

INVITED ARTICLE



Rationale for combination of radiation therapy and immune checkpoint blockers to improve cancer treatment

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ABSTRACT

Radiation therapy for cancer is considered to be immunosuppressive. However, the cellular response after radiation therapy may stimulate or suppress an immune response. The effect may vary with the tumor type and occasionally tumor regressions have been observed outside the irradiated volume, both in animal studies and in the clinic. A renewed interest in the role of immunity for the observed effect of radiation came with the current recognized role of immune checkpoint blockers (ICBs) for control of selected cancer types. We therefore here review preclinical studies and clinical reports on the interaction of ICBs and radiation as a basis for further clinical trials. Some tumor types where the combination of these modalities seems especially promising are also proposed.

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Introduction

The 5-year survival is currently about 70% for all cancers [1,2]. Cure is generally ascribed to surgery (49%), radiation therapy (40%) and drugs (11%), but several modalities are often used simultaneously and therefore the role of each modality can be difficult to define. Radiation therapy is considered necessary for about 50% of all cancer patients [3]. The technology for administering radiation is rapidly expanding [4,5] and the implementation of particle radiation (proton and carbon ion therapy) is ongoing [6–8].

Immune checkpoint blockers (ICBs) are now approved for treatment of malignant melanomas, Hodgkin's lymphoma, lung cancer, renal cancer and tumors with microsatellite instability such as colorectal cancer [9]. Despite many durable responses, the effect is varying within the same tumor entities and only a subset of these tumors respond to ICB treatment. In this paper we review published experimental and clinical studies which have addressed the possibility for combining radiation therapy with ICBs to increase the tumor response.

Methods

The literature was searched for English papers using PubMed with search words 'immune surveillance', 'immunotherapy', 'immune checkpoint blockers', 'immune checkpoint inhibitors', 'immunologic cell death', 'radiation', 'radiation therapy', 'immunotherapy', 'immunology' or combinations of these terms. Specific terms like 'apoptosis', 'autophagy' and 'abscopal response' related to the topics addressed were also used. The reference lists of the selected papers were then

scrutinized and all abstracts for relevant papers checked for references used in this narrative review.

Basic radiobiology

Cell killing

Classic radiobiology describes the inactivation of living cells by radiation therapy as clonogenic cell death. The radiation effect in cell lines is given by the surviving fraction, the percentage of cells with ability to form colonies of at least 50 daughter cells which is considered to imply a capacity for unlimited proliferation [10]. The underlying biological mechanisms are considered to be induction of damage caused directly by ionization of DNA or indirectly via reactions of aqueous free radicals formed around DNA [11,12]. It has been calculated that an X-ray dose of 1 Gray (Gy) produces about 3000 damaged bases, 1000 single-strand breaks (SSBs) and 40 double-strand breaks (DSBs) per single cell [13]. However, essentially all the base damage and SSBs are repaired by specialized base repair enzymes or the base excision repair (BER) system, while the majority DSBs are repaired by non-homologous end joining (NHEJ) and homologous recombination (HR) [14].

Radiation influences plasma membranes especially at doses above 10 Gy by dose-dependent activation of acid sphingomyelinase which hydrolyzes sphingomyelin to ceramide, a second messenger which induces apoptosis, and by de novo mitochondrial synthesis of ceramide [15]. In summary, the effects of radiation are production of reactive oxygen species (ROS) which in addition to the effect on DNA interact with numerous cellular targets which regulate the fate of the

cell through changes in vital structures controlled by p53 and DNA damage checkpoint control kinases (CHK1 and CHK2) [11,16]. After irradiation, cell growth is arrested so that the damage can be repaired, otherwise, cell death occurs. It is possible to measure this phenomenon by counting stained vital cells and dead cells. The cellular death is the result of several mechanisms such as apoptosis [17,18], autophagy [19,20], mitotic catastrophe [21–23], necrosis and senescence [24–26]. Most reviews find that the final cause of death is apoptosis or a form of necrosis, both may be interrelated [27]. Thus mitotic catastrophe may cause a prolonged growth arrest leading to apoptosis or necrosis dependent on the mutation status of p53 [21,23] or death in senescence [22]. While it was previously considered that apoptosis led to a tolerogenic immunologic effect and only necrosis could expose intracellular antigens, it is now assumed that there may be some immunological stimulation after radiation by both death types, but not strong enough to induce immunological clearance of an established cancer.

