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Immunotherapy and stereotactic ablative radiotherapy (ISABR): a curative approach?

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Abstract

Conventional radiotherapy, in addition to its well-established tumoricidal effects, can also activate the host immune system. Radiation therapy modulates tumour phenotypes, enhances antigen presentation and tumour immunogenicity, increases production of cytokines and alters the tumour microenvironment, enabling destruction of the tumour by the immune system. Investigating the combination of radiotherapy with immunotherapeutic agents, which also promote the host antitumour immune response is, therefore, a logical progression. As the spectrum of clinical use of stereotactic radiotherapy continues to broaden, the question arose as to whether the ablative radiation doses used also stimulate immune responses and, if so, whether we can amplify these effects by combining immunotherapy and stereotactic ablative radiotherapy (SABR). In this Perspectives article, we explore the preclinical and clinical evidence supporting activation of the immune system following SABR. We then examine studies that provide data on the effectiveness of combining these two techniques — immunotherapy and SABR — in an approach that we have termed 'ISABR.' Lastly, we provide general guiding principles for the development of future clinical trials to investigate the efficacy of ISABR in the hope of generating further interest in these exciting developments.

Radiation therapy has been used as a predominant treatment option for nearly all types of cancer in the definitive, adjuvant and palliative settings. Traditional medical teaching has focused on the ability of locally applied radiation to directly kill tumour cells within the target volume by causing irreparable DNA damage, which irreversibly damages the tumour cells and prevents them from engaging in further replication and division (FIG. 1). In 2010, data were published indicating that radiotherapy can damage epithelial cells of small blood vessels by reducing sprouting, migration and proliferative capacities, and causing premature senescence, thereby starving cancer cells of nutrients [1,2]. More interestingly, a substantial

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amount of data have emerged showing that locally applied radiation can also stimulate systemic immune responses, thus leading to enhanced tumour cell recognition by the immune system and death of the tumour cells (FIG. 1). A number of investigators have reported that, following irradiation, tumour cells release a large amount of antigens, referred to as tumour-associated antigens (TAAs), in the form of necrotic and apoptotic tumour cells and debris[3–5]. The substantial increase in number and diversity of TAAs can enable antigen-presenting cells and dendritic cells to stimulate a tumour-specific immune response (FIG. 1). In addition to tumour cells acting as the trigger, the destruction of the tumour-supporting stroma that often results from radiotherapy can also potentiate immune recognition[6]. Other reports have focused on the release of 'danger' signals following radiotherapy, which might promote the transition from nonspecific immune responses to adaptive immunity[7,8]. Several other mechanisms of tumour sensitization following radiotherapy, including increased expression of cytokines and modulation of tumour phenotypes, have also been associated with promising outcomes (FIG. 1)[9–11]. Termed 'immunogenic modulation', these processes encompass a spectrum of radiation-induced molecular alterations in the biology of the cancer cell that either independently or collectively make the tumour more amenable to cytotoxic-T-lymphocyte-mediated destruction. These mechanisms have been reviewed in detail elsewhere[12], and include the following: downregulation of antiapoptotic and/or prosurvival genes [12,13]; modulation of antigen-processing machinery components [14,15]; and translocation of calreticulin to the cell surface of the tumour[14,16],. These radiation-induced changes can be exploited to provide synergistic clinical benefits when the radiation treatment is followed by, or given concurrently with, an immunotherapy regimen.

Technological advances that enable the delivery of higher doses of localized radiation to tumour targets with stereotactic ablative radiotherapy (SABR), also known as stereotactic body radiotherapy (SBRT), have been widely implemented in curing patients with early stage cancers of the lung and liver, and its role as a treatment for patients with metastatic disease is being actively investigated[17–19]. SABR involves treatment of tumours with radiation doses that often exceed 5 Gy per fraction with an exceedingly high level of conformality and sharp dose fall-off to spare the surrounding organs at risk. Investigators in many previous studies have focused on the effects of conventional fractionation regimens on the immune system; however, preliminary data suggest that radiation-induced immune responses might be dose-dependent [20,21]. In fact, using radiation doses in the 'ablative' range can not only effectively destroy tumour cells directly, but might also encourage these SABR-killed cells to function as a vaccine *in situ*[22,23].

Herein, we provide a definitive description of ISABR (immunotherapy and SABR), whereby exposure of tumour cells to higher doses of radiation delivered in a limited number of fractions promotes productive interactions between tumours and the immune system, which can be further exploited and/or augmented using active immunotherapy (FIG. 1, BOX 1). This Perspectives article is focused on the available data regarding the relationship between SABR and the initiation of antitumour immune responses. Furthermore, we discuss the early clinical benefits of incorporating immunotherapeutic strategies with SABR, and finally propose novel ways of bridging the gap from bench to bedside with this approach to cancer treatment.

