Systematic review

Radiotherapy and immune checkpoints inhibitors for advanced melanoma

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Abstract

Introduction: The therapeutic landscape of metastatic melanoma drastically changed after the introduction of targeted therapies and immunotherapy, in particular immune checkpoints inhibitors (ICI). In recent years, positive effects on the immune system associated to radiotherapy (RT) were discovered, and radiation has been tested in combination with ICI in both pre-clinical and clinical studies (many of them still ongoing). We here summarize the rationale and the preliminary clinical results of this approach.

Materials and methods: In the first part of this review article, redacted with narrative non-systematic methodology, we describe the clinical results of immune checkpoints blockade in melanoma as well as the biological basis for the combination of ICI with RT; in the second part, we systematically review scientific publications reporting on the clinical results of the combination of ICI and RT for advanced melanoma.

Results: The biological and mechanistic rationale behind the combination of ICI and radiation is well supported by several preclinical findings. Retrospective observational series and few prospective trials support the potential synergistic effect between radiation and ICI for metastatic melanoma.

Conclusion: RT may potentiate anti-melanoma activity of ICI by enhancing response on both target and non-target lesions. Several prospective trials are ongoing with the aim of further exploring this combination in the clinical setting, hopefully confirming initial observations and opening a new therapeutic window for advanced melanoma patients.

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The main role of the immune system is to restore normal tissues’ homeostasis when altered by pathologic processes, including neoplastic transformation [1]. The immune system is often successful in eliminating neoplastic cells. Thus, all established tumours need to overcome immunity to progress and grow, and most of them successfully escape immune control, through different mechanisms [2,3]. Until recently, attempts in developing and applying immunotherapeutic strategies aimed to enhance innate and adaptive immune response failed in controlling most of solid tumours, with some exception represented by melanoma and renal cancer, where different forms of immunomodulation have been used for years. The clinical scenario drastically changed after the introduction of immune checkpoints inhibitors (ICI), a new class of targeted drugs able to activate the immune system against cancer cells, and showing efficacy for both solid tumours and haematological malignancies, with striking results leading to unexpected survival gains for advanced/unresectable melanoma [4]. Melanoma is actually the first cancer subtype where these immune-activating agents showed an advantage in survival over standard chemotherapy, and data from large clinical trials confirmed a substantial benefit with prolonged survival [5].

Over the last decade, it was also hypothesized that the combined effects of radiation therapy (RT) and immunotherapy in metastatic tumours might be synergistic, and this research field is currently one of the most stimulating and potentially practice-changing topics in radiation oncology. Several mechanisms have been proposed for explaining the interaction between RT and the immune system. Among them, microenvironment modification, cytokine and danger signals release, pro-inflammatory effect and immunogenic cell death pattern [6−8]; one of the most attractive experimental hypotheses is that ionizing radiations may act as an “in situ” vaccination in cancer patients, enhancing what has been called the “abscopal” effect after RT [9]. Such effect has been occasionally observed in patients undergoing palliative RT.
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(especially in melanoma), and consists in a response of untreated (distant, outside radiation volumes) lesions following a radiation cycle to one lesion [10]. According to preclinical models, the “abscopal” effect is immune-mediated [9], and thus may be enhanced by the combined use of ionizing radiation and immune-modulating drugs of different classes, for example the immune checkpoint inhibitor ipilimumab, an anti-CTLA-4 monoclonal antibody which binds to T cells [11].

Ipilimumab activates T cells by blocking the inhibitory signal mediated by CTLA-4 through the interaction with surface receptors on antigen presenting cells (APC). Retrospective clinical reports showed that the combination of RT and ipilimumab was able to trigger the abscopal effect in a proportion of melanoma patients, and that this effect might prolong survival [12–14]. Additionally, experimental data provided the proof of principle that radiation and immunotherapy may favourably interact to enhance abscopal anti-tumour effects, and radiation may be used as a way to potentiate the effects of immunotherapy [15]. At the same time, new insights into the mechanisms leading to resistance to the combination of radiation and ICI and possible approaches for overcoming this phenomenon have been discovered [16]. As a consequence of the prolonged survival time achievable with new generation systemic therapies, RT to one or few metastatic sites (either in conventional fractionation or delivering high dose in few fractions) is now widely in use as a local therapy especially for oligo-metastatic disease [17,18].

Aim of this review is to focus on the potential therapeutic partnership between RT and ICI in advanced melanoma, discussing the most relevant pre-clinical and clinical findings, current research and future challenges.

Materials and methods

A narrative methodology was used for selecting and reporting studies on the clinical use of ICI for advanced melanoma, and for describing the biological and mechanistic basis of the combination of radiation and ICI. A systematic review was then performed according to validated guidelines [18,19] for selecting clinical studies reporting on the combination of RT and ICI for advanced melanoma. For this second part, we searched for English-language full length articles published from January 2000 to December 2015 using PubMed, and only studies reporting clinical outcomes following the combination of ICI and RT for metastatic melanoma were included. Studies were excluded if: (a) they were review articles and (b) they were not the most recently published outcomes, in instances of multiple publications from the same study cohort. The search strategy was “metastatic melanoma” OR “advanced melanoma” AND “radiotherapy” OR “radiosurgery” OR “stereotactic body radiotherapy” AND “ipilimumab” OR “ pembrolizumab” OR “nivolumab”, which identified 560 articles. Two clinicians reviewed these records to determine which were suitable for inclusion according to the pre-defined criteria, selecting 11 reports. Five more articles were added from reference lists of the selected publications, for a total of 16 reports on the clinical outcomes of the combined treatment.

Immune checkpoints inhibitors in melanoma: state of the art and new challenges

Until recently, the medical management of unresectable metastatic melanoma was based on the use of chemotherapy, either as single agent (dacarbazine, temozolomide or fotemustine) or multi-drug associations, with or without biotherapies such as interferon or interleukin-2. Despite the optimistic results coming from mainly single centre phase II trials suggesting a potential benefit of chemotherapy plus interferon and/or interleukin-2, a series of randomized trials did not demonstrate any advantage [20]. Two large meta-analyses clearly showed that even if bio-chemotherapy may increase the percentage of responses, this does not result in any improvement of survival, while is coupled with a higher toxicity [21,22]. The unsatisfactory results obtained by (bio)chemotherapy were recently summarized in a study analysing the clinical data of more than 2000 patients enrolled onto 42 phase II trials since 1975. An overall 1-year survival of 25.5% and a median survival of 6.2 months were achieved, without any significant improvement over the last 30 years [23].

