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REVIEW ARTICLE

Combining radiotherapy with immunotherapy: the past, the present and the future

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ABSTRACT

The advent of immunotherapy is currently revolutionizing the field of oncology, where different drugs are used to stimulate different steps in a failing cancer immune response chain. This review gives a basic overview of the immune response against cancer, as well as the historical and current evidence on the interaction of radiotherapy with the immune system and the different forms of immunotherapy. Furthermore the review elaborates on the many open questions on how to exploit this interaction to the full extent in clinical practice.

INTRODUCTION

Only recently, it was noticed that radiotherapy and immunotherapy together can lead to a more effective anti-tumour response than each of the both modalities apart. The interactions between radiation and immune system have become a new area of intense research within cancer research programmes. The goal of this review is to provide the reader an overview of the new strategy combining radiotherapy with immunotherapy, including its earlier development, its current state and the next steps required to bring this new approach to a success in general clinical practice.

## BASIC OVERVIEW OF THE ANTI-TUMOUR IMMUNE RESPONSES

A cancer cell is characterized by the loss of its normal regulatory processes, which gives rise to uncontrolled cell growth and formation of metastases.[1] 90% of cancer deaths are not related to primary tumour but rather attributable to metastatic disease.[2] The molecular basis of this distinct behaviour is governed by aberrant proteins, also called oncoproteins, regulating several biological processes such as cytostasis and differentiation, viability and apoptosis, proliferation and motility, gene regulation, deoxyribonucleic acid repair etc.[1,3,4]

Normally, the immune system protects the host against the formation of cancer, following a process known as the cancer immunity cycle.[5] First, specific antigens are released by the cancer cells which are picked up by antigen-presenting dendritic cells to activate and prime naïve T lymphocytes. Hereby, a very specific reactivity against antigens from the tumour cells is generated. Subsequently, these activated T cells infiltrate a tumour to recognize and destroy the cancerous cells. Then, dendritic cells pick up antigens of dying cancer cells again, restarting the whole process (Figure 1).

Among several subclasses of T lymphocytes, there are two major subtypes governing the cellular immunity against cancer: the cytotoxic or $CD8^+$ T cells and the T-helper (Th) or $CD4^+$ cells. Progenitor Th cells can differentiate in two different subtypes: (a) the effector Th (Th1) cell, which stimulates the dendritic cells and the cytotoxic T cells using surface receptors and by producing ligands such as CD40L and IL2, respectively,[6–8] and (b) the regulatory Th cell or Treg, recognized by nuclear FOXp3 expression,[9,10] which hamper the immune response. The physiological function of the latter is to protect against autoimmunity.[11]

In cancer, however, the above-described normal immune response is deregulated, allowing cancer cells to escape