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Box 1

Overview of immune checkpoints in cancer treatment[70]

Function

Inhibition of certain signaling pathways is necessary for maintaining self-tolerance and to prevent autoimmunity

This inhibition of certain pathways protects tissues from damage during activation of the immune system

Role in cancer

Dysregulation by tumours results in diminished cancer-cell recognition by the host immune system and evasion of an immune-mediated attack

As targets for cancer therapy

Targeted inhibition of these inhibitory receptors results in activation of the immune system and amplification of antigen-specific T-cell responses

Clinical application

CTLA-4 (cytotoxic-T-lymphocyte-associated antigen 4) counteracts the activity of T-cell stimulation by interfering with the co-stimulatory receptor CD28, thereby dampening the amplitude of T-cell activation

The FDA-approved agent Ipilimumab is an anti-CTLA-4 monoclonal antibody for treatment of patients with melanoma and is currently in phase II/III trials for efficacy as a treatment of various other forms of cancers

PD-1 (programmed cell death protein 1) limits T-cell activity in peripheral tissues, thus limiting the extent of inflammatory responses and autoimmune reactions. PD-1 is also expressed on regulatory T cells, which function as inhibitors of an immune response

PD-L1 (programmed cell death 1 ligand 1) is able to interact with CD80 on T cells, thereby delivering inhibitory signals, mainly owing to the PD-1:PD-L1 interaction

Pembrolizumab is an anti-PD-1 monoclonal antibody that acts as an immunomodulator by blocking activation of the PD-1 receptor on activated T-cells by PD-L1 on other cell types, including some cancer cells. This agent is approved by the FDA for the treatment of patients with advanced-stage melanoma and PD-L1-positive NSCLC, and is also undergoing extensive investigations for application in other forms of cancer.

Nivolumab is another anti-PD-1 monoclonal antibody that is approved by the FDA for treatment of patients with unresectable or metastatic melanoma, as well as for the treatment of non-small-cell lung cancer.

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Preclinical evidence

Data from several preclinical studies have demonstrated activation of an immune response following treatment with SABR. In a mouse model, investigators demonstrated increased T-cell priming in draining lymph nodes, leading to $CD8^+$ T-cell-dependent size reductions or eradication of primary tumours and distant metastases after a single fraction of radiation doses of between 15–25 Gy [24]. Interestingly, the investigators observed that the radiation-induced immune responses and reductions in tumour burden following SABR were abrogated with use of conventional fractionation, thus mirroring the $CD8^+$-depleted condition. In a similar study, antitumour immune responses were evaluated in mice after treatment of OVA-expressing B16–F0 tumours with single (15 Gy) or fractionated (3 Gy x 5 fractions) doses of radiation[25]. Use of either fractionation schedule facilitated antigen presentation and priming of T cells in draining lymph nodes. Once primed, these tumour-antigen-specific T cells had an enhanced ability to traffic to and infiltrate tumours. Both regimens were successful in stimulating the immune system, although use of 15 Gy single-dose irradiation resulted in a greater number of host immune cells infiltrating tumours, compared with the 3 Gy x 5 fractionated schedule[25]. This important difference in the immune response following irradiation with varying radiation fraction sizes was further highlighted elsewhere[22]: Mice bearing B16-OVA murine melanoma were treated up to 15 Gy radiation in various fraction sizes, and tumour growth followed. Researchers showed effective immune stimulation with doses of 7.5 Gy and 10 Gy, but not 5 Gy. Conversely, use of higher doses of radiation, namely ≥15 Gy, increased the fraction of splenic regulatory T (T_{REG}) cells, which function to suppress tumour-specific immunity [26]. The importance of the radiation dose and fractionation schedule used was further corroborated in a study showing activation of immune-response-related genes, radiation-induced damage-associated molecular pattern molecules (DAMPs), and inflammatory cytokines in human prostate cancer cells when exposed to radiation in the range of 8–10 Gy [27]. Thus, these data suggest the existence of a threshold dose below which immune stimulation might be suboptimal and above which immunosuppression prevails. Lastly, data from a study by our group, published in 2010[29], further support not only the importance of fraction size with regards to activation of the immune system, but also the longevity of the immune response following irradiation. We analyzed changes in tumour-cell phenotype in prostate cancer cell lines following single-fraction SABR and found that co-stimulatory and co-inhibitory T-cell signalling molecules can be modulated to promote productive antitumour immune responses following treatment with at least 10 Gy doses of radiation. In an attempt to find a potential therapeutic window for the addition of immunotherapeutic treatments, we analysed changes in tumour phenotypes at several time points following SABR. Whereas increased expression of immunostimulatory markers, including OX-40 ligand and 41BB ligand, was evident 72 hours after SABR, decreased expression of PD-L1 (programmed cell death 1 ligand 1), an inhibitor of T-cell expansion and function[28], for example, was detect up to 144 hours after SABR[20].

