[53]

Radiation and stimulator of interferon gene agonists

The stimulator of interferon genes (STING) is the primary mediator of type 1 interferon upregulation upon radiation treatment and performs this function through the recognition of cyclic dinucleotides (CDN) that are derived from cytosolic DNA. Radiation and CDNs have been combined using concurrent and post-RT dosing of CDN for enhanced efficacy, analogous to CDNs as an adjuvant to the radiation vaccine (Fig 1).[38] This timing may be due to the reported dependency on STING and type 1 interferon upregulation signaling for immune stimulation by radiation.[54–56]

Recent work evaluated an additional mechanism by which combination radiation and anti-CTLA4 enhances immune-mediated tumor clearance via the STING pathway.[57] Interestingly, the results also report that dose-dependent upregulation of 3 prime repair exonuclease 1 (Trex1) expression leads to the degradation of cytosolic DNA and blunting STING activity. Together, these data suggest that very high dose radiation may be poorly immunogenic because of Trex1 degradation of CDNs. A more in-depth discussion of radiation and DNA sensing can be found in another review in this special issue. However, Trex1 expression may explain previous observations that fractionated doses of radiation better support CTLA4 combinatorial efficacy.[33]

Radiation and cytokines

Radiation may serve not only as an enhancer of tumor antigen cross-presentation,[58] but also as a driver of polarized cytokine microenvironments. Stressed and dying cells are abundant sources of danger signals such as high mobility group box 1, heat shock proteins, adenosine 5′-triphosphate, reactive oxygen species, and double stranded DNA.[19] Resident phagocytes such as dendritic cells and macrophages are keen sensors of these DAMP, primarily to scavenge off of pathogens but also repair damaged tissue. The default genetic programming of phagocytes after the recognition of DAMPs is to generate inflammation within the corresponding tissue.

However, in response to granulocytic inflammation, macrophages quickly convert to T-cell immunosuppression, tissue remodeling, and the promotion of endothelial and epithelial cell proliferation. Dendritic cells and macrophages can be skewed to prolong tissue inflammation, antigen presentation, and epithelial cell cytostasis if they are compelled toward phenotypes more commonly found in acutely infected or inflamed tissues. These observed phenotypic states are marked by high expression levels of tumor necrosis factor alpha, interferon alpha/beta, inducible nitric-oxide synthase, antigen presentation machinery, costimulatory molecules, and the T-effector cell chemokines CCL5 and CXCL9/10/11.[59–62]

Invariably, in the context of RT, these environments lead to enhanced tumor rejection accompanied by greater residency of activated T cells and often incite an abscopal response that is mediated by the immunologic memory of tumor-associated antigens. However, radiation often leads to a wound-healing, immunosuppressive, cytokine environment that is characterized by a robust M2 macrophage infiltrate, transforming growth factor beta (TGF-β), and adenosine signaling. The optimal window for intervention targeting these suppressive pathways might be concurrent or after RT when immunosuppression arises. In that regard, TGF-β1 is a master regulator of wound repair and immunosuppression by promoting fibroblast proliferation, blocking naïve T-cell proliferation, and inducing Treg and pericyte differentiation.

Two groups have independently verified the rationale of combining TGF-β1 inhibition with RT in mice and humans.[34,35,63,64] Of note, the therapeutic reagents differed in both studies from small molecule antagonism of TGF-βR1 signaling and antibody neutralization of TGF-β1 cytokine, but both preclinical studies delivered treatment in the same sequence (TGF-β1 blockade followed by radiation; Fig 1). Because of increased interferon expression after TGF-β1 inhibition and radiation leading to the upregulation of PD-L1, the addition of checkpoint blockade to this regimen has

proven effective.[35,65,66] Contrarily, macrophage depletion before chemotherapy and radiation in breast cancer mouse models uniformly demonstrated enhanced cytotoxic responses,[17,67] which suggests tumor tissue type and perhaps oncogenic signaling may affect sequencing decisions.

