Universiteit Leiden
The Netherlands

Exploring and modulating the tumor immune microenvironment: Towards improving patient outcomes of immunotherapy in lung cancer

Theelen, W.S.M.E.

Citation
Theelen, W. S. M. E. (2020, October 21). *Exploring and modulating the tumor immune microenvironment: Towards improving patient outcomes of immunotherapy in lung cancer*. Retrieved from https://hdl.handle.net/1887/137007

Version:	Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/137007

Note: To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/137007 holds various files of this Leiden University dissertation.

Author: Theelen, W.S.M.E.
Title: Exploring and modulating the tumor immune microenvironment: Towards improving patient outcomes of immunotherapy in lung cancer
Issue date: 2020-10-21

PART II.

Modulating the tumor immune microenvironment

Willemijn S.M.E. Theelen[1], Monique C. de Jong[2], Paul Baas[1]
[1] Department of Thoracic Oncology, The Netherlands Cancer Institute, Amsterdam; [2] Department of Radiation Oncology, The Netherlands Cancer Institute, Amsterdam

CHAPTER 4

Synergizing systemic responses by combining immunotherapy with radiotherapy in metastatic non-small cell lung cancer: the potential of the abscopal effect

Lung Cancer. 2020 Apr;142:106-113

HIGHLIGHTS

- Immune checkpoint inhibitors are the new cornerstone of metastatic NSCLC treatment
- Several tumor immune escape mechanisms causing failure to ICIs have been postulated
- The immunoediting effect of radiotherapy may overcome some of these mechanisms
- (Pre-)clinical evidence supports augmentation of ICI efficacy when combined with radiotherapy

ABSTRACT

Immunotherapy has obtained a secure place in the treatment of metastatic non-small cell lung cancer (NSCLC) and has made a great impact on prognosis of responders. Unfortunately, not all NSCLC patients derive benefit from this treatment. Several immune escape mechanisms have been postulated, explaining failure of tumor immune attack. A better understanding of these mechanisms helps us to seek treatment strategies to overcome resistance to immunotherapy. Radiotherapy has immunomodulatory qualities capable of enhancing the anti-cancer immune response by tackling a number of these tumor escape mechanisms. In this review, we focus on mechanisms of off-target effects of radiotherapy, the so-called abscopal effect, by describing the current role of immune checkpoint inhibitors (ICIs) in NSCLC, the possible reasons for its failures and evidence on how radiotherapy may be able to counteract these mechanisms. An oversight of pre-clinical and clinical data supporting augmentation of abscopal events by radiotherapy when combined with ICIs is presented. As much remains unclear regarding optimal dose, fractionation, target volume or timing of radiation therapy, future research will need to focus on implementing data from pre-clinical and translational findings in the development of new clinical trials in order to help optimizing the potential of the combination of immunotherapy with radiotherapy.

1. Introduction

In recent years, treatment and prognosis for patients with metastatic non-small cell lung cancer (NSCLC) has changed profoundly due to introduction of immunotherapy. Blocking the programmed death-ligand 1 (PD-L1)/programmed death 1 (PD-1) pathway has become a new cornerstone in the treatment of advanced NSCLC patients, especially for those without targetable mutations. PD-L1 is mainly expressed by macrophages and endothelial cells, but also in a wide variety of solid tumors [1-3]. Binding of PD-L1 to its receptor PD-1 on T cells or antigen presenting cells (APC) activates an inhibitory signal leading to apoptosis or inactivation of these immune cells, thereby allowing tumors to evade the host immune response. To a lesser extent, interventions in another immune checkpoint mechanism, the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) pathway, have shown efficacy in NSCLC. CTLA-4 is expressed on naïve T cells and by binding to its ligands B7-1 or B7-2 expressed by APCs in lymph nodes, CTLA-4 transmits an inhibitory signal disabling priming of new T cell responses [4].

1.1 Current clinical setting of immune checkpoint inhibitors in NSCLC

Immune checkpoint inhibitors (ICIs) gained their first approval by the US Food and Drug Administration and the European Medicines Agency for the treatment of advanced NSCLC based on two phase III trials in second-line setting comparing the PD-1 antibody nivolumab to standard of care (SoC) chemotherapy, i.e. docetaxel. In both non-squamous and squamous NSCLC, nivolumab showed an improvement in overall survival (OS) over docetaxel [5, 6]. Shortly after, registration followed for the PD-1 antibody pembrolizumab and the PD-L1 antibody atezolizumab due to improved OS over docetaxel in second-line as well [7, 8]. Higher expression of PD-L1 as assessed by immunohistochemistry (IHC) has consistently been reported to be associated with higher response rates to anti-PD-(L)1 treatment. Objective response rates (ORR) varied from 8% in PD-L1 negative tumors up to approximately 30% in tumors with high PD-L1 expression [5, 7, 8]. Nivolumab and atezolizumab were approved as second-line treatment irrespective of PD-L1 expression and pembrolizumab for PD-L1 expression ≥1% only as PD-L1 negative tumors were excluded from the registration trial [7].

