

Synergistic effects of Radiotherapy and Immune Checkpoint Inhibitors in Cancer Treatment



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Jørg Vik, MK-11

Supervisor

**Professor Inigo Z. Martinez
Institute for Clinical Medicine
The Arctic University of Norway**

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Abstract

Background: Use of immune checkpoint inhibitors have improved survival in patients suffering from cancer types such as advanced melanoma and NSCLC. Through increased understanding of immunological reactions associated with radiotherapy, a potential for synergy of radiotherapy and immune checkpoint inhibitors has emerged. Preclinical proof-of-concept studies have confirmed synergy of the treatments in mouse models.

Objectives: The primary objective was to identify proof-of-concepts in human data supporting combinations of radiotherapy and immune checkpoint inhibition, and identify characteristics of potential examples. A secondary objective was to assess if larger patient materials supported synergistic effects of such treatment regimens.

Methods: A literature search in PubMed was conducted to identify original data on patients treated with combinations of radiotherapy and immune checkpoint inhibition.

Results and discussion: Six case reports described impressive treatment responses following radiotherapy and immune checkpoint inhibition. A large double blinded RCT, including 799 patients, failed to present evidence for improved median survival in metastatic castration resistant prostate cancer (11,2 months for radiotherapy and Ipilimumab vs. 10,0 months for radiotherapy and placebo). Seven retrospective studies on patients (n=200) with advanced melanoma showed median survival over the expected, while two (n=38) failed to do so. Three of the retrospective studies specifically looked radiographically for abscopal responses outside the irradiated field, noting unusually frequent responses. A single small prospective trial (n=22) on advanced melanoma did not find a median survival over the expected. The studies varied in characteristics of the patient populations, methodologies and in radiotherapy regimens.

Conclusion: Case reports and retrospective studies investigating abscopal responses after radiation presented circumstantial evidence for synergy of radiotherapy and immune checkpoint inhibition. The single identified randomized controlled trial failed to prove increased survival of a relevant combination regimen in metastatic castration resistant prostate cancer, while retrospective studies in advanced melanoma were more encouraging.

Abbreviations

CD – cluster of differentiation

T_H cells – T helper cells

APC – antigen presenting cell

TCR – T cell receptor

MHC – major histocompatibility complex

IL – interleukin

CTLA-4 – cytotoxic T lymphocyte-associated protein 4

T_{regs} – regulatory T cells

PD-1 – programmed cell death 1

TGFβ – transforming growth factor β

PD-L1/2 – programmed cell death ligand 1/2

mAb – monoclonal antibody

MS – median survival

NSCLC – non-small-cell lung cancer

FDA – U.S. Food and Drug Administration

ATP – adenosine triphosphate

RT – radiotherapy

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses

OF – out of radiation field

IF – in radiation field

CR – complete response

PR – partial response

SD – stable disease

MeSH - Medical Subject Headings

mCRPC – metastatic castration resistant prostate cancer

IMRT – intensity modulated radiotherapy

WBRT – whole brain radiotherapy

SRS – stereotactic radiosurgery

SABR - Stereotactic ablative radiotherapy

Gy – Gray (unit)

CT – computed tomography

PET-CT – positron emission tomography – computed tomography

CNS – central nervous system

HR – hazard ratio

ICOS – inducible T cell costimulator

HLA-DR – human leukocyte antigen – antigen D related

FoxP3 – Forkhead box P3

Nomenclature

Radiation Regimens:

The American National Cancer Institute defines *stereotactic radiosurgery* as (1); “A type of external radiation therapy that uses special equipment to position the patient and precisely give a single large dose of radiation to a tumor. It is used to treat brain tumors and other brain disorders that cannot be treated by regular surgery.”

The American National Cancer Institute defines *intensity-modulated radiation therapy* as (1); “A type of 3-dimensional radiation therapy that uses computer-generated images to show the size and shape of the tumor. Thin beams of radiation of different intensities are aimed at the tumor from many angles. This type of radiation therapy reduces the damage to healthy tissue near the tumor.”

The American National Cancer Institute defines *stereotactic body radiation therapy* (also known as *stereotactic ablative radiation therapy* (2), which is the term that will be used in the present review) as (1); “A type of external radiation therapy that uses special equipment to position a patient and precisely deliver radiation to tumors in the body (except the brain). The total dose of radiation is divided into smaller doses given over several days. This type of radiation therapy helps spare normal tissue.”

Abscopal:

The term derives from greek, ab: away from, scopus: target, abscopal: away from target. The word describes effects of radiation in non-irradiated tissue distant from the irradiated tissue (3).

Immune Checkpoint Inhibitors:

The American National Cancer Institute describe immune checkpoint inhibitors as (1); “A type of drug that blocks certain proteins made by some types of immune system cells, such as T cells, and some cancer cells. These proteins help keep immune responses in check and can keep T cells from killing cancer cells. When these proteins are blocked, the “brakes” on the immune system are released and T cells are able to kill cancer cells better. Examples of checkpoint proteins found on T cells or cancer cells include PD-1/PD-L1 and CTLA-4/B7-1/B7-2. Some immune checkpoint inhibitors are used to treat cancer.”

Measures of Treatment Response:

The American National Cancer Institute defines *response* as (1); “In medicine, an improvement related to treatment.” The corresponding definition of *objective response* is; “A measurable response.”

To make reporting of results of cancer treatments more similar, several sets of *response criteria* exist for consistent reporting of *objective responses* measured radiographically. WHO-criteria (4), Response Evaluation Criteria in Solid Tumors (RECIST) (5) and Immune-Related Response Criteria (6) are examples of such criteria. A *complete response* is a complete disappearance of target lesions. A *partial response* is a shrinkage in measurements of tumor size over a certain defined threshold, while *stable disease* is small changes in size in either direction. *Progressive disease* an increase in measurements of tumor size over a certain defined threshold. The exact measurements and thresholds varies somewhat between the three mentioned criteria. Of note, the Immune-Related Response Criteria is designed for following responses to immune-checkpoint inhibitors, due to unusual response patterns, with so-called initial pseudoprogression of tumors sometimes occurring, followed by remission (6).

Introduction

Tumor immunology and immune checkpoints

While the hypothesis of the immune system protecting against cancer is more than 100 years old, with Paul Erlich as an early proponent in 1909 (7), solid evidence for the theory did not emerge before the end of the 20th century. In a paper published in 2004, Dunn et al reviewed the process of accumulating evidence leading up to the resurrection of *the cancer immunosurveillance theory* (8). Not only did the new evidence indicate protective anti-tumor effects of the immune system, but preclinical evidence also suggested that the immune system shaped the properties of the cancer. When tumors were transplanted from immune deficient mice to immunocompetent ones, the new hosts rejected the tumor transplants far more often than they rejected tumor transplants originally grown in immunocompetent hosts (9-11). This suggested that mice with a competent immune system was exerting a selective pressure on the tumor, leading to the evolution of less immunogenic tumor phenotypes in these mice. The realization that the immune system was not only protecting the mice from cancer, but also shaping the properties of the cancer, led Dunn et al to propose a refined model, which they termed *cancer immunoediting* (8). If the immune system had effective mechanisms for killing cancer cells and suppressing tumor growth, it followed logically that any clinically recognized cancer would somehow have been “edited” and subsequently escaped from this suppression (8).

A major focus in tumor immunology has therefore been to identify the mechanisms underlying the ability of cancers to avoid destruction by immune system. Due to the centrality of the adaptive immune system in forming specific responses, T-cell suppressive mechanisms have received special interest, reviewed in ref. (12). The ability of T cells to monitor the internal contents of cells by interacting very specifically with antigen peptides presented on major histocompatibility complexes (MHC) makes them ideal candidates to detect and respond to the fine-grained differences between normal cells and transformed cancer cells. Both cytotoxic cluster of differentiation (CD) 8 positive T cells and CD4 helper 1 T cells (T_H1 cells) are effector cells implicated in eliciting anti-tumor immunity (12).