Survival response after radiation

In addition to inducing DNA damage which can be lethal unless repaired, radiation also induce a strong pro-survival response. Radiation may therefore simultaneously both kill cells and stimulate a survival response [28,29]. Later a similar response has been documented also after chemotherapy [30,31].

Autophagy induced by radiation is considered as a mechanism promoting tumor survival, growth, invasion and spread [19]. Autophagy provides recycling of molecules for energy production and molecules necessary for growth [20]. However, whether autophagy contributes to tumor growth is still not settled [32]. Another early pro-survival effect is the triggered stimulation of hypoxia-inducible factor 1 α (HIF-1 α)

which is a transcription factor for proteins involved in cell metabolism associated with tumor progression (marked red in Figure 1) [33]. Survivin is another activated molecule which inhibits apoptosis and thereby cell death [34].

RT and immunosuppression

By previous clinical radiation techniques, large volumes of normal tissues were included in the treatment volumes to cover the macroscopic and microscopic tumor deposits. Often the blood counts, hemoglobin and leucocytes decreased during therapy, and radiation has therefore been considered an immunosuppressive treatment [35–37]. Total body (lymph node) irradiation used to control hematological malignancies is also immunosuppressive. Radiation stimulates production of cytokines, of which transforming growth factor β 1 (TGF- β 1) has many effects, including inactivation of natural killer cells (NK cells) and stimulation of expression of programmed death receptor ligand 1 (PD-L1), thus protecting the damaged cancer cell from immune surveillance [38]. The protective effect is also mediated through secretion of TGF- β 1, Toll-like receptors, nitrogen oxide (NO), different cytokines like interleukin (IL)-10 [38–42].

Microenvironment

It is well known that a low oxygen level can reduce the cellular sensitivity for photon radiation by a factor of about 3. Hypoxic tumors are therefore less likely to be cured by radiation. It is of interest that HIF-1 α which increases under hypoxic conditions, in turn, increases cellular expression of PD-L1 which dampens the activity of CD8 T-cells by binding to PD-1 receptors on the surface of these cells [43]. Other factors like IL-1 β , IL-6, tumor necrosis factor α (TNF α) and vascular endothelial growth factor (VEGF) produced in the tumor

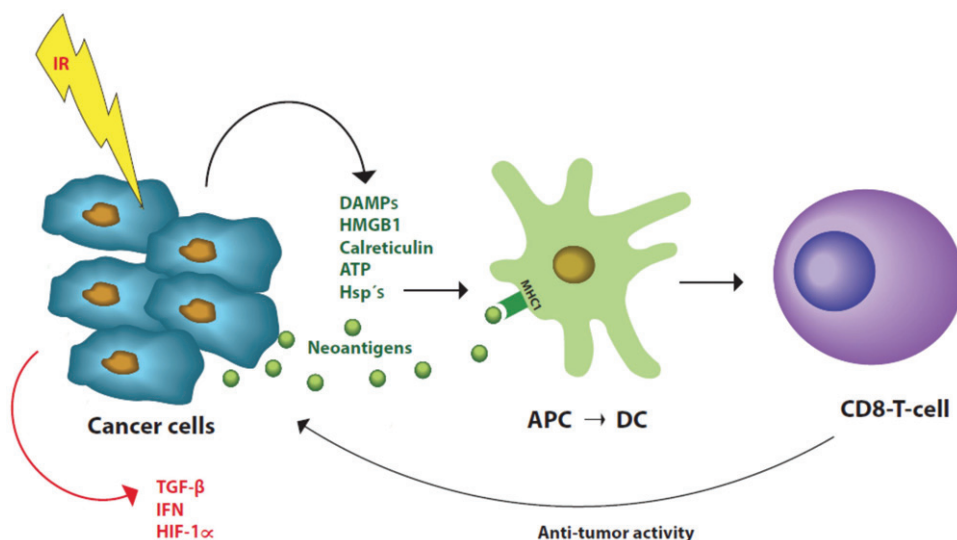


Figure 1. Mechanisms for stimulation of cytotoxic T-cells (CD8 T-cell) by radiation (IR). Immune stimulating factors marked green, inhibition marked red. After radiation tumor cells release danger or damage associated molecules (DAMPs), high mobility group B 1 (HMGB1), calreticulin, ATP and heat shock proteins (Hsp's) together with neoantigens. The neoantigens bind to major histocompatibility complex type I (MHC1) on immature antigen presenting cells (APC) which then moves to the lymph nodes where it as dendritic cell (DC) present the neoantigens to T-cell receptors on CD8 T-cells. After stimulation, the CD8 T-cells then move systemically and can attach tumor cells with the specific neoantigens which triggered the CD8 T-cells. Tumor cells also express transforming growth factor β (TGF- β), interferons (IFN) and hypoxia inducible factor 1- α (HIF 1- α) which protects against immune control. (Illustration: Kristin Risa).

environment stimulates growth of vessels thus improving growth conditions for the tumor cells [44]. Type 2 macrophages, regulatory T-cells (CD4 T-cells) and bone marrow-derived mononuclear suppressor cells surrounding a tumor may also limit the access of infiltrating CD8 T-cells to reach the tumor cells [45].