Figure 1. This figure shows the effects of radiotherapy in relation to the cancer immune cycle. Radiotherapy affects the immune response by induction of immunogenic cell death releasing new antigens to the components of the immune system. This subsequently leads to improved priming and activation of effector T cells. Radiotherapy further leads to increased expression of surface molecules on the irradiated cancer cells making them more vulnerable to cytotoxic T-cell-mediated cell killing. Finally, radiotherapy leads to the release of cytokines attracting T cells towards the irradiated tumour. Improved influx of effector T cells and improved T-cell killing of cancer cells could result in new antigen presented to the components of the immune system.
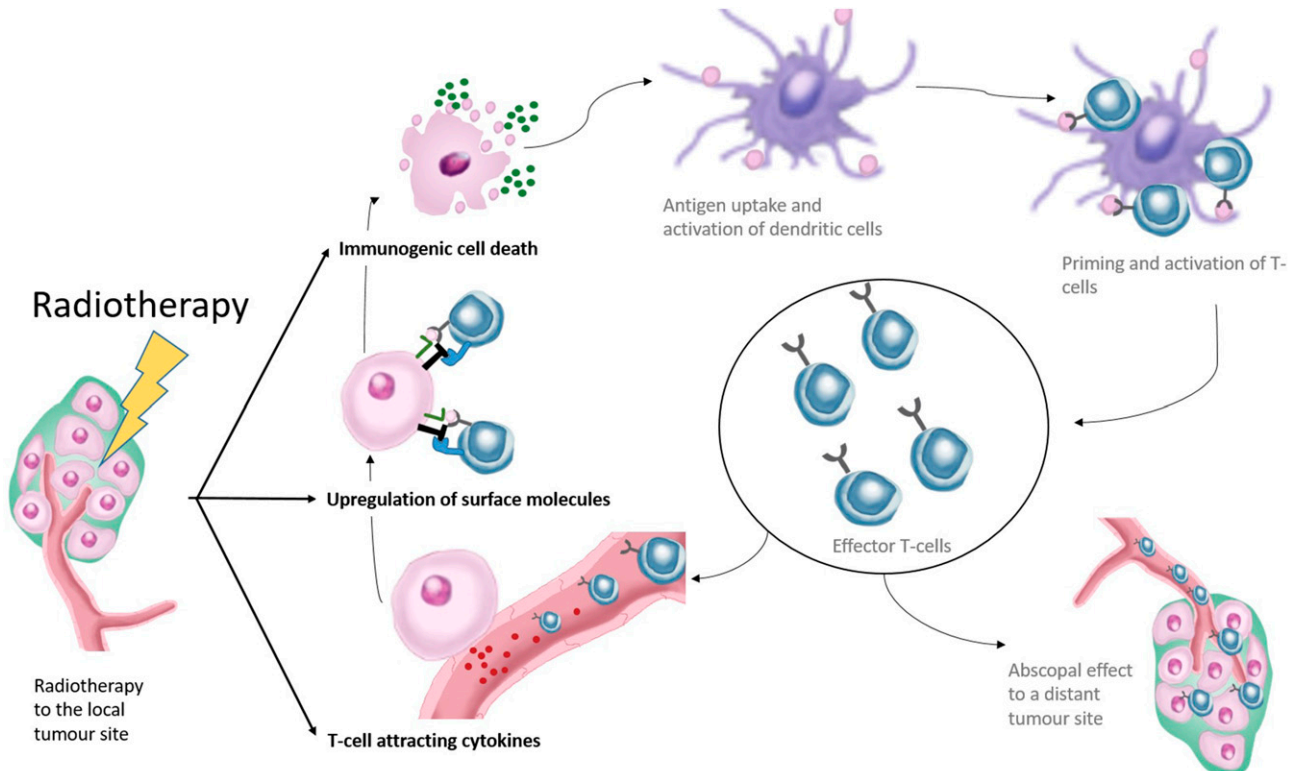
from the immune system and survive. As the cancer immunity cycle is a very complex sequential process, it can fail when at least one essential link is disrupted. The reason for a failed immune response can be diverse and several mechanisms allowing the tumour to escape exist. Such possibilities are, for example, "tumour foreignness" questioning whether there are enough neoantigens that make it possible for the tumour to be recognized as foreign; general immune status of the patients defining if there are enough lymphocytes to combat the tumour; hampered intratumoural immune cell infiltration; the presence of immune T-cell checkpoints and ligands blocking cytotoxic T-cell activity, among many others (for more details, see the cancer immunogram[12]).

THE PAST: FIRST INDICATIONS OF AN INTERACTION BETWEEN RADIOTHERAPY AND THE IMMUNE SYSTEM

A strong relation between the radiosensitivity of the irradiated tumour (murine fibrosarcoma) and the immunocompetence of the host has already been described in a pre-clinical research article dating back to 1979. The dose needed to control the tumour in 50% (TCD50) of immunosuppressed mice was about double the dose that was needed in immunocompetent mice.[13] Consistent with these results, chemoradiotherapy in mice bearing tumours from tumourigenic tonsillar epithelial cells was

more efficient in immunocompetent C57BL/6 mice than in their immunosuppressed C57BL/6 rag-1-deficient counterparts.[14]