These studies focused on the immunomodulatory effects of SABR; however, the successful combination of SABR with immunotherapy regimens, resulting in synergistic anti-tumour effects, has also been reported in the preclinical setting. Cytotoxic-T-lymphocyte-associated

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antigen 4 (CTLA-4), similarly to PD-L1, functions to inhibit T-cell activation and suppress antitumour immune responses[29]. Both of these molecules have prominent roles in immune-checkpoints pathways that, when active, maintain self-tolerance and inhibit autoimmune reactions. Data now confirm that many tumours activate certain immune-checkpoint pathways to evade the host immune response and promote resistance. Thus, the development of checkpoint inhibitors has emerged as a prominent treatment strategy that enables stimulation of antitumour immune responses (BOX 1). Data from one elegant study[31] demonstrated regression of the primary irradiated tumour and distant metastases following radiotherapy (two fractions of 12 Gy) combined with CTLA-4 blockade. Not surprisingly, the substantially improved local and distant tumour control translated into longer survival. Further analyses confirmed that these effects were elicited by $CD8^+$ T-cell-dependent antitumour immunity[30]. Data from a similar study by the same group demonstrated that the use of different SABR regimens (20 Gy x 1, 8 Gy x 3, or 6 Gy x 5) in combination with anti-CTLA-4 antibody therapy again resulted in enhanced or complete regression of the primary tumour compared with use of single-modality therapy. Interestingly, substantial inhibition of tumour growth outside of the radiation field was seen only when immunotherapy was added to the fractionated SABR schedule and not the single-dose regimen[31]. As seen in a previous study from the same research group, the amount of $CD8^+$ T cells demostrating tumour-specific IFN-γ production was proportional to the inhibition of the secondary tumour. Lastly, in the previously mentioned report[25], investigators observed that ablative radiotherapy (15–25 Gy x 1) alone generated robust $CD8^+$ T-cell-dependent immunity, leading to reductions in tumour burden, reduced relapse of the primary tumour, and eradication of metastases. These investigators further showed that treatment combining two consecutive doses of 12 Gy radiation with ad-LIGHT, an immunotherapeutic agent and member of the tumour necrosis factor superfamily — composed of a ligand of the stromal-cell-expressed lymphotoxin β receptor and T-cell-expressed herpes viral entry mediator — resulted in prolonged survival compared with treatment with either modality alone[24].

Treatment with other types of immunotherapy has also been shown to augment antitumour responses when combined with high-dose radiotherapy. Combinations of clinically relevant monoclonal antibodies designed to stimulate antitumour immunity (such as anti-CD137 and anti-CD40 antibodies) or relieve immunosuppression (anti-PD-1 antibodies) with single (12 Gy) or fractionated (4–5 Gy x 4) radiotherapy have also been investigated[33]. Single-fraction treatment combined with anti-CD137 and anti-PD-1 therapy was found to result in enhanced host immune responsiveness to tumours, with a tumour rejection rate of up to 40% in mouse models[32]. Similarly, the fractionated radiotherapy regimens in combination with anti-CD137 and/or anti-PD-1 antibodies were more effective in controlling tumour growth than either treatment alone. Notably, radiotherapy did not deplete, but rather enriched tumours for functionally active, tumour-specific immune effector cells[32]. Data from an elegant study, with results published in 2015[34], corroborated these observations that perhaps combining immunotherapy techniques that have different mechanisms of action might yield better outcomes. In this study[34], despite an initial tumour response, resistance was common when radiation was combined with anti-CTLA-4 antibodies. Resistance correlated with upregulation of PD-L1 and T-cell exhaustion; however, the addition of PD-L1 blockade to this regimen reversed T-cell exhaustion, and, as also promoted by anti-CTLA-4, further

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improved the $CD8^+$ T cell: T_{REG} cell ratio, and further enhanced expansion of the T-cell population and diversification of the T-cell-receptor repertoire [33]. Lastly, treatment with single fractions of 10 Gy radiation in combination with L19–IL-2, a fusion protein designed to selectively deliver IL-2 to cancer cells, targeting tumour neovascularization, resulted in 75% cure rates and increased the percentage of antigen-specific $CD8^+$ cytotoxic T cells[34].

Taken together, these data demonstrate not only that effective immune stimulation can be achieved following SABR monotherapy, but also that addition of immunotherapeutic strategies to SABR therapy results in improved outcomes compared with treatment with either modality alone. These promising preclinical results served as the basis for testing this combination in the clinical setting.