Immunological effects of radiation therapy

Radiation exposure can induce danger signals on the tumor cell surface which may activate immune cells and macrophages [46]. After radiation exposure immune stimulating molecules appear on the surface of tumor cells termed danger or damage associated molecular pattern molecules (DAMPs) which function as signals for the immune system's immature antigen presenting cells (APC) [47–49]. Typical DAMPs are calreticulin, high mobility group box 1 (HMGB1), heat shock protein 70 and 90 (Hsp 70, Hsp 90), extracellular adenosine triphosphate (ATP), S100A4 and uric acid [48–52] (Figure 1). DAMPs act also as chemo-attractants, which activate immature dendritic cells and attracts macrophages, granulocytes and eosinophils [53–55]. Radiation damage may cause release of double-strand DNA to the cytoplasm, with production of interferon type I via cyclic GMP-AMP synthase (cGAS) and stimulator of interferon genes (STING) pathways, leading to activation of dendritic cells and priming of CD8 T-cells [56,57]. However, if the amount of double-stranded DNA is high in the cytoplasm it activates three prime repair exonuclease (Trex 1) which is an endonuclease that inhibits the immune stimulation [58]. Interferon also stimulates the CD4 T-cells (regulatory T-cells) which generally inhibit CD8 T-cells, but seems to stimulate dendritic cells and the priming of CD8 T-cells in an initial phase. It is also documented that high dose radiation inhibits immune suppression by depletion of myeloid-derived mononuclear suppressor cells, type 2 macrophages, cancer-associated fibroblasts and regulatory T-cells and increase infiltration of CD8 T-cells [59–62]. Low dose radiation can normalize aberrant vasculature and reprogram macrophages from type M2 to type M1 in the tumor microenvironment [62]. The mechanisms described may be tissue and tumor specific and also depend on the dose and fractionation regimens.

Radiation therapy is generally considered to be a local treatment. However, occasionally response in tumor deposits outside the treated volume has been reported, thus suggesting a systemic effect [63–66]. The phenomenon has been described as an abscopal (out of field) effect [67–69]. This rare response can be experimentally shown when mice are treated by radiation and immune modulating therapy [70]. For instance, when Fms-related tyrosine kinase 3 ligand (Flt-3L), a stimulator of hematological cells, was administered to mice inoculated with a mammary carcinoma in both flanks, an abscopal response was observed also in the un-irradiated tumor after radiation to the contralateral flank, but only in immunocompetent mice [71]. The results indicate that the abscopal response is an immunological effect which needs at least functional cytotoxic CD8 T-cells to control the unirradiated tumors [72,73]. In the clinic, abscopal responses have

been observed when radiation was administered as palliation after failure of tumor control by ipilimumab mostly in melanoma patients [74–76]. In reviews, the abscopal response occurred 5–6 (range 0–24) months after radiation with a mean duration of 15–19 months [76–78]. Such case reports indicate that combination of inhibitors of CTLA-4 and PD-1/PD-L1 with radiation can make the rare abscopal response a more common phenomenon [70,79,80].

Preclinical combination of radiation and ICBs

Many tumors do not respond to monotherapy with ICBs, therefore researchers have tried to combine these drugs with vaccination regimens in several animal experiments [81,82]. Radiation can be seen as an *in situ* vaccination. Due to the known activation of DAMPs by radiation, this modality was used in combination with antibodies against CTLA-4, PD-1 and PD-L1 with success in mice (Table 1) [83]. Not only was a clear growth delay documented, but also a significantly improved survival of the mice, as well as effect on secondary tumors outside the radiated field. Re-challenge with the original tumor cells in mice after a complete response failed to form new tumors. In separate studies, it was documented that radiation increased the expression of major histocompatibility complex 1 (MHC-1) in tumor cells [83–86], stimulated maturation of dendritic cells and increased danger signals and tumor-associated antigens (neoantigens) and adhesion molecules [87,88], and changes in the microenvironment [89]. Radiation seems also to enhance the expression of T-cell receptors in CD8 T-cells [90]. Radiation can modulate both stimulatory and inhibitory signaling molecules for CD8 T-cells [91]. The animal studies include the first generation of experimental triple therapy (two antibodies and radiation) which further increase the response in murine tumors [92,93]. Several new candidate targets for ICBs are identified [94,95], and other factors like the TNF system (CD137), natural killer cells and complement may also modulate the combined effect [96–98].