Radiotherapy has always been regarded as a highly effective but local therapy for cancer. However, >20 case reports in the whole medical literature describe tumour regressions outside of the radiation treatment fields.[15] This effect was referred to as the abscopal effect, derived from the Latin word *ab scopus* meaning on a distant site. The underlying mechanism of these off-target effects remained obscure, and because of their rareness, these events were regarded as medically not relevant. In the early days, several pre-clinical studies evaluated the effect of local irradiation on distant tumour sites, but the results were not consistent; both inhibition and acceleration of the non-irradiated tumour sites were seen.[16]

Although these findings were interesting, they went without much attention within the scientific community. That is, until the breakthrough of immunotherapy within the field of oncology, which started in 2010 with a Phase III trial investigating ipilimumab in patients with metastatic melanoma, showing for the first time a survival benefit.[17] The following period was a rollercoaster of clinical successes, with immunotherapy being declared the scientific breakthrough of the year in 2013 by the *Science* magazine.[18] During this period, two case reports were

published of a patient progressive under ipilimumab, now showing responses to radiotherapy outside the irradiated area which were linked to a response of the immune system.[19,20] The events described in these case reports were consistent with earlier pre-clinical work demonstrating a synergy between CTLA-4 blockade and radiotherapy.[21,22] Also, at the same time, a Phase I trial was published combining stereotactic body radiation therapy together with high dose of IL2 in renal cell carcinoma and melanoma. The authors found a response rate that was much higher than what would be expected based on historical data from IL2-based treatment alone.[23] The promising results of these clinical studies raised a lot of interest in the field of immunotherapy including in combination with radiation.

THE PRESENT: IMMUNOTHERAPY AS STANDARD OF CARE, AND EXTENSIVE PRE-CLINICAL EVIDENCE ON THE INTERACTION OF RADIOTHERAPY WITH IMMUNOTHERAPY

Over the past few years numerous Phase III trials have demonstrated immunotherapy to confer an overall survival benefit in advanced, recurrent or metastatic melanoma,[17,24] non-small-cell lung cancer,[25–29] renal cell cancer,[30] head and neck cancer,[31] transitional cell carcinoma of the bladder[32] and prostate cancer.[33] In general, these therapies did show a favourable toxicity profile. Clearly, based on these results one can say that a corner has been turned and a new era in oncology has begun. However despite these successes, not surprisingly, these immunotherapies show only benefit in a limited number of patients because they affect only very specific points/processes in the cancer immunity cycle, and in addition, resistance mechanisms by tumour cells may be acquired during therapy or may be elicited by the tumour microenvironment. Apart from Sipuleucel-T (Provenge®) in prostate cancer, which is an active cellular-based vaccine,[33] the other forms of immunotherapy depend on checkpoint blockade. These are very specific antibody-based checkpoint inhibitors such as CTLA-4-directed ipilumumab or PD-1/PD-L1 axis inhibitors such as pembrolizumab, nivolumab and atezolizumab. In essence, CLTA-4 inhibitors work by blocking a specific inhibitory interaction between the dendritic cell and the T cell.[34] PD-1/PD-L1 inhibitors block a specific inhibitory interaction between the effector T cell and the cancer and/or dendritic cell, which normally leads to inhibition of T-cell growth and loss of effector functions.[35]