An important checkpoint in activation of a specific T cell response is the interaction of a professional antigen-presenting cell (APC) and the naïve T-cell (12). Interaction between a

specific T cell receptor (TCR) and a peptide antigen on a MHC-II molecule is not in itself enough for activation and subsequent proliferation of the T cell, and additional co-stimulatory signaling is required. This process is a complicated interplay between a number of ligands and receptors. One of these co-stimulatory signaling pathways is mediated by the ligands B7.1 and B7.2. These are expressed by APCs, and interact with the receptor CD28 on the T cell surface, presenting a stimulatory signal. If the stimulation is strong enough, the T cell clone will produce copious amounts of interleukin (IL) 2 and proliferate, reviewed in ref. (13). However, the B7 ligands also serve as ligands for the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). On CD4⁺ T-cells and CD8⁺ T cells, CTLA-4 is induced on the surface after TCR and CD28 engagement, where it serves as a regulator of negative feedback, inhibiting proliferation, reviewed in ref. (14). CTLA-4 is expressed constitutively on T regulatory cells (T_{regs}) (15, 16), and through CTLA-4-mediated activity these cells can suppress effector T cell activity and proliferation (14). One mechanism for this suppression, explored in vitro and in mice models, is downregulation of B7.1/2 on APCs by “stealing” of these ligands by endocytosis of the CTLA-4/ligand complex, into the lymphocyte bearing CTLA-4 following binding (17). T_{regs} are also mediating potent immunosuppressive effects on effector T cells via secretion of cytokines such as TGFβ, induced by activation of CTLA-4 (18).

Another inhibitory molecule is the programmed cell death 1 receptor (PD-1), reviewed in ref. (19). It is found on T cells, natural killer cells, B cells and myeloid-derived cells. By interacting with the programmed death ligand 1 (PD-L1) or programmed death ligand 2 (PD-L2), signaling through this receptor lead to T cell exhaustion and unresponsiveness, inhibiting effective T cell mediated responses. Unlike the CTLA-4 axis, where ligands are found on APCs, the ligands for the PD-1 receptor can be found in a wider range of cells, including cancer cells (20, 21).

Preclinical experiments as well as clinical evidence have implicated that CTLA-4 and PD-1 mediated signaling is indeed of importance in shielding cancers from attack by effector T cells, as reviewed in ref. (22), and collectively these types of signal molecules that can turn down (or up) signals necessary for an immune response are called *immune checkpoints* (14).

Immune checkpoint inhibitors

Since hijacking of the CTLA-4 and PD-1 signaling axes are important means for tumors to escape *immunosurveillance*, these receptors, and in the case of PD-1 its ligands, presented interesting targets for blockade with monoclonal antibodies (mAb). In two landmark clinical trials, inhibition of CTLA-4 with the mAb Ipilimumab, led to a significant increase in median survival (MS) in patients treated with Ipilimumab compared to control groups (23, 24). This was the first time a drug had been shown to consistently increase survival in advanced melanoma, and some patients have experienced very durable responses. A pooled analysis of survival data from 1861 patients found a plateau on the Kaplan-Meier survival curve starting from 3 years, extending for several additional years for some patients. The 3-year survival rate was 22% (25).

Later, randomized controlled trials have shown treatment benefits of the anti-PD-1 mAb Nivolumab and the anti-PD-L1/2 mAb Pembrolizumab in advanced melanoma (26-28), non-small-cell lung carcinoma (NSCLC) (29-31), and in the case of Nivolumab in advanced renal cell carcinoma (32). A randomized controlled trial has also shown increased progression free survival when treating patients with a combination of Nivolumab and Ipilimumab compared to monotherapies of each (33).

While there has been a number of clinical trials showing benefits of immune checkpoint inhibitors in different types of advanced cancer, responses are limited to a subset of patients. In the two landmark trials with patients treated with Ipilimumab, the percentage of patients with partial or complete responses, or stable disease, was modest at a rate of 28,5% in one of the trials and 33,2% in the other (23, 24). Therefore, while the success of immune checkpoint inhibitors is a major breakthrough, there is still a need to increase the number of patients experiencing durable anti-tumor responses.

Radiotherapy and tumor immunology

In 1953, Mole coined the term abscopal effect to describe distant biological effects of radiation outside of the irradiated tissue field (3). Throughout the years, a number of case reports have described patients suffering from cancer, where irradiation of one tumor is followed by remission of tumor masses outside of the irradiated field, as reviewed in these articles (34, 35). Direct tumoricidal effects of the radiation can obviously not cause these

responses, and evidence points to the immune system as the mediator of these abscopal effects, as reviewed by Formenti and Demaria in 2013 (36). Direct killing of cancer cells by radiation lead to the translocation of the immunogenic marker calreticulin to the surface of the dying cell, as well as release of immunological danger signals such as high-mobility group protein B1 and ATP. With propagation of these signals, the cancer cells die in a manner that has been termed immunogenic cell death, due to the ability of these signals to trigger an immune response (37-39). Killing of cancer cells also expose tumor antigens, which can form the basis of recognition of the cancer cells as so-called non-self by the adaptive immune system, as opposed to the immunologically tolerated self (40). Presentation of these antigens by dendritic cells activated by danger signals can prime tumor specific T cells (41). Thus, radiation can provide a vaccine-like effect (36).

As local effects, radiation can also induce increased expression of MHC-I, which allows for better monitoring of tumor antigens by cytotoxic CD8⁺ T cells (42, 43). Adhesion molecules and stress-induced ligands have also been found to have increased expression on the surface of cancer cells following irradiation (44, 45). In addition, experimental models have shown increased production of chemokines attracting effector T cells (46, 47). Altogether, this might lead to increased responsiveness to immunological attack. However, the fact that abscopal responses are rare, suggests that most clinically detectable cancers have developed tumor microenvironments that are so immunosuppressive, that even the aforementioned vaccine-like effect of radiotherapy (RT) is not enough to elicit an effective adaptive immune response on its own (36).

Intersection between radiotherapy and immune checkpoint inhibition

Based on the ability of RT to provide a vaccine-like boost to the immune system, a rationale emerges for combining RT with immune checkpoint inhibitors, which have the potential to remove suppressive brakes inhibiting the immune system. Thereby a paper published in 2005 by Demaria et al. (48) described a set of experiments presenting the first preclinical proof-of-principle for synergism between RT and immune checkpoint inhibition. They injected the mammary cancer cell line 4T1 subcutaneously into the flank of mice, from where metastases spread to the lungs. By comparing mice receiving either a) no treatment, b) local RT in a single dose to the primary tumor, c) an inhibitory monoclonal antibody (mAb) against CTLA-4, or d) a combination of local RT and inhibition of CTLA-4, the authors found a

significant survival benefit in the mice receiving combination therapy. However, the researchers also noticed that growth of the irradiated primary tumor was not significantly inhibited by the combination therapy, compared to RT alone. Reasoning that the survival benefit of the combination therapy was due to an increased systemic control of metastases, they used a dissection microscope to count the number of metastases present in the lungs of their mice post-sacrifice. Consistent with the survival benefit, the combination regimen did indeed lead to a significant decrease in metastases.

This elegant study design, with controls for both the effect of radiotherapy alone and immune checkpoint inhibition alone, strongly suggested that the observed treatment effect was more than simply the sum of the individual parts. In subsequent years, a small number of clinical studies have been published showing dramatic abscopal treatment responses after radiotherapy and inhibition of CTLA-4, which could represent similar phenomena in humans.

Objectives of the review

This study aims to review the available clinical evidence of synergistic effects of combinatorial treatment regimens of radiotherapy and immune checkpoint inhibitors. The main goal is to identify proof-of-concepts in human data supporting such combination regimens, assess what characterizes these regimens, and discuss how they fit together with preclinical data. A secondary goal is to assess whether any available larger patient materials show trends supporting synergism of these two treatment modalities. The layout of this thesis is based on the guidelines present in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (49), with some modifications. The review will span distinct fields of evidence, with several different types of cancers, and different drugs and radiation regimens. Direct quantitative synthesis of the results is therefore not possible, and thus meta-analysis of the results will not be performed. The goal is to provide a qualitative synthesis of results, with a discussion of possible future directions of research. Preclinical data will be used to inform the discussion, but will not be reviewed thoroughly.