Timing and scheduling

In experimental studies, positive results have been shown after giving various ICBs and radiation (10–30 Gy) concurrent (± 1 day) and after radiation (10–15 Gy) [92,97,99–104]. Best effect was observed when the drug was administered on day 1 compared to day 5 in a 2 Gy x 5 fractionated regimen or 7 days after radiation [105]. In another study inhibition of CTLA-4 was most effective when administered 7 days before 20 Gy radiation in a breast carcinoma model [106]. High doses (20 Gy or 12 Gy) were better than 3 Gy x 5 in other experiments [39,145].

Concurrent radiation and immunotherapy have been the standard for assessing abscopal responses in animals. In the clinic, the abscopal response has been observed when local radiation has been given after ipilimumab or PD-1/PD-L1 in patients with metastases. It is difficult to separate an abscopal response from an expected systemic effect of an ICB after radiation. A phase I trial showed longer than expected

Table 1. Experimental studies on combination of radiation and immune checkpoint blockers.

Tumour	Radiation	Immune therapy	Major finding	Year	Reference
Breast 4T1	12 Gyx1	α -CTLA-4	Improved survival by combined therapy	2005	Demaria et al. [100]
Breast 4T1	12 Gyx2	α -CTLA-4	Reduction of natural killer cells improved combined response	2009	Pilones et al. [97]
Breast TSA	20 Gyx1, 1.8 Gyx3, Gyx5	α -CTLA-4	Fractionated radiation and α -CTLA-4 enhanced tumor response in irradiated and non-irradiated tumors (abscopal effect)	2009	Dewan et al. [143]
Glioblastoma GL261	10 Gyx1	α -CTLA-4 α -4-1BB	α -CTLA-4 and radiation improved survival, marked improvement by triple therapy	2014	Belcaid et al. [99]
Lymphoma EL4	30 Gy	α -CTLA-4	Combined therapy improved survival	2014	Yoshimoto et al. [144]
Breast 4T1 and AT-3	12 Gyx1 and 4 and 5 Gyx4	α -PD-1 α -CD137 (TNF)	Combination of α -PD-1 and α -CD137 with radiation cured tumors	2012	Verbrugge et al. [98]
Glioblastoma GL261	10 Gyx1	α -PD-1	Improved survival after combined treatment which increased infiltration of CD8 T-cells	2013	Zeng et al. [104]
Breast TUBO Colon adenocarcinoma MC38	12 Gyx1 20 Gyx1	α -PD-L1	Delayed growth in both cell lines by combined therapy. Reduced myeloid-derived suppressor cells by combined treatment	2014	Deng et al. [102]
Colon carcinoma CT26 4434 cells	2 Gyx5, 4 Gyx5,	α -PD-1 α -PD-L1	High survival after 2Gyx5 radiation in combination with both antibodies	2014	Dovedi et al. [105]
Renal cell carcinoma RENCA Breast 4T1	15 Gyx1	α -PD-1	Improved local growth delay and survival in all cell lines, and 66% growth delay in secondary tumors (abscopal effect).	2015	Park et al. [101]
Melanoma B16-OVA	12 Gy	α -PD-L1	Growth delay increased from 15.2 to 23.0 days after combination therapy	2016	Wu et al. [103]
Bladder cancer MB49	10 Gyx1	α -PD-1 α -TIM-3 α	Stereotactic radiation was dependent on functional T-cells. α -TIM-3 α and radiation improved survival (50%) while combination of both antibodies and radiation yielded 100% survival after 100 days.	2017	Kim et al. [92]
Glioblastoma GL261					
Colorectal	20 Gyx1	α -CTLA-4	CTLA-4 ineffective alone, improved response including controls when combined	2016	Young et al. [106]
Pancreatic adenocarcinoma	High dose: 12 Gy, 5 Gyx3, 20 Gy; Low dose: 6 Gy, 2 Gy x 5	α -PD-L1	No alteration of radiosensitivity in vitro. In vivo addition of α -PD-L1 significantly improved tumor response	2017	Azad et al. [145]