Clinical evidence for ISABR

The most well-known success story of combining SABR with immunotherapy was detailed in a case report published in 2012[36]. In conjunction with ipilimumab, an anti-CTLA-4 monoclonal antibody, SABR (28.5 Gy delivered in three fractions) was successfully used to treat a painful metastatic paraspinal lesion in a patient suffering from metastatic melanoma. The findings of post-SABR CT scans confirmed not only a local response, but also substantial regression of distant lesions located outside of the radiation field[35]. Local radiation in combination with anti CTLA-4 immunotherapy resulted in systemic antitumor activity, termed the 'abscopal effect'[36], which seemed to be mediated by the immune system[36]. In a similar case study, authors reported clinically significant improvements in the outcome of a patient with metastatic melanoma. Following SABR (54 Gy in three fractions) treatment of two of seven metastatic liver lesions, a complete systemic response occurred, despite disease progression on ipilimumab alone[37]. A third case report of an abscopal effect of ipilimumab in a patient with metastatic, non-small-cell lung cancer (NSCLC) was published in 2013[39]. While undergoing ipilimumab immunotherapy, the most metabolically-active liver metastasis was selected as the target for SABR and was treated with a total radiation dose of 30 Gy in five fractions. Post-treatment scans showed an objective response within the radiation field as well as resolution of non-irradiated foci in the liver, bone and lung [38]. Lastly, in an intriguing retrospective study with results published in 2013[40], investigators analyzed clinical and radiographic records of patients with melanoma who were treated with ipilimumab and either whole-brain radiotherapy (WBRT) or stereotactic radiosurgery (SRS) for brain metastases. The median survival of patients who received WBRT and ipilimumab was 3.1 months compared with 19.9 months in patients who received SRS and ipilimumab therapy. Both treatment with ipilimumab and treatment with SRS were significant predictors of improved overall survival (HR 0.43 and HR 0.45, with $P = 0.005$ and 0.008, respectively. Neither SRS nor ipilimumab treatment individually appeared to account for the prolonged survival seen in the analysis[39]. These findings were corroborated in another case report of a patient with metastatic melanoma, in whom a systemic complete response in the skin and lymph nodes was observed following treatment with ipilimumab and SRS for brain metastases[40]. Additional studies investigating the combination of immunotherapies with SABR, with similar findings to those studies discussed in this section, have also been published (Table 1)[41–44].

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These remarkable results have set the stage for the initiation of several clinical trials investigating the combination of SABR with immunotherapy. Currently, investigators at Johns Hopkins University are enrolling patients with metastatic melanoma with newly diagnosed metastases to the brain or spine; patients will receive an intravenous dose of ipilimumab, followed by CyberKnife® (Accuray Ltd, California, USA) SABR a week later, and three more doses of ipilimumab, to test the safety of this combination[46]. A similar trial, named RADVAX and led by investigators of the Abramson Cancer Center at the University of Pennsylvania, is a stratified phase I/II dose-escalation trial designed to investigate SABR followed by ipilimumab, also in patients with previously treated or untreated metastatic melanoma[47]. A phase I/II trial at MD Anderson Cancer Center currently recruiting participants will investigate the safety and efficacy of the combination of ipilimumab and SABR in patients with advanced-stage solid tumours. Patients will be randomly assigned to receive either concurrent (early) SABR starting on day 1 of ipilimumab therapy, or sequential (late) SABR beginning on day 29[48]. Patients with metastatic cancer and at least one metastatic or primary lesion in the liver, lung or adrenal gland are eligible for enrolment. A range of other clinical studies in this area are currently published or ongoing (Tables 1 and 2)[49]. Results of the ongoing trials we have described, which are anticipated to become available in the next few years, will hopefully provide further insight into the appropriate selection of patients that will benefit from ISABR. Until then, the information in the subsequent section might provide some guiding principles for future investigations.

Future directions

On the basis of the preclinical and clinical data presented in this Perpsectives, sufficient evidence exists to support continued exploration of the combination of immunotherapy and SABR. Nevertheless, several considerations need to be adequately addressed prior to the development of a clinical trial designed to test the efficacy of ISABR.

Firstly, appropriate patient selection remains of paramount importance. In nearly all clinical scenarios, factors including tumour site, stage and type will all affect any relevant outcomes. Currently, SABR is most-frequently used in the setting of metastatic disease and in patients with stage I NSCLC. Findings of randomized trials have confirmed that for patients with stage I NSCLC, SABR alone results in ≥95% local tumour control within the irradiated field[50,51]. The rates of microscopic or distant spread in the early stage disease scenario are low: about 5–10% of patients will develop regional lymph-node recurrences, and up to 15% will have distant metastases[52]. SABR only targets primary lesions, although rates of lymph-node recurrence and distant failures are comparable to those seen following surgical resection of the affected lobe and regional lymph-node dissection. Despite the previous assumption that removal of the visible tumour burden in the lung and dissection of draining lymph nodes would result in lower incidences of regional and distant metastases, this theory has not held true based on results of phase II prospective studies[53], randomized studies[54] and a patient-population study[55]. Furthermore, in a phase III trial, treatment with an antigen-specific immunotherapeutic vaccine, termed MAGE-A3, did not result in any benefit compared with placebo for patients with resected stage IB, II, and IIIA NSCLC, thus failing to meet the primary outcome[56]. One can hypothesize, on the basis of these observations, that the combination of this tumour-specific therapeutic vaccine with SABR might result in the

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generation of the aforementioned *in situ* vaccine with subsequent stimulation of an effective systemic immune response (TABLE 2). Collectively, these findings suggest that localized SABR alone might stimulate the immune system to prevent tumour recurrence and/or metastases. Adding active immunotherapy to SABR might further reduce lymph-node involvement and distant disease, potentially leading to even higher cure rates.