Methods

Eligibility criteria

The primary criteria for inclusion of articles in the analysis were that the article should include original clinical data dealing with combinations of RT and immune checkpoint inhibitors. According to the main objective, identifying proof-of-concepts from clinical data, case reports were considered most relevant, given the more comprehensive reporting on individual patients than what is typically found in larger clinical studies. To decide what could potentially constitute a proof-of-concept, the following set of criteria was defined for **inclusion of case reports**:

1. The clinical data should show abscopal effects, indicating that any anti-tumor activity is not simply a local effect of the RT. This abscopal effect might be: A complete or partial response or unusually long stable disease, as defined by the WHO Response Criteria, the Response Evaluation Criteria in Solid Tumors (RECIST) or the Immune related Response Criteria (4-6). Trying to disentangle synergistic systemic effects from effects of the immunotherapy alone, or abscopal effects of RT alone, is far more difficult, and will be assessed in the discussion rather than at the eligibility level.
2. There must be a close temporal relationship between the two treatment modalities. One systematic review of abscopal effects after radiation reviewing 46 case reports reported a median time of 2 months before the effects manifested (34). Another systematic review of 23 case reports plus one retrospective study reported a median time of 5 months (35). Based on this, for articles to be included in the present study I have set a cut-off of 6 months from RT to treatment with immune checkpoint inhibitors.
3. Lack of confounding treatment: Studies describing patients treated with concurrent additional cytotoxic or targeted therapy were excluded. Subsequent cytotoxic or targeted therapy were also a cause for exclusion, unless a response appeared before the subsequent treatment.

Since my secondary objective was to review whether any larger patient materials published show a trend towards synergism of the two treatment modalities, studies fulfilling the

primary criteria and including several patients were also analyzed. Papers published after 01.01.2005 were included. Only full text papers written in English were reviewed.

Search strategy

(OR	OR	OR	OR	OR)AND	NOT
CTLA-4 antigen	Programmed cell death 1 receptor	CD274	ipilimumab	nivolumab	pembrolizumab	radiotherapy	Review[ptyp]
CTLA-4 antigen	Programmed cell death 1 receptor	CD274	ipilimumab	nivolumab	pembrolizumab	abscopal	Review[ptyp]

Table 1: Search strategy with Boolean operators (AND, OR NOT). Parentheses () are placed where they appeared in the search. In addition, the filters English and Publication date from 2005/01/01 to 2016/12/31 were used.

All searches were conducted in PubMed, after an iterative process of identifying relevant search-words. Finally the terms “abscopal”, “radiotherapy”, “CTLA-4 antigen”, “programmed cell death 1 receptor”, “CD274”, “ipilimumab”, “nivolumab” and “pembrolizumab” were combined in the searches shown in Table 1. Reviews were excluded, and the last search was run on the 13.05.2016. The three FDA-approved immune checkpoint inhibitors Ipilimumab, Nivolumab and Pembrolizumab (50), as well as their targets in the CTLA-4 or PD-1/PD-L1 axis, were searched for.

A conscious choice was made not to use Medical Subject Headings (MeSH), after preliminary searches with and without MeSH terms had identified inconsistent or absent tagging of relevant articles. A screening of titles showed that no articles were lost when not using MeSH terms, while several relevant articles turned up in the search without them.

Article selection

Articles were selected in a three-phased process, with initial screening of titles, thereafter screening of abstracts and finally a review of the full text articles passing the title and abstract screening. Articles deemed not eligible were excluded at each phase of the process.

The process

In the requirements for the 5. year thesis, it is stated that one should comment on the work process. The workflow consisted of 4 phases: 1) planning, 2) literature search and screening, 3) reading and summarizing and finally 4) analysis and writing. Planning started at the end of 2014, when my supervisor, Professor Inigo Z. Martinez, sent me a number of interesting

papers dealing with the immunomodulatory effects of RT. After some discussions about the subject with professor Martinez, this phase culminated when I wrote the project description in early 2015. Originally, the plan was to review clinical data on combinations of immunotherapy in general and radiotherapy. After early searches and screening in phase two, I made the decision to sharpen the focus of the thesis to deal with immune checkpoint inhibitors in particular, combined with radiotherapy. Early searches pointed to a relatively large amount of data available on this specific combination regimen, and a more thorough discussion is possible with a sharpened focus. Throughout my research year in autumn 2015 and spring 2016, I read a number of papers dealing with subjects relevant to my 5th year thesis. My forskerlinje-project is an experimental project that deals with radiation and biological responses in cancer-associated fibroblasts. So while the projects are completely separate, both deal with cancer in general, and I have taken both a 10 study point master course, as well as a 10 study point PhD level course in cancer biology. Due to the earlier than planned immersion into relevant literature, I decided to deliver the thesis in spring 2016 instead of 2017. Phase 2 and 3 overlapped during autumn 2015 and spring 2016, with a final search algorithm constructed in spring 2016, followed by systematic screening and reading of papers. After reading and summarizing the identified papers, the last phase consisted of analyzing the implications and potential future directions and writing the thesis. Professor Martinez gave feedback during the writing process.

I have already been moved to MK-12 administratively, due to my research year. However on the front page, I have written MK-11, since I have registered for delivery of the 5th year thesis together with my old class (and in accordance with the old study plan). This was also confirmed by email correspondence with study consultant Elin Holm.

Results

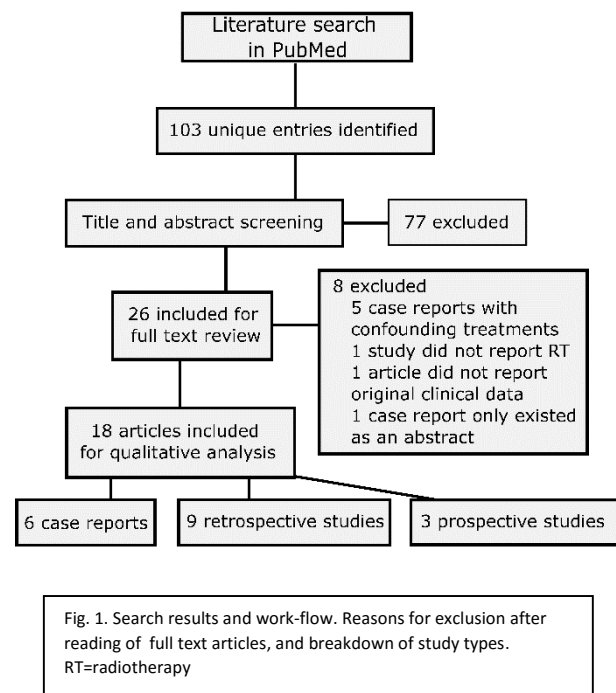
The searches identified 103 unique entries in PubMed, but after application of eligibility criteria, 18 articles with clinical material for analysis were left; six case reports, nine retrospective studies and three prospective studies. The process of article screening is illustrated in Figure 1.

In the identified case reports five patients suffered from advanced melanoma (51-55), and one patient from non-small cell lung carcinoma (NSCLC) (56). The nine retrospective studies all dealt with advanced

melanoma, including altogether 238 patients (range: 13-46 in each study) on relevant treatment regimens (57-65). An additional small prospective study (phase I) included 22 melanoma patients (66). The two other prospective studies described patients with metastatic Castration Resistant Prostate Cancer (mCRPC). One of these studies was a combined phase I/II multicenter, open-label, non-randomized trial (67), while the other was a large phase III multi-center, double blinded, randomized controlled trial (68). The phase I/II trial included 71 patients, with 41 of them receiving combination treatment (67), while the phase III trial included 799 patients in the intention-to-treat analysis divided evenly between a) a combination regimen, and b) RT and placebo (68).

The CTLA-4-inhibitor Ipilimumab was the only immune checkpoint inhibitor employed in all the reviewed studies. Doses ranged from 3-10 mg/kg of body weight, given in induction cycles every 3 weeks with a goal of four doses, and in some cases, maintenance doses every 12 weeks, or re-induction (exact numbers of patients in each category are not available).