α -CTLA-4: Antibodies against CTLA-4; α -PD-L1: antibodies against PD-L1; α -PD-1: antibody against program death receptor 1; α -CD137: antibody against CD137 is a receptor for; TNF: tumor necrosis factor.

progression-free survival after previous radiation in advanced non-small cell lung cancer (NSCLC) patients treated with pembrolizumab [107]. Treatment with durvalumab 1–42 days after chemoradiotherapy resulted in significantly longer progression-free survival (16.8 versus 5.6 months) and overall survival (2-year survival 66.3% versus 55.6%, respectively) in a large randomized trial [108,109]. These important studies have changed the treatment for advanced NSCLC. In malignant melanoma concurrent radiation and ipilimumab improved overall survival to 19 months compared to 10 months after ipilimumab alone [110]. At present different schedules are under investigation, the best timing and radiation dose, volume and fractionation may depend on the tumor type and site as well as the type of ICB.

Particle therapy

As with photon therapy, tumor cells surviving proton radiation display immunogenic modulation by increasing surface molecules like calmodulin, MHC 1 (HLA complex) and tumor-associated antigens which improve their sensitivity for CD8 T-cells [111]. Local treatment with high dose (30 Gy) carbon ions alone or 6 Gy combined with stimulated dendritic cells reduced the number of lung metastases in a mouse model [112]. Both proton and carbon ion radiation decreased migration, invasion and spread of human fibrosarcoma cells *in vitro*, in contrast to photon radiation [113]. In another study carbon ion radiation controlled most squamous cell carcinoma tumors in immune competent C3H mice and restricted growth of secondary tumor inoculations, while tumor growth continued in nude mice [114]. The good local control by particle therapy may further benefit from improved control of distant metastases by combination with ICBs [115].

Radiation, hyperthermia and immune therapy

It is well documented in clinical studies that hyperthermia increases the effect of radiation [116]. Multiple mechanisms are involved, like increased DNA damage, change in micro-environment and immune mechanisms, i.e., surface expression of interferon- γ and Hsp70, HMGB1, interferon- γ and Hsp70 which present antigens to and stimulate immature dendritic cells and activate natural killer cells [116–118]. Hyperthermia may therefore potentially contribute to more effective immune response if combined with radiation and ICBs.

Clinical results of combining ICB and radiation

As presented above the introduction of ICBs is a paradigm shift in immunotherapy which represents an important improvement for tumor control in a limited number of tumors which until now have been difficult to treat. Radiation and surgery represent the major curative modalities for local tumors. Based on the positive experimental findings by combining radiation and ICBs, it seems a logical step to test the combination in the clinic. ICBs alone have been most widely used in melanoma patients which is also

reflected in the first clinical results for combination of ICBs and radiation. It should be noted that most reports are retrospective studies where it is unclear why single drug therapy or radiation was selected, thus the selection to different therapy groups may be biased. The first clinical observation of response to combined treatment was noted in a metastatic melanoma patient who had progressed on ipilimumab, where successive radiation led to regression in both irradiated and non-irradiated sites [76]. A study then documented prolonged survival from 5 to 21 months when radiation alone was compared with radiation and ipilimumab in 77 patients with brain metastases from malignant melanoma [119]. As shown in Table 2 several studies have reported similar findings, but two studies could not document any difference. When the anti-PD-L1 antibodies pembrolizumab or nivolumab (group 1) were compared with the anti-CTLA-4 antibody ipilimumab (group 2), a combination of a BRAF inhibitor and a MEK inhibitor (group 3), BRAF inhibitor alone (group 4) or chemotherapy (group 5) in patients treated with single session stereotactic brain radiation for brain metastases from malignant melanoma, the distant brain control rate at 12 months was 38%, 21%, 20%, 8% and 5%, respectively [120]. The first three groups also exhibited improved overall survival. Currently, many clinical trials combining ICBs and radiation are ongoing [121–123].

Seventy-five clinical trials where radiation is combined with ICBs are registered at ClinicalTrials.gov (Figure 2). The main focus is on lung cancer, head and neck cancers and melanomas, but many cancer types are included in the trials. Most studies seem to be a continuation of the sporadic reported clinical combined treatments presented in Table 2.