Subsequently, anti-PD-(L)1 treatment has found its way into first-line. The KEYNOTE-024 study compared pembrolizumab to platinum-based chemotherapy in patients with previously untreated advanced NSCLC with a PD-L1 expression of ≥50% and found a convincing improvement of OS for pembrolizumab in this group [9]. KEYNOTE-189 and KEYNOTE-407 showed that combining platinum-based chemotherapy with pembrolizumab as first-line regime is superior to chemotherapy monotherapy irrespective of PD-L1 expression [10, 11]. These results have established immunotherapy as the new cornerstone in first-line treatment of NSCLC patients. Two other first-line studies both met their co-primary endpoint of progression free survival (PFS) and OS benefit for the addition of atezolizumab to carboplatin and nab-paclitaxel (IMpower 130) and for the addition of atezolizumab to carboplatin, paclitaxel and bevacizumab (IMpower 150) in non-squamous NSCLC [12, 13].

No phase III trials with CTLA-4 inhibitor monotherapy have been performed in NSCLC. In the multi-arm CheckMate-227 study, the combination of nivolumab with the CTLA-4 antibody ipilimumab improved OS compared to platinum-based chemotherapy in first-line NSCLC [14]. This was significant in both the PD-

L1 ≥1% subgroup, which was the primary endpoint of this study, and in the PD-L1 negative subgroup. Another immunotherapy combination of the PD-L1 antibody durvalumab and anti-CTLA-4 drug tremelimumab showed no PFS or OS benefit over first-line chemotherapy in patients with advanced NSCLC [15]. The role of the addition of CTLA-4 antibodies in advanced NSCLC therefore remains unclear, and no approval of anti-CTLA-4 treatment in NSCLC has been granted to date.

Due to the success of PD-(L)1 inhibition in stage IV disease, immunotherapy was also tested in earlier and curable stages of NSCLC. In stage III NSCLC, the PACIFIC-trial compared one-year adjuvant durvalumab to placebo for patients that had not developed progression after concurrent chemoradiation (CRT). Patients in the adjuvant durvalumab arm experienced improvement of PFS and OS over placebo and this adjuvant treatment is now SoC [16]. Recently published studies in this setting were mainly focusing on safety and translational issues. Other trials with neo-adjuvant and adjuvant treatment for early stage NSCLC are ongoing, so more specific data on efficacy is eagerly awaited.

The introduction of ICIs has made a great impact on clinical outcomes for patients with advanced NSCLC as long-lasting anti-tumor immune responses on monotherapy have been described [17, 18]. The combination with chemotherapy in first-line setting increased response rates to an impressive 48-58% depending on histology, leading to further improvements in survival for advanced NSCLC patients [10, 11]. The addition of adjuvant immunotherapy to CRT transferred benefits to curable stage III patients [16]. Unfortunately, primary –as well as secondary- resistance to immunotherapy is still common and no clear second-line systemic treatment option has momentarily been established.

1.2 What may cause failure to immune checkpoint blockade?

In recent years, many insights were obtained in the interaction between the immune system and solid tumors. Alterations gained in tumor DNA may lead to expression of mutated proteins. Some of these mutated antigens can serve as so-called neoantigens. They can be recognized as non-self by APCs and when phagocytized and presented to circulating T cells a tumor specific immune reaction can be induced. Apparently, mechanisms of tumor immune escape have to be in existence in order to allow tumor growth to occur. Evasion of immunological destruction has now been recognized as an emerging hallmark of cancer [19]. Several of these immune escape mechanisms have been postulated (Table 1): 1) low tumor mutational burden (TMB) may prevent the presence of adequate neoantigens for recognition by APCs or T cells; 2) a low spill of neoantigens due to lack of excessive cell death, for example in slow progressing tumors, could compromise the induction of an immune response; 3) a lack of penetration of APCs into the tumor bed will prevent the ability of antigen presentation; 4) tumor cells may create a hostile environment to prevent infiltration of cytotoxic T cells into the tumor bed; 5) in order to become activated, T cells require inflammatory stimuli like danger signals after immunogenic cell death. In immune ‘cold’ tumors these stimuli are often absent; 6) recognition of neoantigens by cytotoxic T cells may be impaired through hampering of neoantigen presentation by oncogenic downregulation of MHC class I molecules on tumor cells or through a lack of diversity of the T cell receptor (TCR) repertoire; 7) presence of immune suppressive cells, like myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs) and T regulator cells (Tregs) or immune suppressive cytokines can disrupt the ongoing anti-cancer immune reaction; 8) upregulation of immune inhibitory pathways

like the PD-L1/PD-1 axis can cause further suppression and secondary tumor immune escape; 9) also, T cell exhaustion or a too large a tumor load for the immune system to handle may eventually lead to renewed tumor progression [20, 21].