In addition to the current patient groups, patients with advanced-stage disease might also achieve important clinical benefits from treatment with ISABR. Patients suffering from oligometastatic disease or those with locally advanced tumours that have a high propensity for metastasis frequently harbour disease that is not routinely detected during laboratory examinations or imaging work-up. Thus, inciting an immune response using a combination of SABR and an immunotherapeutic approach can address the visible disease burden and also target cancer cells that have, thus far, evaded detection using traditional diagnostic approaches. Building upon findings of basic research[35], a clinical trial is currently ongoing, with the aim of investigating the effectiveness of combining high-dose radiotherapy and the L19–IL-2 fusion protein in patients with oligometastatic solid tumours[57] (NCT02086721). In these clinical situations, it remains unclear whether all of the disease needs to be treated, or if SABR targeting just a fraction of the index lesion being treated can nevertheless incite an effective systemic immune response against all oligometastases.

Similarly, patients with a more substantial disease burden might also benefit from ISABR using the same approach. An accessible metastatic lesion targeted with SABR can initiate an immune response, thereby enabling an effective immune-based attack on other metastases located outside of the radiation field. In this scenario, the SABR-treated lesion acts as an *in situ* tumour vaccine. However, the subsequent immune response following radiation alone is often insufficient to address the distant macroscopic or microscopic disease burden. Additionally, patients with advanced-stage or metastatic disease are frequently treated with several systemic agents, most of which are immunosuppressive. In these instances, in which chemotherapy is used to combat oligometastatic and/or occult metastatic disease, an unanswered question exists concerning whether the use of upfront chemotherapy reduces the recruitment of effector T cells for activation within the irradiated tumour microenvironment — where antigen elaboration occurs. Thus, implementation of an immunotherapeutic strategy, in addition to SABR, might generate a more robust and effective immune response. IASBR relies on the SABR-treated tumour to stimulate a personalized, tumour-specific immune response; therefore, choosing patients with the most appropriate tumour histology, location, and stage might have a less important role in clinical trials investigating this approach.

The optimal timing of the two IASBR treatment modalities is a second important aspect that needs addressing before a trial is embarked upon. Some investigators have proposed that immunotherapeutic treatments should be administered after radiotherapy. One theory hypothesizes that the activation of an immune response and augmentation of this response by immunotherapy might be less effective if radiation has not already generated *de novo* tumour antigens and broken any pre-existing peripheral immune tolerance of the tumour[58]. Additionally, treatment with SABR following the activation of immune cells could be detrimental to an effective antitumour cellular response, owing to the cytotoxic and ablative

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nature of this radiotherapy technique[58]. Conversely, administration of SABR after immunotherapy does offer certain advantages: stimulating antigen-presenting cells and effector T cells prior to SABR will allow these cells to be readily available to respond to the efflux of tumour antigens generated as a result of radiation treatment; similarly, having an active immunoadjuvant within the tumour microenvironment at the time of SABR could maximize its therapeutic effects[58].

Despite these general principles, the immunotherapy agent of choice most probably dictates the optimal sequencing of SABR. For instance, in the example of adoptive T-cell transfer immunotherapy, SABR as the latter therapy would, presumably, interfere with the immune response at the tumour site. Therapeutic cancer vaccine therapy, on the other hand, might require SABR in order to release tumour antigens, which are necessary for activation of antigen presentation and immune-mediated cell killing. To help shed further light on this issue, in a study with results published in abstract form in 2014, investigators administered an anti-CTLA-4 antibody or OX40 agonist antibody either before or after a single radiation dose of 20 Gy to subcutaneous colorectal adenocarcinomas in a mouse model[59]. SABR delivered to the altered tumour microenvironment created by anti-CTLA-4 antibody administration resulted in 100% tumour clearance as opposed to only 50% clearance when an anti-CTLA-4 antibody was sequenced after radiotherapy. Consistent with the notion that the optimal timing of treatment modalities might be determined by the immunotherapy agent selected, administration of the OX40 agonist antibody increased the numbers of activated $CD8^+$ T cells and was optimal when delivered one day after single-fraction radiotherapy[59]. Collectively, taking into account the different specific mechanisms of action of immunotherapeutic strategies might help to dictate the most-appropriate timing of immunotherapuetic interventions in relation to SABR. Thus, these data suggest than an umbrella recommendation regarding the optimal sequencing of immunotherapy and SABR might be misleading, and possibly inappropriate. Rather, obtaining a solid understanding of the mechanism of action of the chosen agent and its role in either stimulating or suppressing a tumour-directed immune response will be more valuable.