Even if it was well known that T-cell response is regulated through a complex balance of inhibitory and activating signals and that the tumour itself can deregulate these pathways leading therefore to an impairment of the immune system activities [3], the relevant new concept which was developed following the failure of cytokine-based immunotherapy was the potential of targeting these inhibitory and activating immunological synapses as a new tool to promote anti-melanoma immune response. Up till now, while the field is rapidly evolving and new drugs are under investigation, two main types of monoclonal antibodies targeting immune checkpoints have been developed and investigated in the treatment of metastatic melanoma. The first targets the cytotoxic T-lymphocyte antigen 4 CTLA-4, the other the Programmed Death 1 (PD-1) pathway. It is of relevance that both compounds physiologically interact with immunological checkpoints leading to inhibitory signals for T cells (priming and effector phases); the blockade of these pathways allows the release of the immune system brake thus fostering, maintaining and stimulating the T-cell response [24].

Anti-CTLA-4

Ipilimumab is a fully humanized monoclonal antibody that binds to CTLA-4, a receptor expressed on the T-cell surface that interacts with CD80 (B7-1) and CD86 (B7-2) on the Antigen-Presenting-Cells (APCs) and down regulates T-cell response. CTLA-4 blockade allows CD28 to bind to B7-1 receptors, leading to immune activation, IL-2 secretion, cytotoxic T-cells expansion and proliferation [25]. The interaction of ipilimumab with the immune system takes place in an early phase of the immune response involving “naive” T lymphocytes and the APCs. This mechanism of action explains the characteristics of the clinical activity as well as the common side effects of this drug, consisting of immune-mediated reactions developing more frequently in the skin, gastro-intestinal tract (mainly diarrhoea), liver and endocrinal glands. Moreover, it gives reason to the delayed occurrence of a relevant clinical response.

After pre-clinical data and pilot studies showing activity, a randomized phase II study compared different dose regimens in metastatic melanoma (0.3, 3 or 10 mg/kg IV every 3 weeks), showing that both 3 and 10 mg/kg induced optimal response, even if the latter dose was coupled with an increase in immune-related adverse effects (irAEs) [26]. In the first phase III trial, ipilimumab ± glycoprotein 100 peptide (gp100) vaccine was compared with gp100 vaccine monotherapy in patients with unresectable stage III or stage IV melanoma. Ipilimumab monotherapy significantly improved median overall survival (OS) compared with gp100 vaccine monotherapy (10.1 months vs. 6.4 months) [27]. In a second randomized phase III trial, the combination of ipilimumab (10 mg/kg) and dacarbazine (850 mg/m²) resulted in significantly superior OS compared to dacarbazine (850 mg/m²) plus placebo (11.2 months vs. 9.1 months) [28].

Notably, ipilimumab produced a plateau in survival curves: a recent pooled analysis of OS data for 1,861 patients enrolled in 10 prospective and 2 retrospective trials, with up to 10 years follow-up, showed that the survival curve began to plateau around
3 years after treatment. Three-year OS rates were 22%, 26%, and 20% for all, treatment-naïve, and previously treated patients, respectively [4]. The results of the ipilimumab expanded access programme (EAP) in Italy were consistent with these data, confirming the activity of the drug also in specific patient's subsets such as the elderly, the mucosal or uveal primaries, and in the presence of brain metastases [29].

Response to ipilimumab can be preceded by an increase in diameter of tumour lesions, reflecting the inflammatory changes and the recall of T-cells to the tumour site. Distinct response patterns have been described: (a) shrinkage in baseline lesions, without new lesions; (b) durable stable disease (in some patients followed by a slow, steady decline in total tumour burden); (c) response after an increase in total tumour burden; and (d) response in the presence of new lesions [30]. This implies the need to adopt specific immune-related criteria to correctly evaluate response, based on the concomitant assessment of both the primary lesions and the appearance of new lesions [31]. Table 1 summarizes the results of phase II/III clinical trials.

**Anti-PD-1**

PD-1 protein is a co-inhibitory receptor expressed on B and T cells, and has been shown to be involved in the negative regulation of T-cell activation [3]. PD-1 ligand (PD-L1) is expressed in different tumours, is associated with a worse prognosis and its expression and interaction with T cells is thought to be one of the main mechanisms underlying the immune system escape [3]. The discovery that tumour cells were able to activate the PD-1/PD-L1 axis, leading to protection from cytotoxic T cells trough exhaustion, led to the development of specific anti-PD-1 inhibitors [32]. The anti-PD-1 monoclonal antibodies nivolumab (a fully human anti-PD-1 IgG4) and pembrolizumab (a humanized anti-PD-1 IgG4) were shown to be highly effective for malignant melanoma, and in 2014 they both have been licensed in the United States as second- or third-line treatment for patients with evidence of disease progression after ipilimumab (in BRAF wild-type melanoma) or ipilimumab and BRAF inhibitors (in BRAF V600-mutated melanoma) [33]. The toxicity profile of anti-PD-1 agents was reported to be similar to anti-CTLA-4 [34]. A randomized phase III study comparing nivolumab vs. dacarbazine in previously untreated melanoma without BRAF mutation demonstrated superior overall response rate (ORR, 40% vs. 13.9%, respectively) and increased 1-year OS (72.9% vs. 42.1%, respectively). Moreover, nivolumab resulted in a better safety profile: treatment-related adverse events occurred in 11.7% of the patients receiving nivolumab and 17.6% of the patients receiving dacarbazine, respectively [35]. In CheckMate 037 phase III trial, patients were randomly assigned 2:1 to receive nivolumab 3 mg/kg every 2 weeks or investigators’ choice chemotherapy (ICC) until progression or unacceptable toxic effects. Primary endpoints were the proportion of patients who had an objective response and overall survival. At first interim analysis on 120 and 47 randomized patients, confirmed objective responses were reported in 31.7% of patients in the nivolumab group vs. 10.6% of patients in the ICC group; no treatment-related deaths occurred [36].

The activity of pembrolizumab for advanced melanoma was firstly shown in 2013 by a phase IB study achieving an ORR of 38% in both ipilimumab pre-treated or not pre-treated patients [37]. Two different doses of pembrolizumab (2 mg/kg and 10 mg/kg) were then investigated and compared with ICC in the Keynote-002 randomized phase II clinical trial. At enrolment, patients had progressive disease after ipilimumab or, if BRAF mutated, after BRAF or MEK inhibitors, or both. Results showed an improvement in progression-free survival (PFS) at 6 months as assessed by independent central review, with HR 0.57 for pembrolizumab 2 mg/kg and 0.50 for 10 mg/kg. Grade 3–4 treatment-related adverse events were more frequent and occurred earlier in patients receiving chemotherapy [38].

