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REVIEW ARTICLE

Stereotactic ablative radiotherapy and immunotherapy combinations: turning the future into systemic therapy?

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ABSTRACT

Radiotherapy (RT) is effective at cytoreducing tumours and until relatively recently the focus in radiobiology has been on the direct effects of RT on the tumour. Increasingly, however, the effect of RT on the tumour vasculature, tumour stroma and immune system are recognized as important to the overall outcome. RT is known to lead to the induction of immunogenic cell death (ICD), which can generate tumour-specific immunity. However, systemic immunity leading to "abscopal effects" resulting in tumour shrinkage outside of the RT treatment field is rare, which is thought to be caused by the immunosuppressive nature of the tumour microenvironment. Recent advances in understanding the nature of this immunosuppression and therapeutics targeting immune checkpoints such as programmed death 1 has led to durable clinical responses in a range of cancer types including malignant melanoma and non-small-cell lung cancer. The effects of RT dose and fraction on the generation of ICD and systemic immunity are largely unknown and are currently under investigation. Stereotactic ablative radiotherapy (SABR) provides an opportunity to deliver single or hypofractionated large doses of RT and potentially increase the amount of ICD and the generation of systemic immunity. Here, we review the interplay of RT and the tumour microenvironment and the rationale for combining SABR with immunomodulatory agents to generate systemic immunity and improve outcomes.

THE INTERPLAY OF RADIOTHERAPY WITH THE TUMOUR MICROENVIRONMENT

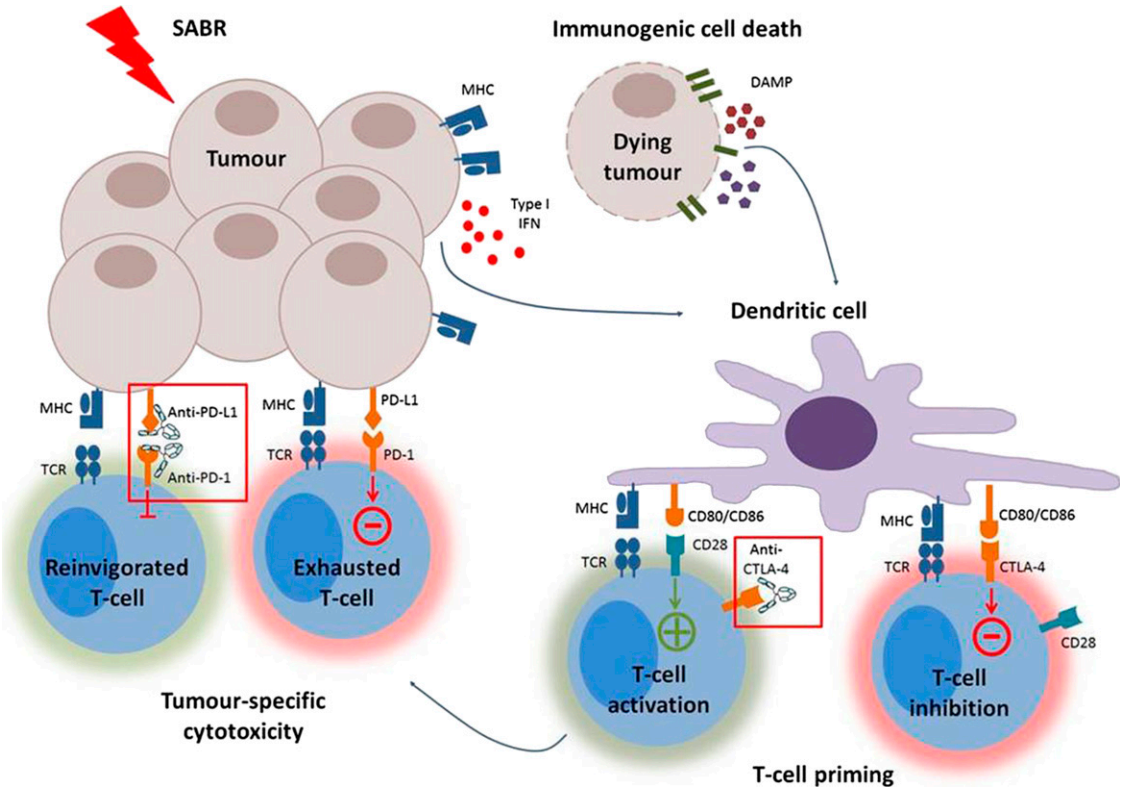
It is estimated that radiotherapy (RT) is required in the treatment of over 50% of all patients with cancer.[1,2] The focus of radiobiology over previous decades has been on the direct cytoreductive effect that RT exerts on cancer cells by inducing DNA damage and only relatively recently have the effects of RT on tumour vasculature, stroma and the immune system received more attention. RT is known to have a number of effects on the generation of tumour-specific immunity, which include enhanced antigen release, expression of Natural killer receptor G2D (NKG2D) ligands, production of Type I Interferon (IFN) and complement, increased major histocompatibility complex and neoantigen expression and the induction of immunogenic cell death (ICD).[3–9]