Radiation for mCRPC was delivered to bone metastases (67, 68). The majority of the melanoma patients (n=183) received RT against brain metastases, divided between whole brain radiation therapy and/or stereotactic radiosurgery. Some (n=32) patients received RT to extracranial sites, including liver, bone, distant lymph nodes, skin, subcutaneous



metastases, and lung (51, 54, 60, 66). One study did not break down the numbers of patients receiving extracranial irradiation and RT against brain metastases (65). Details from the case reports and studies are summarized in Tables 2, 3 and 4.

Case reports

Cancer type	Age and sex	Radiation regimen, dose/fractions	Target	Checkpoint inhibitor	Sequence	Objective responses	Follow up	Ref.
Metastatic melanoma	33 F	IMRT: 28,5 Gy/3	Paraspinal	Ipilimumab: 10 mg/kg	Ipilimumab then RT, then Ipilimumab	PR: both IF and OF	SD 10 months after RT	Postow et al 2012 (51)
	63 F	WBRT: 20 Gy/5	Leptomeninges	Ipilimumab: 3 mg/kg	RT then Ipilimumab	CR: IF PR: OF	SD 16 months after Ipilimumab	Bot et al 2012 (52)
	44 M	SRS: 20 Gy/1	Brain	Ipilimumab: 3 mg/kg	RT then Ipilimumab	CR: OF PR: IF	Alive 18 months after Ipilimumab	Du Four et al 2012 (53)
	57 M	SABR: 54 Gy/3	Liver	Ipilimumab: 3 mg/kg	Concurrent	CR: IF and OF	No evidence of disease after 1 year	Hiniker et al 2012 (54)
	67 M	SRS	Brain	Ipilimumab		CR: IF and OF	No evidence of disease after 4 years*	Stamell et al 2012 (55)
Metastatic NSCLC	64 M	IMRT: 30 Gy/5	Liver	Ipilimumab 3 mg/kg	Concurrent	CR: IF and OF	No evidence of disease after 1 year**	Golden et al 2013 (56)

Table 2: Summary of findings in case reports with patients who has received combination therapies of immune checkpoint inhibitors and radiotherapy, and experienced abscopal responses. Abbreviations: M male, F female, IMRT intensity modulated radiation therapy, WBRT whole brain radiation therapy, SRS stereotactic radiosurgery, SABR stereotactic ablative radiotherapy, Gy Gray, RT radiotherapy, CR complete response, PR partial response, SD stable disease, IF in radiation field, OF out of radiation field

*Had one recurrence managed by surgery

**Underwent an additional re-induction with Ipilimumab between the combination therapy and the complete regression

In case report published in 2012, Postow et al. described a woman who started induction therapy with Ipilimumab against metastatic melanoma in September 2009, and who by the end of 2011 was in remission (51). She did not respond to induction therapy, but continued on maintenance therapy. Throughout 2010 she experienced radiographic worsening, with a paraspinal tumor mass undergoing progressive growth, and several new splenic lesions were detected in November 2010. Due to back pain, she received palliative intensity-modulated RT to her paraspinal mass in December 2010, delivered in a dose of 28,5 Gy divided in 3 fractions. A CT scan one month later, in January 2011, did not show responses in the irradiated site or any of the metastases. After an additional dose of Ipilimumab in February 2011, a subsequent CT scan in April 2011 did showed a significant regression of her irradiated paraspinal mass, and in addition also regression of her other known metastases outside the radiation field. Ten months of follow-up after the RT, in December 2010, showed no subsequent progression of the tumor masses in CT-scans.

Another case report from 2012, by Hiniker et al, reported a complete systemic response in a female patient with melanoma with metastases to the liver and a subcutaneous metastasis in the left arm (54). After detection of liver metastases on a PET-CT scan in May 2011, her

treatment team prospectively decided to combine CTLA-4 inhibition with radiotherapy to try to provoke an immunological anti-tumor response. PET-CT scans in August 2011 after the patient had received two doses of Ipilimumab, revealed an initial progression of the liver metastases, as well as development of several new ones. At this time, stereotactic radiotherapy of two of her liver metastases was delivered in a dose of 54 Gy divided in 3 fractions. Initially a subcutaneous metastasis on her left arm rapidly appeared during the last cycles of Ipilimumab, but a PET-CT scan in December 2011 showed no uptake of 18F-fluorodeoxyglucose in her liver metastases, indicating no metabolic activity in the tumor, and in late February 2012 the subcutaneous lesion had resolved as well. A PET-CT scan 1 year after RT showed a complete regression of all liver metastases, including the non-irradiated ones, as well as the lesion in the left arm, and the patient showed no evidence of any metabolically active malignancy.

Complete systemic responses were also reported to occur twice in a male patient that first underwent fractionated RT in a palliative setting against melanoma of the head and neck, reported by Stamell et al. in 2013 (55). Within 8 months of RT, both his irradiated tumor and all his additional known metastases had resolved completely. However, after 36 months brain and nodal metastases were detected, and the patient was treated with stereotactic radiosurgery to the brain and Ipilimumab. Subsequently a remarkable complete remission occurred, with the patient being alive 4 years after the combination therapy. A recurrence in a cervical lymph node was managed surgically, but no other metastases appeared in the period of follow-up.

Treatment with stereotactic radiosurgery and Ipilimumab also preceded a treatment response in a male melanoma patient with brain metastases, described by Du Four et al. in 2012 (53). The patient received stereotactic radiosurgery against two lesions in the brain, followed by extracranial progression detected three months later. Ipilimumab induction was started three months later, and a complete response outside the brain subsequently developed, as documented with PET-CT scans. The irradiated brain lesions underwent gradual regression, and one lesion disappeared completely. However, two years after stereotactic radiosurgery a lesion surrounded by edema reappeared in the same spot, accompanied by headache. This led to a surgical resection of the lesion, and histology revealed necrosis and gliosis, with no viable melanoma cells or inflammatory cells. Eighteen

months after Ipilimumab initiation no extracranial recurrence was detected, the remaining CNS lesions were stable, and the patient stayed in a stable neurological condition. The authors published this case report together with two other cases, including two females with melanoma with brain metastasis, where the patients also experienced radiation necrosis after RT and Ipilimumab, and the focus of the paper was on radiation necrosis. All three patients responded favorably to treatment. However, the last two patients did receive potentially confounding chemotherapy and dendritic cell based immunotherapy and are therefore not discussed thoroughly in the present thesis.

Whole brain RT in combination with Ipilimumab was reported by Bot et al. to precede a response in a female patient with lung metastases and leptomeningeal metastases of melanoma, exhibiting morning headache, nausea and vomiting (52). She received whole brain RT in 4 fractions followed by 4 courses of Ipilimumab. Already after the first course of Ipilimumab her headache, nausea and vomiting disappeared, and after all four courses the radiological signs of leptomeningeal metastases had disappeared. Her non-irradiated lung metastases underwent a partial response, and in the last follow-up 1,5 years after the start of Ipilimumab treatment the lung tumors were stable. Magnetic resonance imaging presented no evidence of leptomeningeal metastases.

While the five case reports described so far all dealt with patients suffering from melanoma, Golden et al published a report in 2013 on a male suffering from NSCLC (56). The patient suffered from widespread metastatic disease, with several lesions in the lungs, liver, bone, and hilar/mediastinal adenopathy and periaortic adenopathy. The patient was offered RT and Ipilimumab with the prospective goal of triggering an abscopal response. Intensity modulated RT in a dose of 30 Gy were delivered in 5 fractions over 10 days, with Ipilimumab induction starting the day after the first dose of radiation. Following the treatment that started in August 2012, posttreatment chest CT and PET-CT scans were conducted in November 2012 and January 2013, respectively. The patient experienced responses in both irradiated lesions, as well as the non-irradiated ones, and the levels of the non-specific tumor-marker carcinoembryonic antigen dropped dramatically. A metastasis in a lymph node was detected and excised, and another additional four cycles of Ipilimumab were administered from June 2013 to August 2013. PET-CT in September 2013, 1 year after combination therapy revealed no evidence of disease.