In a large randomized phase III study, 834 patients with advanced melanoma were treated either with pembrolizumab at a dose of 10 mg/kg every 2 or every 3 weeks or with 4 doses of ipilimumab (3 mg/kg every 3 weeks). The estimated 6-months PFS rates were 47.3% for pembrolizumab every 2 weeks, 46.4% for pembrolizumab every 3 weeks, and 26.5% for ipilimumab, respectively. Estimated 1-year OS rates were 74.1%, 68.4%, and 58.2%, respectively. The response rate was improved when pembrolizumab was administered either every 2 or every 3 weeks, as compared with ipilimumab. Treatment-related adverse events of grade 3–5 severity were lower in the pembrolizumab groups (13.3% and 10.1%) [39]. Details on phase II-III trials testing anti-PD-1 agents nivolumab or pembrolizumab in advanced melanoma are shown in Table 2.

**Combination of anti-CTLA-4 and anti PD-1 agents**

The rapid increase of these multiple immunotherapy options opened a new challenging field for metastatic melanoma, with the primary aim being the definition of the best combination strategy in order to improve effectiveness without compromising

<table>
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<tr>
<th>Table 1</th>
<th>Summary of the most relevant clinical trials on ipilimumab for advanced melanoma.</th>
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<tr>
<td>Author</td>
<td>Study design</td>
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<tr>
<td>Wolchuck et al., 2010 [26]</td>
<td>Randomized, double-blind, phase 2 trial</td>
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<tr>
<td>Hodi et al., 2010 [27]</td>
<td>Randomized, double-blind phase 3 trial</td>
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<tr>
<td>Robert et al., 2011 [28]</td>
<td>Randomized, double-blind phase 3 trial</td>
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<tr>
<td>Schadendorf et al., 2015 [4]</td>
<td>Pooled analysis (10 prospective and 2 retrospective trials)</td>
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**Abbreviations:** ORR = overall response rate; DCR = disease control rate; OS = overall survival; NR = not reported.

* Including two phase 3 trials (Refs. [24] and [25]).
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Summary of the most relevant clinical trials on nivolumab or pembrolizumab either alone or in combination with ipilimumab for advanced melanoma.

Robert et al., 2014 [35] Randomized, double-blind Phase 3 trial Nivolumab 210 40 72.9 5.1
Weber et al., 2015 [36] Randomize, controlled, open-label, Phase 3 trial Nivolumab 120 31.7 NR NR
Ribas et al., 2015 [38] Randomized, double-blind Phase 2 Pembrolizumab 2 mg/kg 180 21 NR 34% at 6 months
Pembrolizumab 10 mg/kg 181 25 NR 38% at 6 months
Ribas et al., 2015 [39] Randomized, double-blind Phase 2 Pembrolizumab Q2W 279 33.7 74.1 5.5
Pembrolizumab Q3W 277 32.9 68.4 4.1
Postow et al., 2015 [41] Randomized, double-blind Phase 1 Ipilimumab 278 11.9 58.2 2.8
Ipilimumab + Nivolumab 722/23 61/52 27 (crude rate) Not reached/ 8.5
Ipilimumab + Placebo * 37/10 § 11/ 10 § 37 (crude rate) 4.4/2.7§
Larkin et al., 2015 [42] Randomized, double-blind Phase 3 Nivolumab + Ipilimumab 314 57.6 11.5
Nivolumab 315 19 2.9
Nivolumab + Nivolumab 310 43.7 6.9
Ipilimumab 72 60 8.9
Ipilimumab + Nivolumab 70 11 4.7
Hodi et al., 2015 [43] Randomized, double-blind Phase 2 Nivolumab + Ipilimumab 68 41.2 NR NR
Hodi et al., 2015 [44] Randomized, open-label Phase 2 Nivolumab 70 20

Abbreviations: ICC = investigator’s choice chemotherapy; ORR = overall response rate; OS = overall survival; PFS = progression-free survival; NR = not reported.
* Per-protocol interim-analysis.
** Intention-to-treat, independent-central review, RECIST v1.1.
§ Followed by Nivolumab or Placebo.
§§ BRAF wild-type/BRAF V600 mutation positive.

Table 2

Safety and efficacy. Recent data demonstrated a substantial improvement in ORR and OS for the combination of ipilimumab and anti-PD-1 agents in different sequence schedules in which patients are treated with both antibodies. A pilot phase I study tested different approaches: patients received concomitantly intravenous doses of nivolumab and ipilimumab every 3 weeks for 4 doses, followed by nivolumab alone every 3 weeks for 4 doses (concurrent regimen). In a sequenced regimen, patients previously treated with ipilimumab received nivolumab alone every 2 weeks for up to 48 doses. A total of 53 patients received concurrent therapy with nivolumab and ipilimumab, and 33 received sequential treatment. ORR for all patients in the concurrent-regimen group was 40%. Evidence of maximum activity was observed in 65% of patients. At the maximum doses that were associated with an acceptable level of adverse events, 53% of patients had an objective response, all with tumour reduction of 80% or more. Grade 3 or 4 adverse events related to therapy occurred in 53% of patients in the concurrent-regimen group, but were similar to previous experience with monotherapy and were generally reversible [40].

In a double-blind study involving 142 patients with previously untreated metastatic melanoma, patients were assigned 2:1 to receive ipilimumab (3 mg/kg) combined with either nivolumab (1 mg/kg) or placebo once every 3 weeks for four doses, followed by nivolumab (3 mg/kg) or placebo every 2 weeks until disease progression or unacceptable toxic effects. Among patients with BRAF wild-type tumours, ORR was 61% (44 of 72 patients) in the group that received both ipilimumab and nivolumab versus 11% in the group that received ipilimumab and placebo. Median PFS was not reached with the combination therapy and was 4.4 months with ipilimumab monotherapy. Similar results for ORR and PFS were observed in 33 patients with BRAF mutated tumours [41]. Another key study showed encouraging data regarding the superiority of the combination over ipilimumab or nivolumab alone. Patients treated with the combination had a PFS of 11.5 months, compared to 2.9 months for those treated with ipilimumab alone and 6 months for those treated with nivolumab alone, respectively; the ORR (57.5%) obtained by the combination was remarkably higher, yet with a higher incidence of grade 3–4 immune-related adverse events (irAEs), frequently involving more than one organ [42]. The combination was also compared with ipilimumab alone in treatment-naive patients in a phase II study: ORR was 60% and 11% for the combination treatment and for ipilimumab alone, respectively. Median PFS was 8.9 months for the combination versus 4.7 months for ipilimumab alone. Grade 3–4 drug-related adverse events were reported in 51% of patients receiving nivolumab plus ipilimumab, and in 20% in those receiving ipilimumab alone [43]. The recently presented data (in abstract form) of the CheckMate 064 trial, comparing the two sequences of ipilimumab followed by nivolumab vs. nivolumab followed by ipilimumab, suggest a better outcome in terms of ORR for the latter (at 25 weeks 41.2% vs. 20%, respectively) [44]. Details on phase II–III trials testing the combination of nivolumab and ipilimumab are integrated in Table 2.