RT-induced ICD leads to increased expression of ecto-calreticulin, as well as the release of damage-associated molecular patterns, including high mobility group box 1 (HMGB1) and Adenosine triphosphate (ATP). The presence of these molecules leads to the recruitment and activation of antigen-presenting cells and subsequent priming of tumour antigen-specific T-cell responses[10–14] (Figure 1). The "abscopal effect", where clinical tumour regression is seen at a site(s) distant to or outside of the RT field, is understood to be caused by systemic antitumour immune responses, but is rarely seen in routine clinical practice.[15] The lack of a systemic immune response is thought to be caused by local immune suppression within the tumour microenvironment. Immune effector cells including myeloid-derived suppressor cells and Forkhead box P3 $(FoxP3)^+$ T regulatory cells (Treg), as well as inhibitory cytokines such as transforming growth factor-β and interleukin-10 and several "immune checkpoint" molecules are thought to cause local T-cell inhibition[16] and suboptimal priming by dendritic cells.

STEREOTACTIC RADIATION—A NOVEL OPPORTUNITY TO IMPROVE RADIOTHERAPY-INDUCED IMMUNE RESPONSE?

Advances in image-guided RT, as well as tumour motion tracking, have allowed the evolution of stereotactic body radiotherapy (SABR), where high doses of radiation can be delivered to a target with excellent precision and accuracy in a small number of fractions.

Figure 1. Treatment with ionizing radiotherapy [stereotactic ablative radiotherapy (SABR)] can enhance the generation of antitumour immunity *via* multiple mechanisms including modulation of the tumour cell phenotype [for example, through upregulation of antigen-presenting machinery and surface major histocompatibility complex (MHC) expression], inducing the production of Type 1 IFN and eliciting immunogenic cell death. Damage-associated molecular patterns (DAMPs) released by dying tumour cells enhance the processing and presentation of tumour-derived antigen by dendritic cells (DC) to cytotoxic $CD8^+$ T-cells. Subsequent $CD8^+$ T-cell activation is mediated by recognition of antigenic peptides in the context of MHC on the surface of DC by the T-cell receptor (TCR) in combination with co-stimulatory signals delivered *via* ligation of CD80/CD86 by CD28. Activation can be inhibited by the immune checkpoint, cytotoxic T-lymphocyte associated protein 4 (CTLA-4), which has high affinity for CD80/CD86 and thus competitively disrupts CD28 ligation. Blockade of CTLA-4 with specific monoclonal antibody (mAb) enhances activation of tumour-specific $CD8^+$ T-cells and promotes priming of tumour-specific responses. However, anticancer T-cell activity is further regulated by numerous additional checkpoints including the programmed death protein 1 (PD-1)/programmed death ligand 1 (PD-L1) pathway. Tumour cells can co-opt this signalling axis as a mechanism of resistance to T-cell killing. Activated $CD8^+$ T-cells express PD-1, and tumour cell expression of PD-L1 is upregulated in response to interferon-γ (IFN-γ) production by cytotoxic CD8+ T-cells. Engagement of PD-1 induces exhaustion or anergy in T-cells, suppressing antitumour activity. Blockade of PD-1 or PD-L1 with mAb reinvigorates T-cells, restoring cytotoxic activity. Thus, combination of SABR with immune checkpoint blockade is a promising strategy to enhance the generation and maintenance of antitumour immunity.