Studies on melanoma patients

Design	Radiation regimen, dose/fractions	Target	Drug	Sequence	Median survival combination treatment (months)	Landmark survival combination treatment	Ref.
Retrospective study (n=27, control: n=50)	SRS (n=77) WBRT (n=25)	Brain	Ipilimumab (n=27)	SRS then Ipilimumab (n=16), Ipilimumab then SRS (n=11)	21,3	2-year survival 47%	Knisely et al. 2012 (57)
Retrospective study (n=25 control: n=23)	SRS: 15-20 Gy (n=58)	Brain	Ipilimumab (n=25): 3 or 10 mg/kg	Concurrent (n=7), SRS then Ipilimumab (n=10), Ipilimumab then SRS (n=4)	NA (survival curve suggest around 5 months)	6-month survival 56%	Mathew et al. 2013 (58)
Retrospective study (n=33, control: n=37)	WBRT (n=37): 30-37 Gy/10-13 SRS (n=33): 14-24 Gy/1-5	Brain	Ipilimumab (n=33): 3 mg/kg	RT then Ipilimumab, Ipilimumab then RT	18,3	NA	Silk et al. 2013 (59)
Retrospective study (n=10 control: n=21)	SRS:	Brain	Ipilimumab (n=31)	SRS then Ipilimumab	16,5	3-year survival 44%	Tazi et al. 2015 (64)
Retrospective study (n=13)	WBRT: 27-37,5 Gy/9-15 (n=13)	Brain	Ipilimumab: 3 mg/kg (n=12), 10 mg/kg (n=1)	Concurrent (n=6), WBRT then Ipilimumab (n=3), Ipilimumab then WBRT (n=4)	4	1-year survival 15,4%	Gerber et al. 2015 (61)
Retrospective study (n=46)	SRS: 15-24 Gy (n=46)	Brain	Ipilimumab: 3 mg/kg or 10 mg (n=46)	Concurrent (n=15) SRS, Ipilimumab (n=19) Ipilimumab, SRS (n=12)	12,4	1-year survival 40-65% depending on timing	Kiess et al. 2015 (62)
Retrospective study (n=16)	WBRT (n=5): 20-36 Gy/10-13 SRS: 18-24 Gy/1-5	Brain	Ipilimumab: 3 mg/kg (n=14), 10 mg/kg (n=2)	Concurrent (n=4) RT then Ipilimumab (n=5), Ipilimumab then RT (n=7)	14,4	1-year survival 54%	Schoenfeld et. al 2015 (63)
Retrospective study (n=47)	WBRT: 30-37,5 Gy/fraction size 2,5-3 SRS: 18-25 Gy/fraction size 5-25 Other RT: 8-66 Gy/fraction size 2-8	Brain Spine Intrathoracic Bone Soft tissue Abdominovisceral	Ipilimumab: 3 mg/kg (n=43), 10 mg/kg (n=4)	RT then Ipilimumab (n=22), Ipilimumab then RT	28 (17 months following WBRT)	5-year survival 20%	Chandra et al. 2015 (65)
Retrospective study (n=21)	WBRT (n=9) SRS (n=4) Other RT: (n=8)	Brain (n=13) Bone (n=4) Lymph node (n=2) Cutaneous (n=2)	Ipilimumab 3 mg/kg (n=21)	RT then Ipilimumab (n=21)	13	NA	Grimaldi et al. 2014 (60)
Phase I clinical trial (n=22)	SABR (n=22): 6-8 Gy/2-3	Lung Bone Liver Subcutaneous	Ipilimumab 3 mg/kg (n=22)	SABR then Ipilimumab	10,7	NA	Twyman-Saint Victor et al. 2015 (66)

Table 3: Summary of findings in studies on advanced melanoma patients who has received combination therapies of immune checkpoint inhibitors and radiotherapy. Abbreviations: N number of patients, SRS stereotactic radiosurgery, WBRT whole brain radiotherapy, RT radiotherapy, SABR stereotactic ablative radiotherapy, Gy Gray, NA not available

In three of the identified studies on metastatic melanoma and combination treatments with RT and Ipilimumab, matched control groups not receiving Ipilimumab were included (57-59). These studies all dealt with RT against brain metastases. The study by Knisely et al. (57) found a MS of 21,3 months in a cohort treated with stereotactic radiosurgery and Ipilimumab (n=27) compared with 4,9 months in a cohort treated with SRS alone (n=50) (HR 0,61 p=0,102). The 2-year survival rate was 47,2% in the Ipilimumab group and 19,7% in the control group. While the cohorts were generally well matched, including in prognostic scores, performance status, and sex, the group receiving Ipilimumab was significantly younger. There was also a trend towards higher use of additional targeted therapy in the group receiving Ipilimumab. In a study published by Silk et al. (59) another cohort of 33 patients who received Ipilimumab together with either stereotactic radiosurgery (n=17) or whole brain RT (n=16) was compared against a cohort who received either stereotactic radiosurgery (n=16) or whole brain radiotherapy (n=21) alone. MS for the group who received RT and Ipilimumab was 18,3 months, as compared with 5,3 months for the group that received RT alone (HR 0,43 p=0,005). There was significantly higher use of inhibitors of the BRAF oncoprotein in the group receiving Ipilimumab and a higher rate of additional RT to the brain, whereas there were trends towards lower performance status and more neurological symptoms in the group of patients that did not receive Ipilimumab. Otherwise, the groups were well matched. The third of these studies compared a cohort treated with stereotactic radiosurgery and Ipilimumab (n=25) to a cohort treated with only stereotactic radiosurgery (n=33). Primary endpoints were 6-month local control of metastases treated with stereotactic radiosurgery, 6-month freedom from new brain metastases and 6-month overall survival. Respective rates for the group treated with Ipilimumab and the group without were 63% and 65% for local control (p=0,55), 35% and 47% for freedom from new brain metastases (p=0,48) and 56% and 45% for overall survival (p=0,18) (58). Thus, there was no significant benefit of adding Ipilimumab to stereotactic radiosurgery in this study. The median survival after RT for both cohorts combined was 5,9 months (58).

Tazi et al. (64) explored a different angle, by comparing patients with brain metastases at treatment start with Ipilimumab (n=10) with patients without brain metastases (n=21). The authors found that patients who received RT and Ipilimumab against brain metastases did not live significantly shorter than advanced melanoma patients without brain metastases

who received Ipilimumab. MS from cycle 1 of Ipilimumab was 16,5 months for the cohort with brain metastases and 24,5 months for the one without (HR 1,05, $p=0,931$), estimated 3-year survival rates were 50% and 39% respectively. Seven patients in the cohort without brain metastases at treatment start with Ipilimumab did subsequently develop them, and six eventually received stereotactic radiosurgery (64).

Two studies did not include control groups that did not receive either RT or Ipilimumab. Gerber et al. (61) explored whole brain RT combined with Ipilimumab. MS was lowest of all identified studies in this cohort ($n=13$), at only 4 months, with 1 year survival rate of 15,4% (61). Kiess et al. (62) published a study with the largest cohort of melanoma patients with brain metastases, including 46 patients who received stereotactic radiosurgery and Ipilimumab. The MS was 12,4 months (62).

Three of the retrospective studies specifically looked for abscopal effects following radiation. Grimaldi et al. (60) identified 21 patients who had received RT 3,4-8 months after initiation of Ipilimumab treatment, 13 of them with radiation against brain metastases and 8 against extracranial sites. 13 patients experienced a local response to RT, while 9 of these experienced a partial response outside of the radiation field and 2 had stable disease for more than 3 months. No patients without a local response had abscopal responses. MS for abscopal responders was 22,4 months vs 8,3 months ($p=0,002$), correlating the responses to a survival benefit. MS overall was 13 months. Median time to abscopal response was 1 month (range 1-4). Schoenfeld et al. (63) identified 16 patients treated with Ipilimumab and stereotactic radiosurgery and/or whole brain RT against brain metastases. By designating the largest extracranial lesion found radiographically on images taken before RT as an index lesion, they followed this lesion for effects after RT. In 35% of instances where images were available before and after RT, the index lesion decreased in size. As a comparison, most of these patients had two consecutive scans available before RT, and only 17% of these showed a regression without RT in between. MS for the entire cohort was 14,4 months. Chandra et al. (65) reported the largest cohort overall for melanoma patients, with 47 patients receiving RT to the brain as whole brain RT or stereotactic radiosurgery, or extracranially to spine, bone, soft tissue, abdominovisceral and intrathoracic. Survival for the entire group was 28 months, with a 5-year survival rate of 20%. By using the same method as Schoenfeld et al. they found abscopal shrinkage of their index lesion in 25% of the cases following RT,

compared 11% of cases without RT in between radiographic scans. Median time to response was <1 month.