Toxicity

Checkpoint inhibition is associated with a unique spectrum of side effects termed immune-related adverse events (irAEs). irAEs include dermatologic, gastrointestinal, hepatic, endocrine, and other less common inflammatory events. irAEs are believed to arise from general immunologic enhancement, and temporary immunosuppression with corticosteroids, tumour necrosis factor-alpha antagonists, mycophenolate mofetil, or other agents can be an ineffective treatment in most cases. Treatment of moderate or severe irAEs may require a temporary interruption of the drug and the use of corticosteroids to reduce the dose or combination therapy. Treatment is based upon the severity of the observed toxicity. In Table 3, we report the most common toxic effects associated to the use of anti-CTLA-4, anti-PD-1 or the combination of both for advanced melanoma [40–44]. As described, the combined use of a double ICIs is more toxic and should be managed with special attention.

RT and immune checkpoints inhibitors in melanoma: rationale and preclinical data

Despite the efficacy of ICIs, still many patients do not respond or initially respond and then progress, and an anti-tumour immune response powerful enough to control a tumour when it re-
emerges by blocking immunosuppressive mechanisms is possible in only a minority of patients [8]. This is the result of multiple mechanisms that obstruct the priming and activation of anti-melanoma T cells, their recruitment to the tumour sites as well as their function. These phenomena represent an arduous challenge to effective tumour rejection [45,46]. Ionizing radiation has long been known for a long time to cause pro-inflammatory effects, but the potential benefits of this pro-inflammatory response only recently emerged. The most recent important findings were that radiation is able to convert the tumour in an “in situ” vaccine, altering the microenvironment towards the development of an “immunogenic hub”: radiation is in fact able to promote both the priming and effector phases of anti-tumour immune response [47]. Among the first observations, Lugade et al. evaluated anti-tumour immune responses in mice after treatment of B16 melanoma tumours with single (15 Gy) or fractionated (5 × 3 Gy) doses of localized ionizing radiation. Irradiated mice had cells with greater capability to present tumour antigens and specific T cells that secreted IFN-γ upon peptide stimulation within tumour-draining lymph nodes. Immune activation in tumour-draining lymph nodes correlated with an increase in the number of CD45+ cells infiltrating single dose irradiated tumours compared with non-irradiated mice. Similarly, irradiated mice had increased numbers of tumour-infiltrating lymphocytes that secreted IFN-γ and lysed tumour cell targets [48].

Other crucial observations regarded both the role of radiation in modulating the peptide repertoire, enhancing MHC class I expression and inducing antitumour immunity [49] and the radiation-induced IFN-gamma production within the tumour microenvironment, that again positively influences antitumour immunity [50].

Researchers from the University of Chicago then showed, on irradiated mice affected with melanoma and pancreatic cancer, that RT in combination with a specific DNA repair inhibitor was able to trigger immune response by releasing immunomodulatory signals from senescent tumour cells; stimulatory cytokines were discovered to activate T lymphocytes and potentiate immune response [51].

The activation of natural anti-tumour T-cell response requires the uptake and cross-presentation of tumour-derived antigens by dendritic cells (DCs) to T cells; this process depends on type I interferon (IFN-I), which is necessary for DCs recruitment to tumours and their activation. In addition, radiation-induced cell death generates three key molecular signals, that have been shown to promote the uptake and presentation of tumour-derived antigens by DCs: (a) calreticulin translocation from the endoplasmic reticulum to the cell surface acts as a signal for uptake of dying cancer cells by DCs; (b) the release of the nuclear protein high-mobility group box-1 (HMGB1), which binds to toll like receptor (TLR)-4 on DCs, promotes antigen cross-presentation and (c) adenosine triphosphate (ATP) activates the inflammasome via the P2XR7 receptor with downstream release of interleukin (IL)-1β [6]. The generation of these signals collectively defines immunogenic cell death (ICD), which is crucial in promoting the priming phase. Moreover, radiation is able to trigger the effector phase by inducing chemokines and cytokines to recruit effector T-cells at the tumour (CXCL 16), through up-regulation of major histocompatibility complex class I (MHC-I), adhesion molecules, death receptors and NKGD2 ligands that enable recognition and elimination of damaged cancer cells [52,53]. These discoveries were essential in re-defining the landscape of the interactions between local RT, tumour response and the immune system.

At the same time, RT may reinforce immunosuppressive pathways that undermine antitumor immune-surveillance by different mechanisms, especially in the priming phase. Radiation can enhance tumour infiltration by myeloid-derived suppressor cells (MDSCs), a subset of bone marrow–derived immature myeloid cells responsible for sustaining chronic immunosuppression [6]. Moreover, regulatory T cells, with a key role in suppression of anti-tumor immunity, seem more radiation resistant than conventional T cells and show a relative increase after radiation. Also CD47 blockade, whose expression on cancer cells suppress innate immunity, may directly enhance tumour immune-surveillance by CD8 T cells; CD47 expression in tumour microenvironment limits the cooperation between anti-tumour T cell immunity and radiation therapy [54]. Recently, it was also shown that radiation induces Langerhans’ cells to migrate from the skin to lymph nodes, where they stimulate regulatory T-cells [55,56]. Additionally, counterbalance of the effector phase induced by RT is the increase expression of PD-L1 on cancer cells after radiation, which may de-activate effector T-cells [6].

As a consequence, the effects of radiation on tumour microenvironment and its interaction with the immune system appear as a complex balance of activating and suppressing signals. The pro-immunogenic “go” signals are countered by immunosuppressive “stop” signals: the balance of stop and go signals determines the development of effective antitumor immune responses. Positive effects of RT should therefore be harnessed to enhance immune activation, especially in combination with immune-activating agents such as immune checkpoint inhibitors [57]. Zegers et al. demonstrated that a radiation-induced antitumor effect can be enhanced by the administration of IL-2 in combination with L19, a selective tumour targeting agent able to improve therapeutic outcome over IL-2 treatment alone. L19 binds to extra domain B, a part of the fibronectin in tumour neovasculature and overexpressed in many solid tumours [58]. Researchers from the same group lately showed that also in tumour models lacking MHC1 expression and depending on natural killer (NK) immune response, the combination of L19-IL2 plus radiation was able to improve tumour growth delay by an additive effect [59].