Several prospective and retrospective series have demonstrated high rates of local control in patients with medically inoperable, peripheral early-stage lung cancer treated with SABR, with 1-, 2- and 3-year local control rates of 78.0, 54.9 and 38.6%, respectively, in one large retrospective study.[17] The National Health Service England currently commissions it in the treatment of non-small-cell lung cancer (NSCLC).[18] Although SABR is currently funded only for patients deemed medically unfit for surgery, recently reported trials suggest that more patients may benefit from this approach in the future.[19]

The UK SABR Consortium offers guidance on using SABR in a number of other tumour sites including liver metastases, prostate cancer and spinal metastases,[20] although treatment of these disease sites is not yet common practice.

In addition to inducing double-strand DNA breaks similar to conventionally fractionated treatment regimes,[21] RT delivered at higher doses per fraction (8–10 Gy) may induce more significant vascular, stromal and antitumour immune responses within the local tumour microenvironment,[22,23] leading to increased cell death and improved efficacy.

A recent study in a murine colorectal tumour model demonstrated that a single dose of 30 Gy induced significantly more Cluster of differentiation 8 $(CD8)^+$ T-cell infiltration in the tumour bed and improved systemic antitumour durable responses compared with doses of 15 and 20 Gy, which led to tumour protection against further tumour rechallenge.[23] In contrast, 10 daily 3-Gy fractions failed to lead to a robust T-cell infiltration in these tumours, resulting in poorer local control.[23] A single high

dose fraction of radiation has also been shown to enhance presentation and T-cell recognition of tumour-associated antigens,[24] but in contrast this phenomenon was not seen in a melanoma model with a RT schedule at 7.5 Gy/fraction.[25]

As more evidence emerges, the specific hypofractionated or ablative radiation schedule required to improve the immune response may turn out to be tumour specific and be dependent on radiosensitivity and the nature and kinetics of the RT-induced ICD. Furthermore, the effect of RT dose and fractionation on release of damage-associated molecular patterns and the subsequent immune effector cell response is undetermined. Ultimately, the development of SABR techniques affords a much larger range of RT doses and fractionations to be delivered safely to patients than previously. There is promising initial evidence, but a significant amount of further mechanistic investigation is required to ascertain whether this new capability of larger single fractions of RT leads to increased immunogenic death and resultant improved systemic immunity and outcomes.

THE EMERGENCE OF IMMUNOMODULATORY THERAPY

Several "immune checkpoints" controlling regulation of antitumour immunity have now been identified. Overexpression of co-inhibitory molecules such as cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), programmed death ligand 1 (PD-L1), lymphocyte activation gene 3 and T-cell immunoglobulin and mucin-domain containing-3 within the tumour microenvironment leads to downregulation of the antitumour immune response. Over the past few years, novel monoclonal antibody treatments blocking these molecules or their ligands, "immune checkpoint inhibitors", have demonstrated unprecedented success in treating several different cancer types.[26–30]

The anti-CTLA-4 monoclonal antibody, ipilumumab, was the first immune checkpoint inhibitor to be successfully developed and led to significant improvement in the success of the treatment of patients with advanced melanoma. In the pivotal phase III trial, patients with advanced melanoma treated with this drug plus a peptide vaccination had a median overall survival of 10.0 months compared with 6.4 months among patients treated with the peptide vaccine alone. More durable responses were also seen with 1-year and 2-year survival rates of 45.6 and 23.5%, respectively.[26]

The CTLA-4 checkpoint principally regulates T-cell activation. In contrast, programmed cell death protein 1 (PD-1) and its ligands programmed death-ligand 1 (PD-L1) and programmed death-ligand 2 (PD-L2) form an immune checkpoint axis which regulates effector T-cell function, as well as B-cell and NK-cell activity, in normal tissues and tumours.[31,32] T-cell apoptosis is caused by PD-1-/PD-L1-mediated inhibition of the T-cell receptor (TCR) complex,[31,32] and multiple tumour types express PD-L1 in order to "evade" the immune system in this way. Indeed, across a range of solid tumour sites, PD-L1 expression may correlate with poor prognosis.[33]