Table 1. Mechanisms of tumor immune escape.

• Low tumor mutational burden and lack of adequate neoantigens
• Low spill or exposure of neoantigens
• Lack of antigen presenting cell penetration into the tumor bed
• Lack of cytotoxic T cell infiltration into the tumor bed
• Absence of inflammatory stimuli for T cell activation
• Oncogenic downregulation of MHC I molecules on tumor cells
• Lack of diversity of the T cell receptor repertoire
• Presence of an immune suppressive tumor microenvironment
• Oncogenic upregulation of immune inhibitory pathways
• Exhaustion of cytotoxic T cells

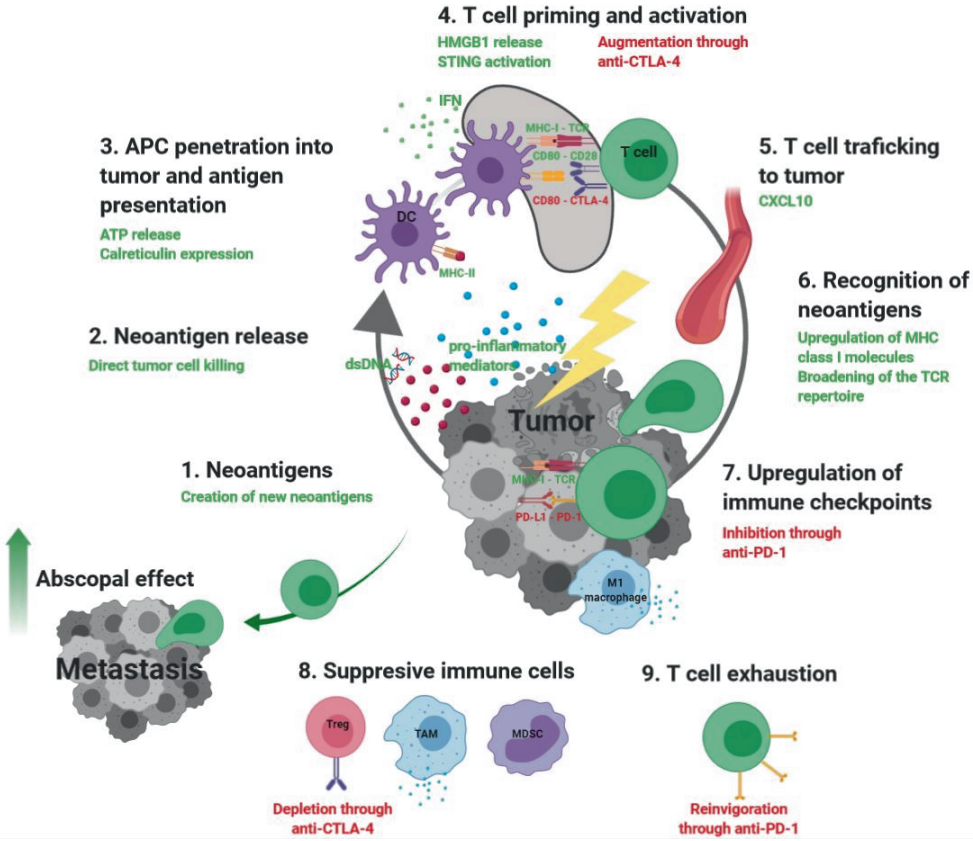
ICIs seem to be able to overcome some of these escape mechanisms, but unfortunately, resistance still forms a big challenge in daily patient care. It is therefore important to explore ways to further enhance the effect of anti-PD-(L)1 therapy to overcome this.