The single prospective study, included in the present review, was a phase I clinical trial (NCT01497808), including 22 patients, combining stereotactic ablative RT regimens of 8 Gy x 2-3 against bone/lung metastases or 6 Gy x 2-3 against liver/subcutaneous lesions with subsequent Ipilimumab 3 mg/kg. Evaluation of non-irradiated lesions revealed partial responses in 18% of the patients, stable disease in 18% and progressive disease in 64%. MS was 10,7 months (66).

In general, combinations of Ipilimumab and RT is reported to give side effects comparable to Ipilimumab alone (23). Some cases of radiation necrosis with surrounding cerebral edema did need surgical management, however (53, 57, 62). Some studies reported intracranial hemorrhages following radiation (58, 61), but apparently this occurs in untreated brain metastases as well (69, 70).

Studies on patients with metastatic castration resistant prostate cancer

Design	Radiation regimen, dose/fractions	Target	Drug	Sequence	Median survival (months) Ipilimumab + RT	Median survival (months) RT + placebo	Ref.
Phase I/II, open-label, multicenter, non-randomized (n=71)	Focal RT (n=42): 8 Gy each for 1-3 lesions	Bone	Ipilimumab(n=70): 3, 5 or 10 mg/kg	RT, Ipilimumab (n=41)	17,4 (overall)	17,4 (overall)	Slovin et al 2013 (67)
Multicenter, randomized, double-blind, phase III trial (intention to treat; n=799)	Focal RT (n=799): 8 Gy each for 1-5 lesions	Bone	Ipilimumab (n=399): 10 mg/kg	RT, Ipilimumab (n=399) RT, placebo (n=400)	11,2	10,0	Kwon et al 2014 (68)

Table 4: Studies on patients with metastatic castration resistant prostate cancer who has received combination therapies of immune checkpoint inhibitors and radiotherapy: Abbreviations: RT radiotherapy

The earliest identified study on mCRPC dealing with RT and Ipilimumab was a non-randomized open-label multicenter phase I/II study (67), with an initial dose escalation phase gradually assigning patients to 3, 5 or 10 mg/kg of Ipilimumab and after accrual 3 or 10 mg/kg and RT. All patients had evidence of progression after discontinuation of anti-androgen therapy. For the phase II part, the cohort of 10 mg/kg with and without RT were expanded to 50 patients, divided into 16 patients that did not receive RT and 34 patients receiving RT. The RT was given as a focal dose of 8 Gy against each of 1-3 bone metastases 24-48 hours before Ipilimumab. Overall survival for all cohorts was 17,4 months, with the

survival for each cohort not reported. In the phase II part there was one complete response (13%) and one instance of stable disease (13%) in the non-irradiated group, and five instances of stable disease (25%) in the irradiated group. The stable disease states lasted from 2,8-6,1 months, while the complete response lasted 11,3 months before censoring.

The second study in mCRPC was a multicenter, randomized, double, blind, phase III trial, where the intention to treat analysis included 799 patients (68). These were divided into two arms comparing RT and placebo against RT combined with 10 mg/kg of Ipilimumab. Again, radiation was directed against bone metastases, with 8 Gy per lesion, delivered within 2 days before Ipilimumab initiation. One to five lesions were irradiated. MS was 11,2 months in the Ipilimumab group and 10,0 in the placebo group. The hazard ratio was 0,85 ($p=0,053$), but the proportional hazards assumption was violated, making it invalid. One year overall survival was 46,8% to 40,4%, respectively. Two year survival was 26,2% to 15,0%, but a large proportion of patients were censored before two years. Prespecified subgroups with good prognostic features did experience a survival benefit in the Ipilimumab arm.

In both studies, adverse effects were common, but mostly managed by corticosteroids, endocrine replacement therapy and other supportive care (67, 68). In the first study one patient died of aspergillosis after 4 months of immunosuppression for managing colitis (67), and less than 2% of the deaths in the second study were considered to be related to Ipilimumab (68).

Discussion

Evidence for synergy

It is difficult to ascertain to what extent the treatment responses described in the case reports represents true synergism between RT and immune checkpoint inhibition. Three of the articles included immunological correlates to the treatment (51, 55, 56). In the case reported by Postow et al. (51), titers of antibodies against the entire protein NY-ESO-1 (a cancer antigen (71)) steadily increased throughout the development of the disease, while beginning to fall concurrent with resolution of the disease. Such a reduction in anti-NY-ESO-1 antibodies concurrent to reductions in tumor burden have been reported earlier (72). Interestingly, immediately following RT titers of antibodies against an epitope or epitopes on the central portion of the protein increased by a factor of over 30, which may indicate a vaccine-like response to RT. However, antibodies against other epitopes on NY-ESO-1 increased before RT, and it is not completely clear what the changes in antibody titers implicate. In another correlate consistent with a vaccine-like effect of RT, analysis of blood samples identified 10 antigens with increased responses mounted against them by antibodies after treatment (51).

In the same patient (51), flow cytometry of peripheral blood monocytes performed at different time points found an increase in CD4⁺ ICOS^{high} cells (ICOS is a marker for T-cell activation (73)) and HLA-DR (a MHC-II molecule) expression on CD14⁺ monocytes (marker found on dendritic cells and macrophages, which could serve as APCs (74)) concurrent with Ipilimumab induction. However, this was followed by a subsequent decrease in these markers, and was not associated with treatment responses. After RT new increases in CD4⁺ ICOS^{high} cells and HLA-DR⁺ CD14⁺ monocytes were detected, but this time also accompanied with a marked decline in myeloid derived suppressor cells. This fall preceded the regression of the tumor masses of the patient. That there were increases in numbers of activated CD4⁺ T cells and HLA-DR⁺ monocytes correlated to both Ipilimumab induction and RT, could suggest that both treatment modalities did elicit desired immunological responses, but for some reason the immunological responses after Ipilimumab induction were ineffective at combating the cancer until RT was applied. Likewise, that the numbers of myeloid derived suppressor cells decreased following RT may be a sign that RT did play a role in driving the

immunological anti-tumor response. Overall, the timing, with the lack of response to initial Ipilimumab-treatment, 14-months of radiographic progression during maintenance therapy, followed by RT and one additional dose of Ipilimumab and then the dramatic response, also presents circumstantial hints of synergy between Ipilimumab and RT. One phase III randomized controlled trial, including Ipilimumab alone in one of the treatment arms, had a mean time to response of 3,18 months (23). However, the same randomized controlled trial as well as another phase III trial also observed changes in responses as late as beyond 6 months (23, 24). One patient described in 2014 showed an anti-tumor response 11 months after therapy initiation with Ipilimumab (75), so one cannot completely dismiss an independent delayed response to Ipilimumab. Ruling out an abscopal effect of radiation, not dependent on Ipilimumab therapy, is also unfortunately impossible based on the available data.

Stamell et al. (55) also reported immunological correlates in their case report. Before combination therapy with RT and Ipilimumab, the patient had already experienced an abscopal response by RT alone, and preexisting antibodies against melanoma antigen A3 (MAGEA3) were already present before combination therapy. Titers of these antibodies measured by enzyme-linked immunosorbent assay (ELISA) showed a marked increase following RT and Ipilimumab treatments, and a new response against the cancer antigen PAS containing domain 1 (PASD-1) was detected. These correlates are consistent with a vaccine-like effect of RT, but due to the lack of markers specifically associated with responses to Ipilimumab, they do not in themselves present conclusive evidence for synergy.