Nivolumab is a fully humanized monoclonal antibody that selectively blocks the PD-1 receptor. A number of trials reporting recently have demonstrated its efficacy compared with standard treatments in several different advanced tumour types. Patients with metastatic melanoma who received this treatment first line had a 1-year survival rate of 72.9%, compared with a rate of 42.1% in patients who were treated with dacarbazine chemotherapy.[27] Another large trial exploring the use of nivolumab compared with standard chemotherapy as second-line treatment for metastatic NSCLC demonstrated an improvement in median and 1-year overall survival rates (9.2 *vs* 6.0 months and 42 *vs* 24%, respectively).[28] In patients with advanced renal cell cancer, treatment with nivolumab again led to improved overall survival rates when compared with a standard treatment (25.0 *vs* 19.6 months).[29]

Pembrolizumab, another monoclonal antibody targeting the PD-1 receptor, has also demonstrated impressive results. When compared with ipilumumab, this drug demonstrated an improvement in the rates of progression-free and 12-month survivals, as well as reduced rates of severe toxicity in patients with advanced melanoma.[30] Several other novel agents targeting the PD-1/PD-L1 axis are being evaluated in early clinical trials.[34] Immunodmodulatory agents have led to very encouraging clinical responses; yet, durable tumour remission rates associated with these treatments are relatively low,[28–30] and strategies using combination therapies are likely to be required to increase the number and duration of long-term response rates.

ENHANCING THE IMMUNOGENIC POTENTIAL OF STEREOTACTIC ABLATIVE RADIOTHERAPY USING IMMUNOMODULATORY AGENTS

In 2012, a case report described a patient with metastatic melanoma, whose disease had progressed despite receiving anti-CTLA-4 therapy. Intriguingly, treatment with palliative RT to a single site of metastatic disease led to a systemic response and treatment with further ipilumumab then caused regression of lung metastases.[35] Subsequent case reports and phase II studies have further increased the research interest, with these initial results suggesting that RT in combination with immunoregulatory agents may lead to "abscopal" responses in some patients, providing optimism that RT can enhance the systemic anti-immune response.[36,37] The underlying principle is that local RT-induced ICD and antitumour immune responses need to be augmented with the addition of immunoregulatory agents which enhance the local and systemic immune response by overcoming the immunosuppressive nature of the local tumour environment, leading to systemic specific antitumour immunity and increasing the possibility of abscopal effects (Figure 1).

Proof-of-principle preclinical studies using combinations of RT and immunoregulatory agents have demonstrated improved durable systemic antitumour immune responses.[4,38–40] In preclinical models, tumour cells have been shown to upregulate the expression of PD-L1 as an adaptive resistance mechanism in response to both fractionated[41] and single high-dose[42] radiation regimes, and this process was dependent on the production of interferon gamma by $CD8^+$ T cells.[41] Furthermore, blockade of PD-1 or PD-L1 enhanced the therapeutic effect of RT and

combination generated protective tumour antigen-specific memory T-cell responses.[41] Another study combining anti-PD-1 therapy with RT in melanoma and breast cancer models also demonstrated that concurrent treatment increases endogenous T-cell infiltration of established tumours, thus significantly improving tumour control.[24]

More recently, the use of dual immune checkpoint inhibitors in combination with RT provided further interesting mechanistic insights.[43] The authors suggested a model whereby anti-CTLA-4 treatment served to increase the $CD8^+$ T-cell to Treg ratio by predominantly inhibiting Treg cells, and RT diversified the repertoire of TCRs on lymphocytes infiltrating the tumours. Together, these treatments resulted in an expanded population of T-cell clones with an extended TCR repertoire. However, elevated PD-L1 on cancer cells led to resistance to combination anti-CTLA-4 and RT treatment owing to T-cell exhaustion, which was reversed by the addition of PD-L1 blockade.[43]

Other classes of novel immunomodulatory agents that are immunostimulatory may also play a role in combination with RT. These include members of the Tumour necrosis factor receptor (TNFR) superfamily such as OX-40, CD40, CD137, Inducible T-cell Costimulator (ICOS) and Glucocorticoid-induced TNFR-related protein (GITR) (beyond the scope of this review), as well the Toll-like receptor (TLR) family. TLR are expressed by antigen-presenting cells (such as dendritic cells and macrophages), as well as effector B, T and natural killer cells and are able to recognize a diverse repertoire of pathogen-associated molecular patterns on foreign pathogens. Signalling through TLRs directs an appropriate specific immune response. In murine models of various solid tumours and lymphoma, RT combined with systemic delivery of a TLR7 agonist led to durable anti-tumour immune responses.[38,44,45] Ongoing and developing clinical studies investigating the combination of RT and immunostimulatory approaches hold great promise and require well-designed trials and associated translational research to understand the mechanisms of response and non-response.