A final point, which also requires consideration, is the ability to identify patients' responses to therapy. As mentioned above, patients with obvious progressive disease in a single location yet potentially also harbouring tumour cells in other locations, which are undetectable using traditional methods, might derive the greatest clinical benefit from ISABR. Thus, measuring responses to treatment using standard laboratory tests or imaging modalities might falsely reveal a lack of systemic disease control. Soley choosing traditional clinical end points, such as tumour resectability, tumour response, disease-free survival, and/or overall survival to assess the efficacy of ISABR may not tell the complete story. Therefore, supplementing with immunological readouts as well to capture disease response is recommended, as they can establish proof-of-principle of activation of the immune system prior to exploration of clinical end points. For instance, measuring the production of inflammatory cytokines in a patient's serum following administration of ISABR might act as a surrogate for the true efficacy of an antitumour immune response. Quantifying the generation of tumour-specific T-cells will help assess the ability of ISABR to elicit a tumour-directed immune response. Furthermore, measuring alterations in the number and function of T_{REG} cells, natural killer cells, and circulating antigen–antibody complexes will

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provide an overall picture of the generation of a productive immune response. Similarly, the presence of neoantigens and a greater mutational load seems to correlate with the cytolytic activity of NK cells and $CD8^+$ T cells and, therefore, might help to predict outcomes following ISABR[60]. Indeed, tumour mutational load, described as the predicted burden of deleterious alleles[61], has been demonstrated to positively correlate with an improved objective response, durable clinical benefit and progression-free survival in patients with NSCLC who received treatment with anti-PD-1 therapy[62]. Lastly, reports published in 2014 indicate that CTLA-4 blockade induces evolution and diversification of the T-cell repertoire, thereby increasing the number of unique T-cell-receptor clonotypes. In this study[63], improved clinical outcomes were associated with less clonotype loss and maintenance of high-frequency clonotypes during treatment[63]; perhaps these features could act as surrogates for clinically-relevant antitumour responses. In fact, these changes in T-cell clonality have been associated with increased overall survival in clinical trials with cohorts of patients with prostate[64] or breast [65] cancer. These, along with other immunological tests, should be used to supplement standard examination criteria in order to more accurately determine the extent of disease response[66]. Data from another study[67] highlight the challenges in choosing the most-appropriate readouts in clinical immunotherapy studies. The investigators reported tumour progression despite induction of very high levels of tumour-antigen-specific $CD8^+$ T cells in patients with melanoma, following vaccination with altered peptide immunogens[67]. Efforts to identify such immune biomarkers in addition to the use of more-traditional measures of disease response should be undertaken so that rational treatment combinations can be designed in terms of intensity, sequencing and maintenance of immune stimulation after combination with SABR. This approach will also enable the possibility of enriching treatment populations in clinical trials with patients who are most likely to respond to treatment and/or tailoring therapy specifically for distinct subsets of patients.

Conclusions

The proposal to combine immunotherapy and radiotherapy is not novel. Many investigators have shown that this bimodality therapeutic approach is not only feasible, but also effective, and that the doses of radiation required fall within the window of conventional fractionation schedules. Within the last two decades, clinicians have taken advantage of technological breakthroughs that enable treatment with higher doses of radiation while maintaining acceptable levels of exposure of the surrounding organs at risk. The popularity of SABR has risen drastically over the past few years and its full potential, no doubt, remains to be realized. Owing to the local efficacy and ablative qualities of SABR as a single modality, SABR is infrequently combined with other treatments, especially immunotherapy. Thus, the goal of this Perspectives was to present the relevant literature supporting the combination of immunotherapy with SABR, described as ‘ISABR’, in the preclinical and clinical settings. Currently, a few clinical trials of this approach are underway, although the results are not expected to become available for several years. The aim of the final section of this Perspectives was, therefore, to provide some general guiding principles to consider regarding the development of, and to promote interest in, future research efforts as we believe the synergistic relationship between SABR and immunotherapy is just beginning to blossom.

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Biographies

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Dr. Sunil Krishnan gained his MD in 1993 from Christian Medical College, Vellore, India. Currently, Dr. Krishnan is the Director of the Center for Radiation Oncology Research at M. D. Anderson Cancer Center and the John E. and Dorothy J. Harris Professor of Gastrointestinal Cancer in the Department of Radiation Oncology. He has co-authored over 150 peer-reviewed scientific publications, co-authored 17 book chapters, and co-edited 2 books, mostly related to radiation therapy. Over the last decade, Dr. Krishnan has developed and advanced novel strategies to improve radiation treatment outcomes of gastrointestinal cancers. This has included combination of radiation therapy with targeted agents, botanicals, and metallic nanoparticles as well as delivery of higher doses of radiation therapy more precisely to tumors via image guidance.

Dr. Joe Y. Chang received his medical degree from Shanghai Medical College, Fudan University, Shanghai, China and holds an MS in Immunology. He later earned his PhD in Cancer Biology from MD Anderson Cancer Center. He completed his Radiation Oncology residency training at Rush-Presbyterian St. Luke Medical Center, Chicago. He is Professor and the Director of Stereotactic Ablative Radiotherapy at MD Anderson Cancer Center. He published more than 200 research articles/books chapters in the field of stereotactic radiotherapy, proton therapy, image-guided radiotherapy, and gene therapy/immunotherapy. He is recognized as a leading authority in the field of stereotactic radiotherapy and proton therapy.