The combination of RT with ICI has been explored on different fronts across years. Preclinical studies have reported increased loco-regional control when radiation is combined with checkpoint blockade immunotherapy in different cancer subtypes [60]. Moreover, increased systemic disease control has been shown by com-

### Table 3

<table>
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<tr>
<th>Side effects</th>
<th>Ipilimumab (%)</th>
<th>Nivolumab (%)</th>
<th>Ipilimumab + Nivolumab (%)</th>
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<tr>
<td>Diarrhoea (any grade)</td>
<td>33–37</td>
<td>19</td>
<td>45</td>
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<tr>
<td>Grade 3–4</td>
<td>6–11</td>
<td>2</td>
<td>9–11</td>
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<tr>
<td>Skin rash (any grade)</td>
<td>17–33</td>
<td>26</td>
<td>16–40</td>
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<td>Grade 3–4</td>
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<td>12–23</td>
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<td>Grade 3–4</td>
<td>7–9</td>
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</table>
bining radiation with both anti-CTLA-4 and anti-PD-1/PD-L1 inhibitors [16]. A key point in this field was the discovery that the manifestation of the abscopal effect, rarely occurring after RT, is immune-mediated [9]. Investigators at Vanderbilt University found that mice that underwent irradiation of a melanoma tumour to 25 Gy in 1 fraction before surgical excision had fewer lung metastases than did mice that underwent excision but no radiation treatment. Greater tumour infiltration by CD8 T cells in mice that had previously received an ex vivo irradiated melanoma vaccine were noted, and the authors suggested that DC-mediated phagocytosis was responsible for the decrease in the frequency of metastases in mice with irradiated tumours [61]. Similarly, investigators at the University of Chicago reported that the efficacy of high-dose ablative radiation therapy in a mouse model of melanoma was immune mediated by CD8 T cells [62]. Sharabi et al., from the University of California, noted improved tumour control when radiation was combined with anti-PD-1 antibody in a mouse model of breast cancer and melanoma, with enhanced proliferation and activation of antigen-specific T cells and effector memory cells in draining lymph nodes. Stereotactic RT resulted in the development of antigen-specific T cell and B cell-mediated immune responses. These immune-stimulating effects were significantly increased when radiation was combined with either anti-PD-1 therapy or regulatory T cell depletion, resulting in improved local tumour control [63]. Park et al. also showed in preclinical melanoma and renal carcinoma models that the combination of stereotactic RT plus PD-1 blockade was able to induce complete regression of the irradiated primary tumour also eliciting a reduction in size of non-irradiated outside the radiation field (abscopal effect). The observed effect was tumour specific and was not dependent on tumour histology or host genetic background, suggesting that RT may induce an abscopal tumour-specific immune response in both the irradiated and non-irradiated tumours, which is potentiated by PD-1 blockade [64].

Taken altogether, these data constitute the experimental basis for the combination of ICI and radiation therapy to one or few lesions, with the aim of enhancing the effects of immunotherapy by using radiation as a powerful tool to overcome resistance and to trigger abscopal effect. For the first time, the results of a prospective “proof of principle” trial, smartly testing the combination of local RT and granulocyte–macrophage colony stimulating factor (GM-CSF), as DCs growth factor, successfully showed in various cancer subtypes (not including melanoma) that RT on metastatic sites could allow the activation of the abscopal effect on non-target lesions in a substantial fraction of patients [65]. Results of this trial are paramount to further support research in this field, particularly when investigating the combination of RT and ICI. A study investigating on the combination of anti-CTLA-4 in both humans and mouse models of metastatic melanoma showed that the induction of the abscopal effect is limited to a fraction of patients due to an acquired resistance to ipilimumab which is PD-1/PD-L1 mediated. The clinical component of this study was a phase I trial testing the combination of RT on a single lesion (6–8 Gy delivered over two or three fractions) followed by ipilimumab (4 cycles, beginning 3–5 days after the last RT fraction), showing a 36% overall abscopal response rate. Non-responding patients had up regulated PD-L1, and genetic elimination of PD-L1 from therapy-resistant melanoma cells dramatically restored response to ipilimumab plus radiation. This study planted a seed for the sequential combination of radiation and both anti-CTLA-4 and anti-PD1/PD-L1 antibodies may combat adaptive immune resistance upon localized radiation plus anti-CTLA-4 therapy, and the superior activity of radiation and dual immune checkpoint blockade is mediated by non-redundant immune mechanisms [66].

Fig. 1 illustrates a schematic model of the possible interaction between radiation, tumour microenvironment and the immune system, summarizing the “in situ” vaccination concept and the “abscopal” effect induced by RT. Fig. 2 illustrates the interactions between RT and ICI, with distinctive clinical combinations and sequences, aiming at different possible endpoints (maximizing response, overcoming resistance).

**RT and immune checkpoint inhibitors in melanoma: clinical data**

In 1975, Kingsley et al. firstly described a case of abscopal effect in a patient treated with radiation therapy for metastatic melanoma [10]. More recently, two pivotal clinical reports showed how the combination of RT and ipilimumab might be efficient in obtaining disease control on the treated site enhancing abscopal effect on un-irradiated sites. Postow et al. [12] described the case of a female patient treated with 4 doses of ipilimumab at 10 mg/kg followed by maintenance every 12 weeks. After 1 year she had progressive disease on both a para-spinal mass and spleen/thoracic lymph nodes, and received palliative fractionated RT on the para-spinal mass, while continuing ipilimumab. After 4 months the targeted mass regressed and, remarkably, also a very good partial response was observed on the hilar lymph nodes and spleen.
lesions, with stable disease at 10 months. The authors performed immunological studies showing an increase in antibody response after RT, consistent with an immune-mediated abscopal effect. Few months later, Hiniker et al. [67] reported on a case of a male patient who first developed a nodal recurrence after resection of the primary (at 3 years) and then had oligo-recurrent metastatic disease with 2 liver metastases at 4 years. He received 2 doses of ipilimumab followed by RT on 2 out of 7 metastases, followed by 2 more doses of ipilimumab. At 5 months, all liver lesions were in complete response, but the patient relapsed at the site of previous surgery (skin): he was simply observed, and the lesion completely resolved after 2 months. Investigators at Memorial Sloan Kettering Cancer Centre (MSKCC) performed a retrospective analysis of 29 patients who received extra-cranial RT in combination with ipilimumab; no significant increase in adverse effects was observed, and patients receiving RT during maintenance had higher OS than those treated during the induction phase [14]. A retrospective observational series on 23 patients treated with palliative RT after ipilimumab reported the occurrence of abscopal responses in 11/23 (52%); median time between ipilimumab and RT was 5 months, and median OS for patients obtaining an abscopal response was significantly higher than for non-responding patients (22.4 vs. 8.3 months) [13]. Chandra et al., on 47 patients, showed in 68% of cases an improved response on index lesions (outside radiation field) [68]. A case of abscopal response after SRS for a brain metastasis and ipilimumab was also reported by Stamell et al. [69]. Barker and Postow recently reviewed the clinical outcomes of the combination of ipilimumab and RT in advanced melanoma, with the aim of: (A) maximizing response upfront (concomitant approach, higher toxicity) (B) overcoming resistance in poor responders (sequential approach) and (C) triggering the restoration of immune response and overcoming acquired resistance after initial response (sequential/concomitant approach) [Refs. 52,16]. For cells’ shapes and colours see Fig. 1. ICI: immune checkpoint inhibitors.