Future prospects

The progress of immunotherapy the last 5 years represents a breakthrough in medical oncology with response rates of $\square$ 10–40%, and even 50–60% by combination of different ICBs, and durable responses for more than 2 years in selected advanced malignancies [124]. Combination of CTLA-4 and PD-1/PDL-1 or PDL-2 inhibitors seems to be particularly effective, reflecting that these drugs have different target sites, CTLA-4 affects activation of CD8 T-cells while PD-1 on activated CD8 T-cells interact with PD-L1 or PD-L2 and therefore reduce the effector function [125]. However, the combination of different ICBs may increase the toxicity. Long-term tumor control can now be obtained in metastatic cancers where we previously had no curative treatment options. The other side of the story is the fact that most tumors cannot be controlled by these drugs due to inherent or acquired resistance. It is important that CD8 T-cells have systemic anti-cancer effects, attacking cancer cells in any site in the body. Experience indicates that most anticancer drugs, as well as immunotherapy, are most effective when the cancer cell burden is limited.

Radiation therapy remains a main curative and palliative modality to treat cancer together with surgery or concomitant chemotherapy/targeted therapy. A limitation to the curative potential of radiation for solid malignancies is that

Table 2. Clinical reports on combination of radiation and immune checkpoint blockers.

Tumour	Number	Radiation	Tumour location	Immune checkpoint inhibitor	Comments	Year	Author
Melanoma	Single case	9.5 Gyx3	Lungs	α -CTLA-4 Ipilimumab	Tumour progression on ipilimumab. Later radiation-induced regression of irradiated and nonirradiated tumors (abscopal response).	2012	Postow et al. [76]
Melanoma	77 (SBRT alone 50, SBRT + α -CTLA-4 27)	SBRT/WBRT, 20% reirradiated. Dose not stated.	Brain	α -CTLA-4 Ipilimumab	Median survival RT alone 4.9 months, combination RT + ipilimumab 21.3 months, 2-year survival 20 % and 47 %, respectively.	2012	Kniseley et al. [119]
Melanoma	Single case	SBRT 8 Gyx3	Skin	α -CTLA-4 Ipilimumab	Primary tumor and metastases regressed after radiation. After 3 years nodal and brain recurrence. CR after combined therapy. Alive after 7 years.	2012	Stamell et al. [146]
Non-small cell lung	Single case	6 Gyx5	Liver	α -CTLA-4 Ipilimumab	CR one year after combined therapy, both radiated and non-irradiated metastases (abscopal effect ?)	2013	Golden et al. [147]
Prostate cancer Castration resistant	71 pts, 41 combination	8 Gyx1	Bone	α -CTLA-4 Ipilimumab	Feasibility study. Of 28 pts. receiving 10 mg/kg +8 Gy, there was 1 CR and 6 had stable disease. PSA decreased >50% in 8 patients.	2013	Slovin et al. [148]
Melanoma	58 (SBRT alone 33, combined 25).	SBRT 20 Gyx1	Brain	α -CTLA-4 Ipilimumab	No difference.	2013	Mathew et al. [149]
Melanoma	29	Median 30 Gy	Non brain	α -CTLA-4 Ipilimumab	Median time to treatment failure was 5 months for RT during induction of α -CTLA-4, 39 months RT during maintenance. Corresponding to overall survival of 9 and 39 months, respectively.	2013	Barker et al. [150]
Melanoma	37 WBRT, 33 WBRT + α -CTLA-4	WBRT 30–37.5 Gy in 2.5–3 Gy fractions	Brain	α -CTLA-4 Ipilimumab	Median survival 5.3 months after WBRT, 18.3 after combined treatment.	2013	Silk et al. [151]
Prostate cancer Castration resistant	799 399 α -CTLA-4 400 Placebo	8 Gy +/- ipilimumab 4 doses	Bone	α -CTLA-4 Ipilimumab	Median OS 11.2 months after combination, 10.0 with placebo. 2-year OS 26.2% vs. 15.0%	2014	Kwon et al. [152]
Melanoma	46	SBRT 21 Gyx1	Brain	α -CTLA-4 Ipilimumab	SBRT before or during treatment with ipilimumab better than after: 1-year overall survival 65%, 56% and 40%, respectively	2015	Kiess et al. [153]
Melanoma	54	SBRT 21–18–15 Gy depending on size	Brain	α -CTLA-4 Ipilimumab	No difference.	2015	Patel et al. [154]
Melanoma	88, 44 α -CTLA-4, 44 RT and α -CTLA-4	Ablative (20 Gy) or palliative	Non brain	α -CTLA-4 Ipilimumab	Ipilimumab before RT caused longer duration of response than after: 75% versus 45% response at 12 months. Ablative RT versus conventional RT significantly improved median overall survival (20 versus 10 months) and OS 80 % and 49 % at one year.	2016	Qin et al. [155]
Melanoma	22	Various	Multiple sites	α -CTLA-4 Ipilimumab	3 CR, 3 PR and 5 stable diseases. Median progression-free survival 26 weeks, overall survival for all 55 weeks.	2016	Hiniker et al. [156]
Melanoma	16	6 Gyx5–6	Multiple sites	α -CTLA-4 Ipilimumab	31 months median local control, of irradiated lesions, 19 months disease control.	2016	Kropp et al. [157]
Advanced cancer with >5% PD-L1 expression	10	20 Gy median (range 6–33 in 1–10 fractions)	Multiple sites	α -PD-L1 Durvalumab	Combination with durvalumab gave 60% objective responses, 2 CR, 4 PR and 4 stable diseases. One year overall survival 44% (median 11.5 months).	2016	Levy et al. [158]
Melanoma	96	SBRT 21 or 24 Gyx1	Brain	α -CTLA-4 (No 25) Ipilimumab α -PD-L1 (No 21) Nivolumab or pembrolizumab Controls: BRAF/MEK inhibitor (No12) BRAFi (No18), Chemotherapy (No20)	12 months distant metastasis control rate was 38% after pembrolizumab or nivolumab, 21% after ipilimumab, 20% after BRAF/MEK inhibitor, and 8% after chemotherapy.	2016	Ahmed et al. [120]
NSCLC	17	SBRT: 18–24 Gx1, 12/5 fx.	Brain	α -PD-L1 Durvalumab α -PD-L1 Nivolumab	Better distant brain tumor control with radiation during or prior to immunotherapy	2017	Ahmed et al. [159]
Melanoma	16		Brain	α -CTLA-4 Ipilimumab		2017	Williams et al. [160]