On the momentum of the success of these new forms of immunotherapies, several efforts were made to study the interaction of radiotherapy and the immune system in more depth, aiming to exploit the current immunotherapy successes even further. How radiotherapy contributes to an enhanced immune response has been reviewed in detail elsewhere.[36–39] A recent and extended review focusing on the molecular pathways involved can be found here.[40] To summarize, radiotherapy has been shown to trigger the immune response by (1) induction of immunogenic cell death (ICD) broadening up the immune repertoire of T cells, (2) recruitment of T cells towards the irradiated tumour and (3) increasing vulnerability towards T-cell-mediated cell killing (Figure 1). ICD is associated with a pre-mortem stress response allowing the cell to attract the attention of the immune system.[39] This process allows the immune

system to distinguish cell death from a pathogenic process (*e.g.* viral infection or cancer) or cell death in the context of normal tissue homeostasis.[39] ICD is characterized by the release of tumour-associated antigens in the form of apoptotic bodies and debris, together with adenosine triphosphate, calreticulin, HBMG-1, which induce dendritic cell recruitment and result in their activation, antigen uptake and maturation.[37–39] By doing so, radiotherapy delivers new antigens to the adaptive immune system, promoting the priming and generation of new anti-tumour T cells. Secondly, radiation recruits effector T cells towards the tumour by releasing T-cell-attracting chemokines such as CXCL-9 and CXCL-10.[41] Interestingly, CXCL-10 secretion has also been linked to the process of ICD through type I interferon signalling.[39] Thirdly, radiotherapy induces a transient overexpression of MHC class I and Fas surface receptors rendering tumour cells more vulnerable to cytotoxic T-cell killing.[42–45] However, obviously, radiotherapy alone is not sufficient to induce curative anti-tumour immune response especially against metastatic cancer, highlighting the need for combinatorial immunotherapy approaches to boost immune system.

Numerous pre-clinical studies reported improvement of the local radiotherapy and/or abscopal response with CTLA-4 inhibitors.[21,22,46–49] Interestingly, a retrospective case series on 101 patients treated with ipilimumab seems to confirm these findings. Of these 101 patients, 70 patients received concurrent radiotherapy. These 70 patients showed a significant increase in overall survival over the patients who did not receive concurrent radiotherapy, as well as increases in response.[50] The interaction of PD-1/PD-L1 inhibition with radiotherapy has also been reported to enhance both local[51–55] and abscopal effects.[53,56] Furthermore, in the context of radiotherapy, there seems to be a clinical rationale to combine PD-1-directed therapy and CTLA-4 as both immunotherapeutic agents activate non-redundant immune mechanisms.[48] IL2 is a cytokine with an essential role in the activation of immune response. Although it also stimulates proliferation of regulatory T cells, it also activates cytotoxic T and natural killer cells resulting in an activation of the immune system augmenting together with radiotherapy local as well as abscopal tumour responses.[57] As IL2 is associated with significant morbidity (capillary leak syndrome, ischaemia, flu-like syndromes),[58] efforts were made to reduce its toxicity by making its delivery more tumour specific.[59] These tumour-targeting "immunocytokines", such as NHS-IL2 (targeting free deoxyribonucleic acid) and L19-IL2 (targeting external domain B (EDB) of fibronectin in newly formed vessels), have shown to enhance local radiotherapy effects.[60–62] L19-IL2 was only effective in tumours expressing EDB, and the effect was greatly dependent on the presence of CD8^{+} cytotoxic T cells.[62,63] However, even in a MHC class I-deficient tumour model, where cancer cytotoxicity is not dependent on specific antigen-targeted activity of the CD8^{+} T cells but rather on natural killer cell activity, an additive effect of radiotherapy over L19-IL2 alone was seen.[60] Our research group has also found an abscopal effect of radiotherapy/L19-IL2 combination on secondary non-irradiated tumours (Personal communication Dr Rekers, presented at ESTRO 35, 2016). These results are in line with an earlier clinical study evaluating high dose IL2 treatment with stereotactic radiotherapy showing much higher than expected clinical response rates for an extended period of time.[23]

These promising data provided a rationale for the start of a multitude of currently running clinical trials (>70), testing combinations of radiotherapy (fractionated or stereotactic body radiation therapy) together with CTLA-4 inhibition, PD-1/PD-L1 inhibition, vaccination or cytokine treatment such as IL2, anti-transforming growth factor-beta or granulocyte-macrophage colony-stimulating factor. Recent overviews of ongoing radiotherapy–immunotherapy trials are provided elsewhere;[64–66] for an up-to-date version, see clinicaltrails.gov.