**Fig. 2.** Different possible therapeutic combinations between RT and ICI for advanced melanoma, with the aim of: (A) maximizing response upfront (concomitant approach, higher toxicity) (B) overcoming resistance in poor responders (sequential approach) and (C) triggering the restoration of immune response and overcoming acquired resistance after initial response (sequential/concomitant approach) [Refs. 52,16]. For cells’ shapes and colours see Fig. 1. ICI: immune checkpoint inhibitors.
melanoma, including brain metastases [70]. A few reports described successful outcomes in patients treated with ipilimumab and whole-brain radiation therapy (WBRT). Early reports included a 49-year-old patient who received ipilimumab 4 weeks after receiving 30 Gy WBRT, with a significant regression of brain metastases at 12 weeks after the initiation of ipilimumab [71], and a woman with lepto-meningeal disease who received 20 Gy WBRT followed by ipilimumab having a complete radiographic response 2–3 months after completing treatment, without symptoms [72]. Gerber et al. reported on 13 patients receiving WBRT and ipilimumab, with a promising overall response rate, yet 10/10 patients with available imaging demonstrated new or increased intracranial bleeding [73]. Investigators from Dana-Farber Cancer Institute reported on 16 melanoma patients who received ipilimumab and either WBRT or stereotactic radiosurgery (SRS), 4 concomitantly. Extra-cranial target lesions achieved a response rate of 35% when comparing pre and post RT imaging [74]. Silk et al. from the University of Michigan compared 33 patients with brain metastases receiving either SRS or WBRT and ipilimumab (before or after RT) vs. 37 not receiving ipilimumab, showing improved survival for the combination of SRS and ipilimumab [75].

Knisely et al. reported on 77 patients with brain metastases treated with SRS, with patients who received ipilimumab having a median survival of 21.3 months vs. 4.9 months for those who did not. Survival was not significantly different whether the drug was given before or after SRS [76]. In a similar study from New York University, on 58 patients treated with brain SRS, no difference in local tumour control, survival, or frequency of intracranial haemorrhage was reported for those who did or did not receive ipilimumab, respectively [77]. Tazi et al. reported on the combination of SRS and ipilimumab on 10 patients, showing promising survival results (comparable to those without brain metastases) [78].

Investigators at Memorial Sloan-Kettering Cancer Centre (MSKCC) also reported on 46 patients treated with ipilimumab and brain SRS: on multivariate analysis, prolonged survival was associated with the delivery of SRS during ipilimumab [79]. An increase in brain metastasis size >150% occurred in 40% of the tumours treated with SRS before or during ipilimumab and, conversely, in 10% of metastases treated with SRS after ipilimumab. Haemorrhage was observed after SRS during ipilimumab in 42% of brain metastases. The potential complications of brain SRS and ipilimumab were studied in a small series of 3 patients: after 20 Gy SRS, radiation necrosis was observed in all, proven radiologically in two and histologically in one patient, respectively [80]. Radionecrosis was also reported by Knisely et al. (3/27 patients) [76] and Kiess et al. (5/46 patients) [79]. The higher rate of increasing lesions as well as radio-necrosis features among patients receiving SRS or WBRT in combination with ICI is a matter of debate, but many researchers believe that these findings could be an expression of greater local immune reactions.

Table 4 summarizes the clinical outcomes reported so far by different series with the combination of RT and ICI in metastatic melanoma.

**Dose/fractionation issues and the role of stereotactic ablative RT**

The technological advances in the field of Radiation Oncology now allow for rapid non-invasive delivery of very high radiation doses to various metastatic sites. Stereotactic Body Radiation Therapy (SBRT) or Stereotactic Ablative RT (SABR) has been extensively investigated in recent years in metastatic patients [17,81], showing high local control rates and promising progression-free survival estimates, at the price of a very limited toxicity. With the term SBRT/SABR, we now refer to a “philosophy” of cancer treatment