(continued)

Table 2. Continued.

Tumour	Number	Radiation	Tumour location	Immune checkpoint inhibitor	Comments	Year	Author
NSCLC	97	WBRT 30 Gy/10fx. SBRT 24-18-15 Gy for ≤ 2 , 2-3 and 3-4 cm tumors Previous RT in 42 patients	Thorax, Brain	α -PD-L1 Pembrolizumab	Median FU WBRT 8.0 months, SBRT 10.5 months. Progression WBRT 5/5, SBRT 9/11, median 2.5 months Median PFS 6.3 months after RT vs. 2.0 months without RT	2017	Shaverdian et al. [107]
Melanoma	101	70 concurrent therapy, 31 ICB alone	Various	α -CTLA-4 Ipilimumab	OS 19 vs. 10 months in combined group vs. Ipilimumab alone	2017	Koller et al. [110]
Melanoma, RCC, NSCLC	137	Palliative RT	Brain	α -PD-1 Pembrolizumab or nivolumab	Prolonged survival, particularly melanoma patients	2017	Pike et al. [161]
NSCLC after Platinum based CT and RT. No progression	713 Randomised	54-66 Gy	Lung	α -PD-L1 Durvalumab	Response rate 28.4% after α -PD-L1, 16.0% after placebo. Median OS 23.2 months vs. 14.6 months, respectively	2017	Antonia et al. [108,109]

α -CTLA-4: Antibodies against CTLA-4; α -PD-L1: antibodies against PD-L1; α -PD-1: antibody against program death receptor 1; OS: overall survival; CR: complete response.

the modality can only be used with curative intent for local disease or regional (and in some cases of oligometastatic) spread due to normal tissue tolerance. The optimal dose and fractionation to expose tumor antigens are not yet finally settled. It seems necessary to induce sufficient damage in the tumor cells to expose danger signals or molecules (calreticulin, Hsps, HMGB1, ATP etc.) which can attract dendritic cells and CD8 T-cells. Experimental data indicate that moderately hypo-fractionated regimens or high doses release more immunogenic stimulatory factors, thus favoring development of mature dendritic cells, and activate CD8 T-cells [126–128]. Radiation alone can induce abscopal responses, but these seem rare [129,130].