The last patient with immunological correlates was the patient reported by Golden et al. (56), who suffered from NSCLC. His absolute lymphocyte counts and eosinophil counts increased significantly during treatment with Ipilimumab and RT. Such increases are associated with prolonged survival in melanoma patients treated with Ipilimumab (76-80), and could suggest that Ipilimumab was implicated in anti-tumor responses. Two lymph node metastases excised in 2010 and 2013, before and after combination treatment, had notably different histological patterns of immune-cell infiltration. In the first biopsy, lymphocytes were concentrated in perivascular areas of the tumor, while in the newly excised one, there was lymphocyte infiltration into tumor nests. They found more CD8 and TIA-1 (marker for cytotoxic granules (81)) positive cells in the latest biopsy, and while FoxP3⁺ (marker for T_{reg}'s

(82)) cells were also more common, the ratio of CD8/FoxP3 cells was higher (56). This pattern of lymphocyte infiltration is consistent with preclinical data on effective CTLA-4 inhibition (83).

Immunological correlates indicating effective anti-tumor responses after CTLA-4 inhibition in NSCLC are interesting, due to earlier data on the lack of effect of CTLA-4 inhibition alone in this cancer type. A phase II trial testing the anti-CTLA-4 mAb Tremelimumab as monotherapy against locally advanced or metastatic NSCLC found no significant benefit in progression-free survival compared with standard platinum-based cytotoxic therapy (84). Another phase II trial compared standard cytotoxic treatment and placebo against cytotoxic treatment and Ipilimumab. A phased regimen of cytotoxic treatment followed by Ipilimumab did achieve a significantly improved immune-related progression free survival, with a concurrent treatment showing no difference from the control (85). One interpretation put forth by the authors of the paper was that CTLA-4 inhibition alone is ineffective against NSCLC, but cytotoxic chemotherapy creates a vaccine effect that primes T cells for action, and synergizes with CTLA-4 inhibition, removes suppression of T cells (85). The impressive effect of RT combined with Ipilimumab in the case report could be consistent with such a model, with the RT filling the role of priming T cells with tumor antigens and danger signals (56). Still, ruling out a therapeutic effect of Ipilimumab alone is not possible. Especially since the study showing no effect of monotherapy used Tremelimumab (85), which could simply be less effective than Ipilimumab, despite inhibiting the same signaling axis.

Despite uncertain causal factors for the anti-tumor responses, all six patients in the reviewed case reports (51-56) experienced impressive regression of advanced cancer with typically bleak prognoses. For instance, a retrospective study on melanoma patients with leptomeningeal metastases, where 77,5% had received treatment with radiation and/or chemotherapy, reported a MS of only 10 weeks, with 1 and 2 year survival rates 7% and 3%, respectively (86). This stands in contrast to the 16 months of radiographically stable disease and clinical remission experienced by the patient described by Bot et al (52). The timing and immunological correlates in the case reports, when available, presents some indications for speculating that there were synergistic effects of RT and immune checkpoint inhibition in some of these cases. Of note, six patients is too few for making many inferences. However, larger patient materials are starting to become available. The seven identified retrospective

studies on advanced melanoma with brain metastases, discussed in the present review, reported a MS ranging from 4-21,3 months, with all but two studies (n=38) (58, 61) reporting a MS over 1 year(57, 59, 62-64). This is generally higher than MS reported in earlier material without combination regimens of RT and immune checkpoint inhibition in patients with brain metastases.

One retrospective study from the Sydney Melanoma Unit, on 686 patients treated between 1985-2000 for melanoma with brain metastases, reported an overall MS of 4,1 months, with a MS of 8,9 months for patients treated with surgery and RT, and 3,4 months for RT alone (87). Other studies confirm the bleak prognosis of melanoma patients with brain metastases (88, 89). Retrospective studies of patients treated with stereotactic radiosurgery reports MS below 8 months (90-92). With the retrospective studies at hand it is difficult to answer conclusively whether the better than expected survival in five of the seven studies on combination treatments with RT and Ipilimumab in melanoma patients with brain metastases represent true treatment benefits, or biases in the studies.

If the improved survival found is indeed generalizable, another difficult question is what the causal mechanisms are. The limited clinical benefit of RT without immune checkpoint inhibition in previously reported cohorts could perhaps support that Ipilimumab is implicated in improving survival. Whether this is a synergistic or even additive effect together with RT is difficult to assess. A systematic review extracting data from 14 studies on melanoma patients with brain metastases treated with immune checkpoint inhibitors, found a MS of 7,0 months in clinical trials and 4,3 months in real world studies (93). This is below the MS reported in most of the studies reviewed here, which could support a benefit of combination therapy over Ipilimumab, but no solid conclusion can be drawn from comparisons between heterogeneous cohorts.

For patients with extracranial metastases, the single identified prospective study on advanced melanoma did not report treatment benefits very different from Ipilimumab without radiation (25, 66). However, the retrospective study of Chandra et al. (65) found a very solid MS of 28 months (also including an unspecified number of patients with brain metastases), compared to 11,4 months from a pooled analysis of patients on Ipilimumab (25).

The three studies on melanoma that specifically looked for abscopal effects after RT, reported rates of 52%, 35% and 25% of included patients with responses outside of the irradiated field (60, 63, 65). In addition, the study in ref. (60) did not only show an increased survival for abscopal responders. Here, a local response to radiation turned out to be highly predictive of such a response, which may suggest that RT is implicated in the improved survival. Patients were also followed for absolute lymphocyte counts from before Ipilimumab initiation. At baseline, the groups were similar, but the abscopal responders had significantly higher absolute lymphocyte counts during Ipilimumab induction and before RT (60). The lymphocyte counts did show patterns associated with improved survival after CTLA-4 inhibition (76-80), which could perhaps suggest a role for Ipilimumab treatment in improving survival as well. The large percentages of abscopal responders also serve as hints that synergy with RT and Ipilimumab is implicated, given the typical rarity of abscopal responses after RT alone (34, 35). As mentioned earlier, two of these studies included internal controls to validate that the rate of abscopal responses found, was not simply due to actively looking for the phenomena (63, 65).

The studies on mCRPC did overall not provide conclusive evidence supporting the combined treatment (67, 68). This does not necessarily need to be evidence against the general soundness of combining immune checkpoint inhibition and RT. It could for instance be that the treatment is not well suited for the cancer type. Data from two studies on melanoma suggests that a high mutational load in the tumor is correlated with, but not necessary for, a clinical response to CTLA-4 inhibition (94, 95). There is evidence that mutational loads, as well as the specific mutations present, varies between cancer types (96). This could present a basis for different responses between melanoma and mCRPC-patients. Another possibility is that the radiation regimen was suboptimal.

Radiation regimen and timing

In both papers on mCRPC the authors state that the dose of 8 Gy in one fraction per lesion was selected due to proven benefits for pain palliation (67, 68). It does not appear that there was a focus on optimizing the radiation for a desired immunological effect. Preclinical experiments in mice with mouse breast carcinoma cells and mouse colon carcinoma cells by Dewan et al. (97) explored the effect of doses and fractionation of RT. They found that CTLA-4 inhibition together with fractionated regimens of 8 Gy x 3 and 6 Gy x 5 exhibited

considerable anti-tumor synergy on both a primary irradiated tumor, and in an abscopal manner on a secondary non-irradiated tumor. A single dose of 20 Gy failed to do so, while 8 Gy x 3 was especially effective. Controls with RT alone and anti-CTLA-4 alone had only local or no effect, respectively. However, it is unclear how transferable dosing and fractionation data is from animals to humans (98).