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**Table 4**

<table>
<thead>
<tr>
<th>Study</th>
<th>No patients</th>
<th>Radiotherapy dose/ fractionation (Type)</th>
<th>Ipilimumab schedule (sequence)</th>
<th>Targeted site</th>
<th>Target site response (%)</th>
<th>Abscopal response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postow et al., 2012 [12]</td>
<td>1</td>
<td>28.5 Gy/3 fr (palliative)</td>
<td>10 mg/kg (sequential)</td>
<td>Para-sapinal metastasis</td>
<td>PR 100</td>
<td>PR 100</td>
</tr>
<tr>
<td>Hiniker et al., 2012 [67]</td>
<td>1</td>
<td>54 Gy/3 fr (SBRT)</td>
<td>3 mg/kg (sequential)</td>
<td>Liver metastasis</td>
<td>CR 100</td>
<td>CR 100</td>
</tr>
<tr>
<td>Barker et al., 2013 [14]</td>
<td>29</td>
<td>30 Gy/10 fr (median) (SBRT/palliative)</td>
<td>3 mg/kg (sequential)</td>
<td>Non-brain lesions</td>
<td>Symptoms relief 77</td>
<td></td>
</tr>
<tr>
<td>Grimaldi et al., 2014 [13]</td>
<td>21</td>
<td>20–24/1; 20 Gy/5fr; 30 Gy/10fr; 50 Gy/25 fr (SBRT/SRS/palliative/ WBRT)</td>
<td>3 mg/kg (sequential)</td>
<td>Brain, bone, lymph-node, cutaneous lesions</td>
<td>PR 62</td>
<td>PR 43 3D 10</td>
</tr>
<tr>
<td>Chandra et al., 2015 [68]</td>
<td>47</td>
<td>26 Gy (median) (SBRT/SRS/palliative/WBRT)</td>
<td>3–10 mg/kg (sequential)</td>
<td>Brain, soft tissue, bone, intrathoracic, abdominovisceral</td>
<td>NR</td>
<td>PR 36</td>
</tr>
<tr>
<td>Stammell et al., 2013 [69]</td>
<td>1</td>
<td>NR (SRS)</td>
<td>NR</td>
<td>Brain</td>
<td>NR</td>
<td>CR 100</td>
</tr>
<tr>
<td>Muller-Brenne et al., 2003 [71]</td>
<td>1</td>
<td>30 Gy/10 fr WBRT</td>
<td>3 mg/kg (sequential)</td>
<td>Brain</td>
<td>CR 100</td>
<td>NR</td>
</tr>
<tr>
<td>Bot et al., 2012 [72]</td>
<td>1</td>
<td>20 Gy/5fr WBRT</td>
<td>3 mg/kg (sequential)</td>
<td>Brain</td>
<td>PR 56.7</td>
<td>NR</td>
</tr>
<tr>
<td>Gerber et al., 2013 [73]</td>
<td>13</td>
<td>30 Gy/10 fr (median) WBRT</td>
<td>3–10 mg/kg (sequential)</td>
<td>Brain</td>
<td>NR</td>
<td>PR 35</td>
</tr>
<tr>
<td>Schoenfeld et al., 2015 [74]</td>
<td>16</td>
<td>22 Gy; 36 Gy (SRS/WBRT)</td>
<td>3 mg/kg (sequential)</td>
<td>Brain</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Silk et al., 2013 [75]</td>
<td>33</td>
<td>14–24 Gy/1–5 fr; 30–37.5 Gy/10–13 fr (SRS/WBRT)</td>
<td>3 mg/kg (sequential)</td>
<td>Brain</td>
<td>NR</td>
<td>PR 56.7</td>
</tr>
<tr>
<td>Knisely et al., 2012 [76]</td>
<td>27</td>
<td>NR (SRS)</td>
<td>NR (sequential)</td>
<td>Brain</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Mathew et al., 2013 [77]</td>
<td>25</td>
<td>20 Gy/1 fr (median) (SRS)</td>
<td>3 mg/kg (sequential)</td>
<td>Brain</td>
<td>LC 63%</td>
<td>NR</td>
</tr>
<tr>
<td>Tazi et al., 2015 [78]</td>
<td>10</td>
<td>NR (SRS)</td>
<td>3 mg/kg (sequential)</td>
<td>Brain</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Kiess et al., 2015 [79]</td>
<td>46</td>
<td>15–24 Gy/1 fr (SRS)</td>
<td>3–10 mg/kg (sequential)</td>
<td>Brain</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Du-Four et al., 2012 [80]</td>
<td>3</td>
<td>20 Gy/1 fr (SRS)</td>
<td>3 mg/kg (sequential)</td>
<td>Brain</td>
<td>CR 100</td>
<td>NR</td>
</tr>
</tbody>
</table>

**Abbreviations:** Gy: Gray; fr: fractions; WBRT: whole-brain radiotherapy; CR: complete response; PR: partial response; SD: stable disease; LC: local control; NR: not reported; SRS: stereotactic radiosurgery; SBRT: stereotactic body radiation therapy.

* Either evaluated with Response Evaluation Criteria in Solid Tumours or with immune-related response criteria.
Table 5
Prospective clinical trials combining either anti-CTLA-4 or anti-PD-1 agents and radiotherapy for advanced melanoma (from www.clinicaltrials.gov, December 2015, in order of estimated completion date).

<table>
<thead>
<tr>
<th>Registration number</th>
<th>Study design</th>
<th>Eligibility criteria</th>
<th>Intervention</th>
<th>Primary endpoint</th>
<th>Estimated enrolment</th>
<th>Estimated study completion date</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT01689974</td>
<td>Phase II</td>
<td>Locally unresectable, metastatic melanoma, with at least 2 distinct measurable metastatic sites, one of at least 1 cm or larger</td>
<td>Arm A: IPI alone Arm B: IPI and RT</td>
<td>Response rate</td>
<td>10</td>
<td>Completed in March 2015</td>
</tr>
<tr>
<td>NCT01497808</td>
<td>Phase I/Ii</td>
<td>Metastatic melanoma</td>
<td>IPI and SBRT</td>
<td>Dose-limiting toxicity</td>
<td>40</td>
<td>June 2015 (ongoing, not recruiting)</td>
</tr>
<tr>
<td>NCT01557114</td>
<td>Phase I</td>
<td>Unresectable locally advanced or metastatic melanoma with at least one melanoma metastasis accessible to radiation therapy</td>
<td>Induction IPI (4 courses), →RT → Maintenance IPI</td>
<td>Maximum Tolerated Dose of RT in combination with IPI</td>
<td>30</td>
<td>March 2016</td>
</tr>
<tr>
<td>NCT01449279</td>
<td>Single institution, open-label, pilot study</td>
<td>Stage IV melanoma</td>
<td>IPI and palliative radiation therapy</td>
<td>Percentage of patients experiencing serious adverse events in the first 4 months of treatment</td>
<td>20</td>
<td>June 2016</td>
</tr>
<tr>
<td>NCT01996202</td>
<td>Phase I</td>
<td>Resected patients at high risk of recurrence/ Neoadjuvant- definitive approach for locally advanced patients</td>
<td>RT and IPI</td>
<td>Incidence of immune related adverse events associated with IPI, acute and late radiation toxicities</td>
<td>24</td>
<td>June 2016</td>
</tr>
<tr>
<td>NCT01970527</td>
<td>Phase II</td>
<td>Index lesion between 1 and 5 cm</td>
<td>SBRT (3 fractions) between days 1 and 13 → IPI every 3 weeks (4 courses)</td>
<td>Late toxicity, immune-related clinical response, immune-related PFS, OS</td>
<td>40</td>
<td>September 2016</td>
</tr>
<tr>
<td>NCT02115139</td>
<td>Phase II</td>
<td>Melanoma brain metastases</td>
<td>Whole brain RT with concurrent IPI</td>
<td>1-year OS</td>
<td>66</td>
<td>October 2016</td>
</tr>
<tr>
<td>NCT02097732</td>
<td>Phase II</td>
<td>Melanoma brain metastases</td>
<td>Standard arm: SRS → IPI (4 cycles) Experimental arm: IPI (2 cycles) → SRS → IPI (2 cycles)</td>
<td>Local control rate</td>
<td>40</td>
<td>May 2017</td>
</tr>
<tr>
<td>NCT02107755</td>
<td>Phase II</td>
<td>Oligo-metastatic melanoma</td>
<td>SBRT with concurrent IPI</td>
<td>PFS Maximum Tolerated dose, with dose-limiting toxicity in 25% of patient OS, safety and tolerability (acute and subacute toxicity)</td>
<td>32</td>
<td>June 2017</td>
</tr>
<tr>
<td>NCT0210406183</td>
<td>Phase I</td>
<td>Metastatic melanoma with at least 3 extra-cranial measurable lesions</td>
<td>SBRT with concurrent IPI</td>
<td>Overall response rate</td>
<td>50</td>
<td>November 2017</td>
</tr>
<tr>
<td>NCT01565837</td>
<td>Phase II</td>
<td>Oligo-metastatic but unresectable melanoma</td>
<td>SBRT with concurrent IPI</td>
<td>Overall response rate</td>
<td>60</td>
<td>December 2018</td>
</tr>
<tr>
<td>NCT02407171</td>
<td>Phase Ia (expansion cohort)</td>
<td>Metastatic melanoma (with at least one site of measurable disease suitable for SBRT)</td>
<td>SBRT (at maximum tolerated dose discovered in phase I) and Pembro (200 mg every 2 weeks)</td>
<td>Abscopal effect</td>
<td>234</td>
<td>October 2019</td>
</tr>
<tr>
<td>NCT02562625</td>
<td>Phase II</td>
<td>Unresectable or stage IV melanoma with 1-3 lesions targets for high dose radiotherapy and at least one other lesion which will not be irradiated to assess the abscopal effect of the treatment</td>
<td>Arm 1: Pembro alone Arm 2: Pembro and RT (24 Gy/ 3 fr)</td>
<td>Maximum tolerated dose of IPI</td>
<td>24</td>
<td>November 2019</td>
</tr>
<tr>
<td>NCT01703507</td>
<td>Phase I</td>
<td>Melanoma brain metastases</td>
<td>Arm A: IPI and WBRT Arm B: IPI and SRS → RT (8 Gy/1 fr∼20 Gy/5 fr) → re-biopsy → Pembro → RT → Pembro</td>
<td>Change in PD-L1 levels</td>
<td>40</td>
<td>January 2020</td>
</tr>
</tbody>
</table>