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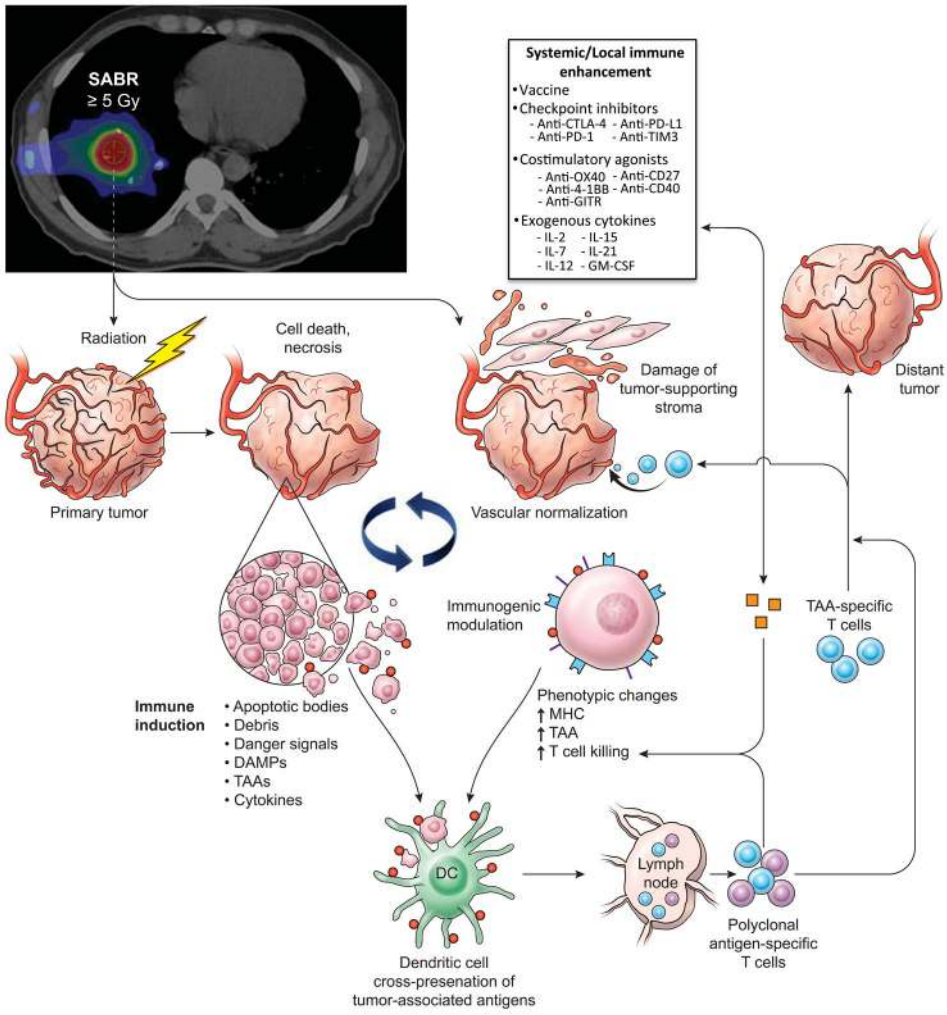

Figure 1. Immune stimulation by SABR

Antitumour effects of stereotactic ablative radiotherapy (SABR). SABR results in immune activation by inducing tumour-cell death, modulating tumour-cell phenotype and normalizing aberrant tumour vasculature to allow for improved oxygen and drug delivery. After cell death, the release of tumour debris with associated danger signals, tumour-associated antigens (TAAs), and inflammatory cytokines are recognized by and activate dendritic cells, promoting antigen presentation to cells of the immune system. Polyclonal antigen-specific T cells are then generated, some of which can attack tumours located within the radiation field, as well as distant tumours; this response can be augmented by the addition of systemic immune-enhancement measures. GM-CSF; granulocyte macrophage colony stimulating factor; IL, interleukin; MHC, major histocompatibility complex.