The clinical results presented in Table 2 show evidence of a positive interaction of radiation and ICBs, but limitations are the retrospective design of most studies and an effect of ICBs alone is difficult to disclose. It is too early to claim a clinical paradigm shift for the combination of ICBs and radiation, but the combination should be seriously addressed. To prove the positive interaction of these two modalities it could be logic to add radiation to patients who do not respond to ICBs or to those who develop resistance [76,131]. Examples of this strategy have been seen in malignant melanoma and lung cancer. Another possibility that might seem attractive in tumor types where the patients tend to develop metastases despite good local tumor control, is to use optimal radiation for best possible local tumor control and simultaneously expose specific tumor antigens for the immune system to successively attack micrometastases. It is presumed that systemic immunotherapy may more efficiently eradicate micrometastases than bulky disease [129]. Locally advanced anal cancer is an example where this strategy may be used. In a series of 41 anal cancer patients, 56% were positive for PD-L1 in archived paraffin blocks from primary tumors [132]. Tumour-infiltrating CD8 T-cells are related to HPV status and infiltration of CD8 T-cells is a good prognostic factor in anal cancer [133,134]. The abscopal response has also been documented after radiation of anal cancer [135]. Recently nivolumab (3 mg/kg) every second week was administered to 37 patients with recurrent

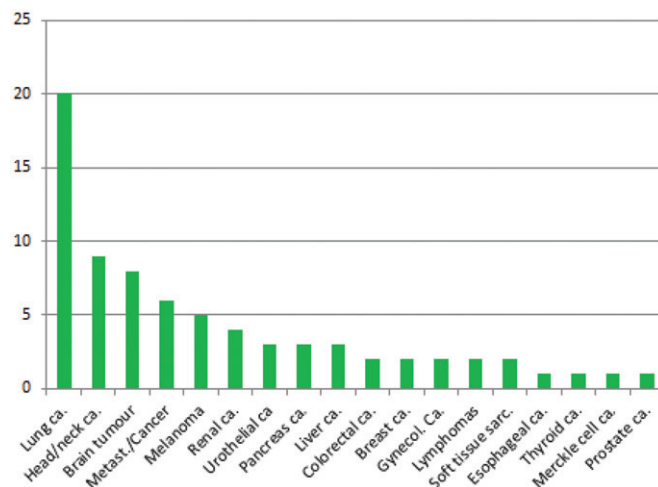


Figure 2. Overview of 75 ongoing clinical studies examining combined treatment with radiation and immune checkpoint blockers (ICBs) registered at ClinicalTrials.gov per March 2018.

anal squamous carcinomas of the anal canal in a phase II study [136]. Two complete responses and 7 partial responses (24% response rate) were reported in this HPV related disease. As a next step, the authors plan to combine nivolumab and ipilimumab. We expect that by selecting high-risk patients without systemic manifested metastases, a better chance for cures can be obtained by this combination of ICB and radiation as initial therapy. Local and systemic failure after radiation with chemotherapy for anal cancer with lymph node metastases without systemic disease is in our experience 30–40% despite an initial complete tumor response [137,138]. Locally advanced anal cancer seems therefore to be a candidate for a randomized study to test whether combination of radiation therapy with immunotherapy will increase tumor control above that of radiation alone.

An abscopal response was documented after use of carbon-ion radiation in recurrent colorectal cancer [139]. Carbon ion radiation seems to expose more tumor antigens for stimulation of CD8 T-cells and can yield an abscopal response. Pancreas cancer has a grim prognosis despite often limited metastases at presentation. Carbon ion radiation suppresses migration and invasiveness of human pancreatic carcinoma cells in contrast to photon radiation in experimental studies [140]. Carbon-ion radiotherapy combined with surgery is used in clinical trials [141]. When carbon-ion therapy was combined with gemcitabine it yielded 83% local control at 2 years, while the patients died of metastases resulting in only 35% 2-year survival [142]. Selecting carbon ion radiation which can control local tumor growth and simultaneously expose tumor antigens which can be attacked by CD8 T-cells released by ICBs, may, therefore, be a new strategy for pancreatic cancer.

Based on the available evidence presented above ICBs aimed at CTLA-4 and the PD-1/PD-L1 axis are actively combined with radiation, but inhibitors of PD-1/PD-L1 yields higher effects. There seem to be more adverse effects associated with CTLA-4 inhibitors, possibly related to the fact that CTLA-4 is also expressed on regulatory T-cells which normally suppress autoimmunity. A combination of both seems to yield the best results with acceptable tolerance despite being hampered with more side effects.

Conclusions

Immune checkpoint inhibition has documented effect in a selected group of cancers, and combination of ICBs with different mechanisms of action is part of our standard armamentarium to control malignant diseases. Experimental studies show that radiation may expose tumor antigens and danger signals which attract immune cells. Both experimental studies and early clinical reports indicate that a combination of radiation with ICBs improves local control of cancer and may influence growth of tumor cells outside the irradiated areas. Hopefully, the high cost of such combined modality therapy will not be a barrier for further controlled clinical studies.

Disclosure statement

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