The scheduling of immunomodulation may also prove to be vital in attaining synergistic effect with RT. Preclinical evidence suggests concomitant rather than sequential scheduling of anti-PD-L1 treatment and RT is required for the generation of an effective antitumour response.[41] Equally, the order of treatments within sequential combination therapeutic strategies needs to be explored further preclinically. SABR delivered first to generate cancer cell-specific antigen with subsequent immunotherapy given a few days later to help prime T-cells may be more efficacious and would potentially avoid cytotoxic effects of RT on T-cells; however, this is only theoretical. Future trials will be required to confirm the role of concurrent treatments and establish optimal scheduling.

CLINICAL TRIALS EVALUATING COMBINATION THERAPY

Clinical studies investigating the potential of combining RT and immunomodulatory therapy in a prospective and randomized manner are beginning to open. The UK-led *PERM* and PEAR studies plan to evaluate the efficacy of combining palliative RT with pembrolizumab in patients with advanced melanoma and lung cancer, respectively.[46,47] A phase I trial, the *PLUMMB* study, will investigate the safety and tolerability of pembrolizumab combined with a hypofractionated radiation schedule for patients with locally advanced or metastatic bladder cancer.[48] Trials specifically investigating the synergistic effect of SABR with immunomodulatory agents given sequentially have also begun, including *PEMBRO-RT*, a phase II trial prospectively testing pembrolizumab after SABR *vs* pembrolizumab alone in patients with advanced NSCLC.[49]

Preclinical evidence has demonstrated the potential efficacy of combining stereotactic RT with novel immunotherapeutic agents. However, the potential increased clinical toxicities with these combinations can be carefully evaluated only in early-phase clinical trials (as above). Trials evaluating the safety of combining SABR concurrently with immune checkpoint inhibitors are currently recruiting in patients with advanced solid tumours[50] and advanced melanoma.[51]

Further studies are investigating other novel immunomodulatory treatments in combination with SABR. A phase II trial using sipuleucel-T[52] as a licensed therapeutic vaccine (US Food and Drug Administration approved, 2010) in combination with SABR is currently recruiting.[53] Another phase II study aims to assess response rates in patients with metastatic renal cancer treated with combination of high-dose interleukin-2 therapy with SABR.[54]

CONCLUSION

Recent advances in RT delivery technology have enabled the evolution of stereotactic RT techniques where large doses of radiation can be delivered to tumour sites in a highly conformal manner. In the radical setting, SABR may ultimately shift the paradigm that surgical techniques should be the primary treatment modality of choice for early-stage disease across most tumour sites. As well as providing local disease control by directly cytoreductive mechanisms, there is some compelling preclinical evidence that hypofractionated/single high-dose RT may illicit a more reliable immune response compared with conventionally fractionated radiation. Currently, for most RT deliveries, this potential immune priming may be largely negated by the highly suppressive nature of the tumour microenvironment, preventing a tumour-specific immune response both locally and at sites distant to the RT field. The advent of an increasing number of immunomodulatory agents, led by the immune checkpoint inhibitors, which have demonstrated durable clinical responses in a variety of hard-to-treat cancers, has provided renewed optimism that the RT-induced local immune response can be converted to increased numbers of systemic antitumour immune responses and improved outcomes. Early clinical trials examining the safety of SABR and immunomodulation concurrently are now beginning to open. Combinations of these treatment modalities have the potential to drive more regular abscopal effects, improving durable response rates in advanced tumours, and eradicating undetectable micrometastases in radically treatable disease improving relapse rates. These studies may herald an exciting new era in oncological management where stereotactic radiation is not only highly effective as local therapy, but in combination with immunomodulatory agents, as an effective enhancer of systemic therapy as well.

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