Fractionated radiation and timing of immunotherapy

The expansion of our knowledge of these systems, both on the tissue and molecular levels, is requisite to the precise timing of immunotherapy administered around traditional cytotoxic approaches. Most of the evidence presented herein relates to 1 to 5 doses of radiation, as opposed to the more common clinical scenario of standard fractionated radiation over weeks to months. How fractionated radiation influences the ideal timing of immunotherapy has been more challenging to address in preclinical models. One concern with regard to combination immunotherapy and standard fractionated radiation is the depletion of T cells in the irradiated field after RT[15] because this is expected to attenuate antitumor immunity. Targeted RT modalities using image-guided conformal RT greatly minimize the field size that is exposed to radiation; thus, blood volume exposure is reduced (discussed elsewhere in this issue).

Altering the dose-to-delivery equivalent to increased bio-equivalent doses in fewer fractions with higher daily doses (hypofractionation) also results in decreased blood volume exposure and more immunogenic tumor cell death. Dose and fractionation influence on antitumor immunity is discussed in depth elsewhere in this special edition as well. There is limited evidence on how best to sequence immunotherapy with a standard fractionated radiation, but analyses of immune parameters provide important information that will impact IO RT trial designs so that the timing of immune therapies can be coordinated within the window of greatest opportunity.

For example, an analysis of peripheral blood samples from 2 independent, nonrandomized, clinical trials indicated that 30 Gy over 3 fractions in 1 week attenuated peripheral lymphopenia compared with standard 50.4 Gy over 28 daily fractions in patients with borderline resectable and locally advanced pancreatic ductal adeno-carcinoma who received neoadjuvant chemoradiation.[68] Importantly, both treatment groups qualified for surgery in comparable proportions on the basis of a radiographic assessment, but the duration to normalization of absolute lymphocyte count was significantly lower in the hypofractionation group, which indicates that bone marrow and secondary lymphoid organ reserves were spared from the depletive effects of RT.

These data suggest that fractionated radiation might not pair well with T-cell targeting therapies such as vaccines, checkpoint inhibitors, and agonist costimulatory antibodies. However, macrophage blockade or antigen-presenting cell adjuvants may have activity and enhance responses to standard fractionated radiation given their relative radioresistance compared with lymphocytes. Of note, T cells are constantly trafficking, and even though peripheral lymphopenia is observed with fractionated RT, that may not reflect the T-cell population in the tumor microenvironment, as evidenced by the recent PACIFIC trial in which peripheral lymphopenia was almost certainly present in patients when they initiated durvalumab, and yet T-cell targeted therapy was still efficacious.

Conclusions

We outline the current knowledge in the field of radiation and IO combinations but admittedly fail to sufficiently address the gap in knowledge of how to correctly sequence these combinations. Indeed, precious few studies have directly compared the efficacy of ordering pre-, post-, and concurrent radiation treatments around various IO modalities (Fig 1). The few studies that have attempted to explore these questions have typically done so with strongly immunogenic implantable mouse models or have observed changes in primary tumor burden, which rarely accounts for cancer-related fatalities.

Conflicting data exist in preclinical models. Differences between study results must be viewed in the context in which these studies are executed. Not only will differing mouse strains dictate unique biology, but so can tissue type, tumor model,[69] vivarium microbiome,[70] and even circadian rhythms,[71] analogous to unique patient responses to immunotherapy.

Further consideration must be given to the over-stimulation of the immune system with combination therapy leading to feedback suppression. For example, one study examined the efficacy of anti-PD-1 checkpoint blockade pre- and postcostimulation with anti-OX40 in a middle T-antigen driven luminal B mammary carcinoma model. Not only did the researchers observe an enhancement of tumor kill when administering anti-PD-1 antibody after anti-OX40, but also reported a direct antagonism of efficacy when concurrently giving both antibodies.[72] The most probabilistic explanation is that when PD-1 signaling is blocked too soon after OX40 co-stimulation, T-cell exhaustion is accelerated and T-cell cytotoxicity is rendered less effective. This clearly has translational relevance to strictly immune-based combinations, but we believe that radiation could mirror the co-stimulatory effects that are provided by therapeutic intervention.

As demonstrated in this review, the mechanism of immune therapy that is used will directly influence the ideal timing in combination with radiation. Further studies are needed to optimize our efforts. We urge the radiation oncology community to embrace these research efforts to rationally design clinical trials based on preclinical data and with consideration for the immunotherapy mechanism of action.

Acknowledgments

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