Abbreviations: SRS: stereotactic radiosurgery; SBRT: stereotactic body radiation therapy; PFS: progression-free survival; OS: overall survival; IPI: ipilimumab; Pembro: pembrolizumab; NA: not applicable; NR: not reported.
with high focused doses in 1 or few sessions (<10), administered with ablative intent. Very few melanoma patients have been included in studies on SABR for extra-cranial metastases, mostly for lung and liver [17]: efficacy and toxicity, when reported, appear similar to any other primary histology.

The best combination strategy, as well as the best dose/fractionation regimen for RT is still under debate. When combined with ipilimumab, preclinical comparisons of different dose fractionation schedules (24 Gy in 3 fractions, 30 Gy in 5 fractions, or 20 Gy in single fraction) in breast cancer models suggest that multifraction approach is superior to single fraction regimens, in terms of abscopal effect induction [82]. Golden et al. reported on the combination of ipilimumab and 30 Gy in 5 fractions on liver lesions in a patient with refractory lung cancer, with complete response of treated and untreated lesions and the patient alive at one year [83].

The clinical data reported so far on melanoma patients appear consistent with these findings, as shown in Table 4. In some reports, ablative fractionation has been used to target visceral lesions, with 18 Gy × 3, in others a slight hypo-fractionation was preferred, delivering 9.5 Gy × 3. However, abscopal effect was also observed after ipilimumab when standard fractionation was used. The only prospective trial testing the combination of radiation therapy and dendritic cell stimulation with GM-CSF in various cancer subtypes employed a 35 Gy/10 fractions approach on 2 index lesions treated subsequently [65]. The biological mechanisms of radiation induced cell death at fraction sizes greater than 8–10 Gy might be different from the classical radiobiology paradigm of conventionally fractionated RT. In addition to DNA damaging events, experimental models suggest that endothelial membrane alterations including sphingomyelin mediated endothelial apoptosis lead to microvaskulature dysfunction [84]. A marked increase in MHC-1 molecules expression was observed after high doses (25 Gy) when irradiating melanoma cells at different dose levels [85]. Lee et al. demonstrated that the reduction of tumour burden after ablative RT depends largely on T-cell responses: ablative radiation may dramatically increase T-cell priming in draining lymphoid tissues, leading to reduction/eradication of the primary tumour or distant metastasis in a cytotoxic T cell-dependent fashion [62]. Smilowitz et al. showed that increasing radiation dose may improve the outcome of immunotherapy when irradiating an advanced intracerebral melanoma model on mice at different dose levels in single fraction (15, 18.75 or 22.5 Gy) [86]. The use of ablative radiation doses on small targets in combination with immunotherapy seems a good strategy also because of draining lymph nodes sparing, allowing for APC migration, T-cell activation, cross priming and in situ effector T cells migration. However, we need to wait for prospective ongoing trials to fully answer to this question.

Likewise, the optimal timing of the combination between RT and immune checkpoints inhibitors is a matter of debate: in melanoma, the establishment of anti-tumour immunity seems to be enhanced when anti-CTLA-4 antibody precedes RT, or when given concomitantly [47]. In a recent Editorial, it has been suggested that radiation should be regarded as a complex “drug”, and its combination with immunotherapy warrants a systematic approach to optimize the efficacy of the radiation component [87]. An alternative approach to the concomitant use of ablative doses and ICI is a strategy where an intervention aimed to increase priming (i.e. GM-CSF or toll-like receptor agonists) is followed by concomitant radiation and anti-CTLA-4, and next by anti-PD1/anti-PD-L1 agents to prevent T cell exhaustion. From experimental data, this approach seems the most promising in advanced melanoma [14], however many combinations might be efficient and ready to be explored in the clinical setting (Fig. 2).

A summary of ongoing clinical trials testing the combination of RT and immune checkpoints inhibitors in melanoma is provided in Table 5.

**Conclusions**

Both preclinical models and clinical data showed that RT to one or few melanoma metastases might trigger and/or enhance the so-called abscopal effect. This effect is amplified in melanoma when combining RT with ICI such as anti-CTLA-4 or anti-PD-1/anti-PD-L1 antibodies, or the concomitant/sequential combination of both. Recent discoveries led to a better understanding of the mechanisms underlying this effect, and clinical data from retrospective observational studies and few prospective studies confirmed this hypothesis, suggesting prolonged response and survival. Currently, several prospective trials are ongoing with the aim of defining which is the best combination strategy, as well the best RT dose/fractionation regimen. Results of these studies will give answers to very important questions, hopefully creating a new window of therapeutic opportunities for metastatic melanoma patients, especially for those who still do not respond to ICI, where radiation to one or few lesions could play a major role in enhancing immune-mediated anti-tumour effects.

**Conflict of interest statement**

The Authors declare no conflict of interest with the material included in this review article.

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**References**