THE FUTURE: INTEGRATING RADIOTHERAPY AND IMMUNOTHERAPY IN THE CLINICAL SETTING

To optimize a radiotherapy regimen or treatment in the context of immunotherapy, several factors need to be considered. These are optimal fractionation schedule and dosing, the timing between radiotherapy and immunotherapy, the radiotherapy technique to deliver the dose, implications for the clinical target volume (CTV), lesion selection and safety.

When considering fractionation and dose to be combined with immunotherapy, one must first consider the goal of the treatment approach: does the treatment aim for an improved local effect or to create an abscopal effect on the non-irradiated (micro) metastasis? As described above, radiotherapy stimulates the immune system by broadening up the immune repertoire of T cells (vaccination effect), by attracting T cells to the irradiated site (homing effect) and by rendering irradiated cells more vulnerable towards T-cell-mediated cell kill (vulnerability effect). It is expected that only the broadened immune repertoire is useful for the immune system to produce a generalized systemic response, meaning that the underlying biology is different and perhaps more critical when one aims for an abscopal response. Several groups have investigated different fractionation schedules and doses. Gandhi et al[67] provided recently a very extensive review on the matter. Several authors describe a dose-dependent increase of cell surface molecules such as FAS, MHC1 or ICAM1[42,44,45] using doses varying between 1 and 50 Gy in humans (HCT116 colorectal carcinoma and Mel JuSo melanoma) and murine (MC38 colon carcinoma) cell lines. As these receptors are important for T-cell vulnerability, they are presumed in the first place to be important for enhancing the local effect of radiotherapy. When comparing fractionated (5 × 3 Gy) and single-dose (15 Gy) radiotherapy in a B16 melanoma model in their capacity to activate dendritic cells in the lymph nodes (as measured by activation/priming of a hybrid reporter T-cell line), it was demonstrated that 15-Gy single dose was more efficient.[41] By contrast, in a similar tumour model (B16-OVA melanoma), another group showed T-cell priming, this time measured by an INFγ Enzyme-Linked ImmunoSpot assay on splenic T cells, to be more efficient with 2 × 7.5 Gy than 15-Gy single dose.[68] Several groups did also investigate the impact of fractionation when combining radiotherapy with immunotherapy. Dewan et al[21] showed that while all fractionation schedules showed comparable local tumour control as monotherapy in combination with CLTA-4 inhibition, 3 × 8 Gy was superior to 5 × 6 or 1 × 12 Gy in a TSA breast and MCA38 colon carcinoma model, with respect to local tumour control and abscopal response. Clinical data from a retrospective review in patients with melanoma receiving ipilimumab suggested fraction doses ≤3 Gy to be associated with abscopal responses.[69] By contrast, work from

our group investigating the interactions of the L19-IL2 immunocytokine with radiotherapy in a C51 tumour model show that a larger dose per fraction is more efficient to induce an abscopal response, as only 1 × 15 Gy and not 5 × 5 or 5 × 2 Gy was able to induce tumour cure of the non-irradiated tumours. However, fractionated irradiation was as efficient as single-dose irradiation in eradication of the primary locally irradiated tumours (unpublished data). To conclude, fractionation and dose are of vital importance to maximize the effect of associated immunotherapy, but no consensus exists on which schedule is optimal. The conflicting results from the literature let us presume that optimal fractionation is highly context dependent, and therefore conclusions should be cautiously drawn. Insights may come from clinical trials, evaluating local and/or abscopal response to (different kinds of) immunotherapy combined with different fractionation schedules. This is, however, a cumbersome procedure and only allows for indirect measurement of the immune-stimulating radiotherapy effect. As tumour response is not only dependent on the radiotherapy schedule but also on the elements in the cancer immune cycle, the impact of choosing the right fractionation schedule when looking at local or abscopal tumour control will be diluted. More elegant and straightforward ways to evaluate the efficiency of different fractionation schedules therefore may rely on innovative new biomarker approaches, such as the release of immunogenic cell death-related chemokines and cytokines[70] in blood or biopsies, or on methods allowing large-scale measurement of extension in the T-cell repertoire—novel techniques based on barcode-labelled peptide-MHC-1 multimers are able to screen >1000 T-cell specificities in a single sample.[71] Such an approach could allow us to compare the impact and efficiency of different radiotherapy schedules on increasing the variety of specific T-cell responses towards different tumoural antigens.