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Table 1

Selected examples of published studies of SABR and Immunotherapy combinations

Study details	SABR dose (Gy)/fractions	SABR Target	Immunotherapy agent	Sequence of treatments	Location of response
Postow *et al.*, (2012)[36]	28.5/3	Paraspinal	Ipilimumab	immunotherapy, then SABR, then immunotherapy	IF and OF
Hiniker *et al.*, (2012)[38]	54/3	Liver	Ipilimumab	immunotherapy, then SABR, then immunotherapy	IF and OF
Golden *et al.*, (2013)[39]	30/5	Liver	Ipilimumab	Concurrent	IF and OF
Silk *et al.*, (2013)[40]	14–24/1–5	Brain	Ipilimumab	immunotherapy then SABR, or SABR then immunotherapy	IF
Stamell *et al.*, (2014)[41]	NR	Brain	Ipilimumab	Concurrent	IF and OF
Karbach *et al.*, (2014)[42]	45/1	Brain	Autologous tumor-lysate-loaded dendritic cells	SABR then immunotherapy	IF and OF
Kiess *et al.*, (2014)[43]	15–24/1	Brain	Ipilimumab	SABR then immunotherapy, or Concurrent treatment, or Immunotherapy then SABR	IF
Kwon *et al.*, (2015)[44]	8/1	Bone	Ipilimumab	SABR then immunotherapy	IF
Seung *et al.*, (2012)[45]	20/1	Any	IL-2	SABR then immunotherapy	IF and OF

SABR, stereotactic ablative radiotherapy; IF, in field; OF, out of field.

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Table 2

Selected ongoing clinical trials investigating the efficacy of ISABR

Institution and study details	SABR dose (Gy)/fraction	SABR Target	Immunotherapy agent	Sequence of treatments	Phase
Johns Hopkins University, NCT01950195[46]	NS	Brain, Spine	Ipilimumab	Immunotherapy, then SABR, then immunotherapy	I
University of Pennsylvania, NCT01497808 (RADVAX)[47]	NS	NS	Ipilimumab	SABR then immunotherapy	I/II
MD Anderson Cancer Center, NCT02239900[48]	50/4 60/10	Liver, Lung, Adrenal	Ipilimumab	Concurrent, or immunotherpy then SABR	I/II
Chiles Research Institute, NCT01862900[71]	15/1 20/1	Lung, Liver	Anti-OX40	Concurrent	I/II
Stanford University, NCT01769222[72]	20/2	Any	Ipilimumab	Concurrent	I/II
New York University, NCT01401062[73]	22.5/3	Any	Fresolimumab	Concurrent	I/II
NIH/NCI, NCT02298946[74]	8/1 24/3	Liver	PD-1 inhibitor	SABR then immunotherapy	I
Thomas Jefferson University, NCT01703507[75]	24/1 21/1 18/1 15/1	Brain	Ipilimumab	Concurrent	I
MD Anderson Cancer Center, NCT02444741[76]	50/4	Lung, Liver	PD-1 inhibitor	Concurrent	I/II

ISABR; Immunotherapy combined and stereotactic ablative radiotherapy; SABR, stereotactic ablative radiotherapy; NCI, National Cancer Institute; NS, not specified.

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Table 3

Proposed ISABR studies

Patient population	SABR dose (Gy)/number of fractions	SABR target	Type of immunotherapy	Sequence	Readout
Stage I NSCLC	50–60/3–5 60–70/8–10	Primary tumor	Vaccine-MAGE * PD-L1	Immunotherapy followed by SABR Concurrent ISABR	PET/CT scan, PD-L1, TIL, Treg, CD8/CD4, Exome micro-RNA, cytokine production, CEA-specific T cells
Early stage hepatocellular carcinoma	40–60/3–5 50–70/8–10	Primary tumor	PD-L1	Concurrent ISABR	MRI scan, PD-L1, TIL, Treg, CD8/CD4, Exome micro-RNA, cytokine production
Stage IV CRC	50–60/3–5 60–70/8–10	Dominant Liver or lung metastasis	Vaccine-CEA PD-1	Immunotherapy followed by SABR Concurrent ISABR	CT scan, MRI of the abdomen, cytokine production, CEA-specific T cells, TILs in treated and off-target metastases, inflammatory cytokine production, PD-L1, Treg, CD8/CD4, Exome micro-RNA
Stage IV NSCLC with spinal/brain metastases	12–25/1 15–24/1	Spinal metastases Brain metastases	PD-L1 PD-1 + ipilumumab	Immunotherapy then SABR Concurrent ISABR	PET/CT, brain MRI, tumour-specific T cells, PD-L1 expression levels, inflammatory cytokine production, TIL, Treg, CD8/CD4, Exome micro-RNA
Stage IV NSCLC	Organ-dependent dose regimen	Oligometastasis	PD-L1 PD-1 + ipilumumab	SABR then immunotherapy Concurrent ISABR	PET/CT scan to monitor regression at distant metastatic sites, PD-L1 expression levels, TILs in treated primary and untreated metastases, Treg, CD8/4, Exome micro-RNA

CEA, carcinoembryonic antigen; CRC, colorectal cancer; ISABR; Immunotherapy combined and stereotactic ablative radiotherapy; MAGE, melanoma antigen E; NSCLC, non-small-cell lung cancer; SABR, stereotactic ablative radiotherapy; TILs, tumour-infiltrating lymphocytes; T-reg, regulatory T-cells; RNA, ribonucleic acid.

*MAGE-tumor-associated antigen is expressed in 50% of patients with NSCLC[68], with no immunohistochemical staining of this antigen observed in normal lung tissue samples[69].