Some of the identified papers described the exact radiation doses and fractionation for altogether 14 patients with complete or partial abscopal responses (51-54, 56, 60). In the identified case reports three of the patients received extracranial regimens of 18 Gy x 3, 9,5 Gy x 3 and 6 Gy x 5 (51, 54, 56), while one received whole brain RT of 4 Gy x 5 (52). Stereotactic radiosurgery was performed with a dose of 20 Gy x 1 in one case (53). Grimaldi et al. (60) described the radiation regimens preceding the 9 abscopal partial responses in their study, which were whole brain RT 3 Gy x 10 in four patients, stereotactic radiosurgery 20-24 Gy x 1 in three patients, and extracranial regimens of 2 Gy x 25 and 4 Gy x 5 in one patient each. So overall, the doses and fractionation varied, with fractionated RT preceding the abscopal response in 10 cases (51, 52, 54, 56, 60) and unfractionated in 4 cases (53, 60). In one case report with a complete systemic response stereotactic radiosurgery is reported, but without any specifications about dose or fractionation (55). In addition to the studies describing exact regimens, Chandra et al. found a significant association between radiation fractions of ≤ 3 Gy and shrinkage of non-irradiated lesions. While most of the abscopal responses occurred after fractionated radiation, some followed single fraction stereotactic radiosurgery, and it is unclear from the data what the optimal radiation regimens should be. This may vary between target tumors and sites, as well. An interesting question is whether immunological responses to radiation are similar in the brain and outside the CNS. Radiation doses and fractions in the articles on melanoma patients are summarized in Tables 2 and 3, when available.

The sequencing could also have ramifications for effective treatment. In their aforementioned preclinical model, Dewan et al also found that concurrent treatment were superior to delayed checkpoint inhibition after RT (97). However, the clinical data is mixed on this. Knisely et al. (57) found no significant difference in MS between Ipilimumab before or after RT, and Mathew et al. (58) reported no significant difference in incidence of new brain metastasis between concurrent therapy and Ipilimumab before RT. Supporting this

Chandra et al. (65) found no impact for timing on the frequency of abscopal responses. Silk et al. (59) found improved survival for patients treated with RT before Ipilimumab compared with after. Kiess et al. (62) reported higher 1-year survival for concurrent treatment or RT before Ipilimumab compared to RT afterwards. Schoenfeld et al. (63) also found longer survival in patients undergoing concurrent treatment or RT prior to Ipilimumab, compared to Ipilimumab prior to stereotactic radiosurgery. They also found a significantly higher rate of abscopal responses when Ipilimumab was delivered within a three months window surrounding radiotherapy. However, in the cohort of Grimaldi and colleagues (60) all patients received RT after Ipilimumab, while many still experienced favorable outcomes. While there might be a trend in the data towards concurrent treatment or radiotherapy first, interpretations could be skewed by selection bias. Patients who have to receive RT after Ipilimumab might experience quicker progression, or they may respond less to immune checkpoint inhibition, as suggested by Kiess et al. (62).

Future prospects

While there is some clinical evidence for apparent synergy between RT and CTLA-4 inhibition with Ipilimumab, in particular from case reports and the studies on abscopal responses following RT, there is still a lot of unknowns with regard to optimal treatment regimens. Increased knowledge about the mechanics of interaction will prove helpful for designing treatment algorithms for dosing and sequencing, and current knowledge about these mechanics has recently been reviewed thoroughly elsewhere (99, 100).

Other interesting avenues of research are the exploration of other immune checkpoint inhibitors than Ipilimumab, and studies on combinations with additional treatments. Zeng et al. found improved survival in a mouse model of glioblastoma multiforme by combining a single fraction of 10 Gy with inhibition of the PD-1 receptor with a mAb (101). Dovedi et al. also explored the PD-1/PD-L1 axis in several mice models, including a colon carcinoma, a melanoma and a breast cancer cell line (102). Radiotherapy delivered as 10 Gy in 5 fractions, here led to an increase in expression of PD-L1 on tumor cells, which could be responsible for resistance to immune attack after RT (102). A small retrospective cohort from humans with head and neck cancer supports these data (103). Chemoradiotherapy was associated with a significantly higher PD-L1 expression, while chemotherapy alone correlated to a decrease in expression, suggesting that RT could be responsible for the increase (103). Dovedi and

colleagues combined the fractionated RT regimen with mAbs against both PD-L1 and PD-1, which led to complete responses in 66% and 80% of the mice (103). In addition, they explored timing of the treatments, and found that delaying checkpoint inhibition for too long abrogated the effects (102). Other preclinical studies have also found both local and abscopal synergism for PD-1/PD-1L inhibition and RT (104-106). Impressive rates of complete responses and improved survival was found in mice models treated with double blockade of both CTLA-4 and PD-1/PD-L1 together with radiotherapy (66). A recently published phase III trial in patients with metastatic melanoma found significantly increased progression-free survival and fewer treatment-related adverse effects in patients receiving the PD-L1/2 inhibitor Pembrolizumab, compared to patients receiving Ipilimumab (28). These results makes possibilities of combination regimens with PD-1 axis inhibition and RT extra exciting.

In yet another preclinical combination regimen Belcaid et al. explored a triple therapy of RT, CTLA-4 inhibitor and a CD137 agonist (a co-stimulatory receptor, also known as BB4-1 (107)) in a glioblastoma model. Survival was improved compared to single therapies of each, or combinations of a) CTLA-inhibition and RT or b) CD137 agonism and RT (108). Another study in a BRAFV600 mutant melanoma model in mice found anti-tumor effects of stereotactic ablative radiotherapy, PD-1 blockade and CD137 agonism (109).

While novel combination therapies offer exciting prospects, randomized controlled trials are needed to conclusively prove the efficacy of a combination regimen. Several clinical trials are recruiting patients for combinations of radiotherapy and PD-1/PD-L1 axis inhibition, including phase II randomized controlled trials exploring stereotactic ablative radiotherapy and Pembrolizumab in NSCLC (NCT02444741 (110)) and stereotactic ablative radiotherapy and Nivolumab in head and neck squamous cell carcinoma (HNSCC) (NCT02684253 (111)). For treatments with Ipilimumab, the University of Michigan Cancer Center is recruiting patients for a phase II RCT for melanoma with brain metastases, comparing stereotactic radiosurgery before Ipilimumab to Ipilimumab before stereotactic radiosurgery (NCT02097732 (112)), which can prove illuminating for sequencing of treatments.

Limitations to the study

This review have dealt with studies including heterogeneous designs, populations and treatment regimens, which made direct comparisons challenging. In addition, responses

were examined with non-homogenous criteria, with different studies using RECIST, WHO-criteria and immune related response criteria (4-6). Where preclinical studies have been mentioned to inform the discussion, differences between animal and human biology can serve as a source of confounding (98). Most of the studies are single case studies and retrospective cohorts. Generalizations from such studies should be approached with caution, due to inherent risks of selection biases in the patients included. Many patients included in several of these studies were enrolled in clinical trials, with frequent follow-ups, which could cause a lead-time bias, with earlier detection of for instance brain metastases, and longer follow-up subsequently misinterpreted as increased survival (57, 62). There might also be publication biases, where material showing interesting correlations is preferentially published over negative results. Selection of articles for the review was performed alone by one author, which could serve as a source of bias. Often several authors do this process, and a single person might unconsciously pick articles in line with personal hypotheses. Finally only a single database has been used for the search, which might lead to the omission of relevant articles.

Conclusion

Several preclinical models have shown clear synergistic effects of combinations of radiotherapy and immune checkpoint inhibition (48, 66, 97, 101, 102, 104-106, 108, 109), and circumstantial evidence from case reports and retrospective studies are accumulating for similar phenomena in humans (51-56, 60, 63, 65). There are still a number of questions left regarding factors such as doses and fractionation of radiotherapy, as well as timing of treatments (99, 100). The single identified large RCT for patients suffering from metastatic castration resistant prostate cancer did not show improved survival for patients treated with radiotherapy and CTLA-4 inhibition (68), but non-randomized studies in melanoma patients are overall more encouraging (57-66). However, to prove conclusively if synergistic treatments with radiotherapy and immune checkpoint inhibitors are feasible in larger patient populations randomized controlled trials with appropriate controls have to be conducted. Further preclinical work may also be needed to illuminate the mechanisms underlying the interplay between radiation and immune checkpoint inhibition, and help inform future clinical trials.

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