Treatment techniques such as volumetric modulated arc therapy better shape the volume around the target tissue but also lead to a low-dose bath to a large part of the body.[72] Lymphocytes are among the most radiosensitive cells in the body with a D_{10} (dose to reduce the total amount of surviving cells to 10% of the initial value) of around 3 Gy only.[73] In this context, the dose to the tumour-draining lymph nodes and the timing of the fractionation may be of importance, especially in daily fractionated schedules. The normal transit time of a naïve helper of cytotoxic T lymphocyte is 12 h to a day.[74] However, when confronted with a dendritic cell-presenting antigen, these T cells remain in contact with the dendritic cell and undergoes a "blasting" transformation. This process again takes another 24 h, even before clonal expansion ensues.[75,76] For cytotoxic T cells, however, it was shown that the stable interaction with the dentritic cell was dispensable, still allowing to undergo successful effector differentiation, but long-lived memory was hampered.[77] It is possible that even a low dose given in short daily intervals to the lymph nodes may interfere with the priming process of T lymphocytes and its memory functions. The impact of the low-dose bath and daily fractionation to date is unknown and needs further investigation.

Integration of radiotherapy within an immunotherapy schedule may also need rethinking of the classical definitions of target

volumes. When, for example, in a more diffusely metastatic patient radiotherapy is added as a form of immune adjuvant, it may not be necessary to expand the target volumes for microscopic tumour extension (CTV or CTV margins) or apply wide margins around the volume to account for deviations in daily treatment setup (planning target volume or planning target volume margins), as it may suffice to irradiate a part of the tumour to induce immune stimulation. The narrower margins would allow for better sparing of the organs at risk, to reduce complication probability. This approach, which is theoretically promising, however, needs validation in clinical trials.

Little is known on the selection of the right target for radiotherapy in the context of creating an abscopal effect together with immunotherapy. Some authors proposed a mathematical model to predict the lesions with the highest potential.[78] This model was based on T-cell trafficking and the assumption that abscopal effects can only be achieved when activated T cells from the irradiated tumour can reach the distant sites in sufficient numbers. However, with no clinical data sets to validate this virtual model, extreme care should be taken before using this model in practice, as it lacks many other important parameters determining abscopal responses.[79]

The timing seems to be of essential importance when embedding radiotherapy into an immunotherapy approach to get the most optimal results. The ideal timing between immunotherapy and radiotherapy depends on the mechanism of action of the specific form of immunotherapy.[52,80] For example, Young et al[80] investigated the optimal timing of radiation in combination with an OX-40 agonist antibody and a CLTA-4 antagonist antibody. Best results were seen when CTLA-4 was given before radiotherapy. Nonetheless, other authors did find synergistic activity also for CTLA-4 inhibition when given concurrently or sequentially with radiotherapy.[21,22] By contrast, OX-40-based immunotherapy worked best when given immediately following radiotherapy.[80] The authors proposed that OX-40 would function by boosting antigen-specific T-cell numbers,[80,81] whereas anti-CTLA-4 would rather function as a downregulator of regulatory T cells.[80,82] Therefore, OX40 inhibition would be most beneficial just following radiation-induced antigen release. It is possible that in the case of CTLA-4 inhibition, antigen release created by radiotherapy is most efficient only when the regulatory T-cell fractions are depleted first. Dovedi et al[52] investigated the ideal treatment sequence for inhibition of the PD-1 axis, and found the best effect was found when the PD-L1 was given concurrently or immediately following the radiotherapy. Delaying the PD-L1 infusions for 1 week abrogated the interaction between radiotherapy and PD-1 axis inhibition.[83] Inhibition of the PD-1 axis increases the lytic activity of the cytotoxic T cells.[35] Therefore, an optimal interaction is expected just at that moment when radiotherapy temporarily induces surface ligands on the cancer cell increasing its vulnerability to T-cell attacks.[42–45] The authors also showed that radiotherapy temporarily induced overexpression of PD-1 axis molecules on the tumour cells as well as on the tumour-infiltrating T cells.[52] Inhibition of the PD-1 axis is therefore expected to be most efficient when it attenuates the radiation-induced immune

response most efficiently, in close temporal relation to the radiation treatment.

Hypoxia is associated with both radioresistance and immune suppression.[84,85] This hypoxia can be quantified by hypoxia positron emission tomography tracers such as HX4, FAZA or F-MISO.[86,87] Unravelling these resistance mechanisms associated with hypoxia could lead to the identification of new therapeutic targets. Furthermore, reducing hypoxia with hypoxia-targeting drugs[88,89] could potentially lead to a reduction of immunosuppresion in the tumour microenvironment. Currently, this is an area of active investigation in our research team.

Finally, only limited clinical information is available on the different combinations of immunotherapy and radiotherapy. It is possible that radiation-induced acute toxicity, which is associated with an inflammatory response, could be aggravated by an activation of the immune system following immunotherapy.[90] Kroeze et al[91] recently reviewed the evidence on stereotactic radiotherapy and concurrent anti-CTLA4 and anti-PD-1/PD-L1. Regarding the combination with anti-CTLA4 and cranial stereotactic irradiation, they seem to show that the approach is safe, although the available studies are small. Very limited data are available on the combination of extracranial stereotactic radiotherapy with anti-CTLA4. Regarding the combination of concurrent anti-PD-1/PD-L1, they concluded that there was insufficient data to allow for conclusions. Following this report Levy et al[92] published the results of a small Phase I/II trial investigating the combination of the PD-L1 inhibitor durvalumab with conventional and stereotactic radiotherapy. The combination was well tolerated. Kwon et al[93] evaluated the combination of 8-Gy conventional radiotherapy followed by ipilimumab *vs* placebo in a large Phase III trial of metastatic castration-resistant patients with prostate cancer. Although the primary end point (overall survival) was negative, they did not see a higher-than-expected toxicity of the radiotherapy–ipilimumab combination than what would be expected from ipilimumab alone. Two small Phase I trials showed that NHS-IL2- or IL2-based immunotherapy could be safely administered following conventional[61] or high-dose stereotactic radiotherapy,[23] respectively. The combination of stereotactic radiotherapy followed by L19-IL2 is currently under evaluation in our Phase I trial (NCT02086721). To summarize, the available data show no indication of induction of excessive toxicity of radioimmunotherapy over immunotherapy alone. However, as data are mostly immature and limited, prudence remains imperative.[90] Therefore, it is advised to test these combinations preferably within the context of a clinical trial.

CONCLUSION

The advent of immunotherapy is currently revolutionizing the field of oncology, where different drugs are used to stimulate different steps in a failing cancer immune response chain. Extensive pre-clinical data have shown that radiotherapy can synergize with these agents by broadening up the immune repertoire in T cells (vaccination effect), by attracting T cells to the irradiated site (homing effect) and by rendering irradiated cells more vulnerable towards T-cell-mediated cell kill (vulnerability effect). There are many open questions on how to integrate radiotherapy into an immune treatment in patients in the most optimal

fashion; these questions are about optimal fractionation and dose, target volume, treatment technique, timing and safety. A plethora of clinical trials are currently ongoing investigating these radiotherapy–immune interactions in patients.

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