1.3 The immunomodulatory effect of radiotherapy

There has been a growing amount of in vitro and *in vivo* evidence that ionizing radiation has strong immunomodulatory potential, which provides a biological rationale that radiotherapy might be successful in making tumors more vulnerable to immune attack. A direct toxic effect of radiotherapy is decreasing tumor burden through the induction of tumor cell death. Furthermore, radiotherapy is able to induce immune responses that can both be pro-inflammatory and antitumor as well as immunosuppressive and protumor [22, 23]. When radiotherapy causes immunogenic cell death however, the subsequent effects may be able to counteract many of the tumor immune escape mechanisms mentioned above (Figure 1). Immunogenic cell death is characterized by the release of tumor antigens, production of pro-inflammatory mediators like ATP and high mobility group box 1 (HMGB1), and enhancement of surface expression of calreticulin [24]. Secretion of large amounts of ATP stimulates recruitment and activation/maturation of APCs. Exposure of calreticulin acts as an ‘eat-me’ signal, hence promoting the uptake of dead cell-associated antigens by APCs [25]. Radiotherapy also has the potential to create novel proteins that can be presented by APCs and thereby increases the pool of neoantigens [26]. This immunogenic cell death leads to increase of antigen presentation in the tumor draining lymph nodes, where tumor-specific T cells can become activated and then make their way to the newly inflamed tumor bed [27, 28]. The activated T cells are guided by the secretion of CXCL10 by tumor cells, a stimulator of T cell recruitment. The release of HMGB1 promotes the synthesis of pro-inflammatory factors including type I interferons (IFN), which are responsible for this CXCL10 production [25].

Also, radiotherapy causes release of double-stranded DNA and RNA, thereby activating the “stimulator of interferon genes” (STING) signaling pathway in dendritic cells through type I IFNs. When STING pathway activation in dendritic cells was blocked, priming of T cells would not occur, suggesting that

Figure 1. Tumor immune escape mechanisms and immunomodulatory effects of radiotherapy.



Mechanism of tumor immune escape are summarized in black text. The immunomodulatory effects of radiotherapy are presented in green text and grouped per escape mechanism. In red text the additive immune stimulatory effects of immune checkpoint inhibition are given. APC = antigen presenting cell; HMGB1 = High mobility group box 1; STING = stimulator of interferon genes; CTLA-4 = cytotoxic T-lymphocyte-associated protein 4; TCR = T cell receptor; PD-1 = programmed death 1; PD-L1 = programmed death-ligand 1; Tregs = T regulator cells; TAM = tumor-associated macrophages; MDSCs = myeloid-derived suppressor cells. The image was constructed using biorender.

STING signaling plays an essential role in generating an adequate immune response [29]. Furthermore, radiotherapy can improve the ability of tumor cell recognition by increasing upregulation of MHC class I molecules on tumor cells and by broadening the TCR repertoire [26, 30].

However, reports on the effect of radiotherapy on the presence of immune suppressive cells, like MDSCs, TAMs and Tregs, have been conflicting. A shift towards a more pro-inflammatory tumor microenvironment has been described as well as an increase in immune suppressive cell populations after radiotherapy [31, 32]. The reason for these discrepancies might be due to differences in tumor models or radiation regimes.

Low-dose radiotherapy has shown to have some specific immunomodulatory effects. Given the high radiosensitivity of leucocytes, low doses of radiotherapy are able to rid a tumor of immunosuppressive cells [33]. Additionally, low-dose radiotherapy keeps the local vasculature intact, while facilitating

extravasation of leucocytes by upregulation of ICAM-1 on endothelial cells is able to differentiate macrophages towards an immune-stimulatory M1 phenotype [34, 35].
Upregulation of immune checkpoints, like PD-L1 or IDO1, by radiotherapy with subsequent rebound immune suppression has been described in several pre-clinical studies [28, 36-38]. This upregulation strengthens the hypothesis that radiotherapy, when applied in the right way, can be an accessible and capable immunomodulator in combination with ICIs.

2 Rationale of combining radiotherapy with immune checkpoint inhibition

Besides a local synergistic antitumor effect between radiotherapy and the immune system, rare cases of systemic phenomena of interaction have also been observed. Local radiotherapy in itself can induce a systemic response by showing tumor shrinkage of untreated cancer lesions in patients [39, 40]. The notion of a systemic response by local radiation treatment is referred to as the abscopal effect; ab scopus meaning away from the target. These findings led to further research as to how localized radiotherapy could induce tumor-specific T cell activity and whether combination with ICIs could create a synergistic effect and even overcome primary resistance to ICIs.

2.1 Pre-clinical evidence of synergy between immuno- and radiotherapy

Several in vitro and *in vivo* studies in solid tumors compared ICIs combined with radiotherapy to either of the regimes alone. In an *in vivo* model of glioblastoma multiforme, the combination of a PD-1 antibody and radiation showed increased infiltration of cytotoxic T cells, reduced Tregs and improvement of tumor control compared to either regime alone [41]. In similar experiments in other *in vivo* solid tumor models, tumor regression was more pronounced when radiation was combined with anti-PD-1 treatment in the radiated lesion as well as in the non-radiated lesion [28, 37, 42, 43]. The synergistic response disappeared after depletion of T cells, suggesting that T cells are mandatory players in the abscopal effect [28]. The authors concluded that the necessity of additional anti-PD-1 treatment to establish a meaningful abscopal effect is because of upregulating of the PD-1/PD-L1 axis as an immune suppressive ‘side effect’ of radiation [37].
Less is known about the optimal dose, fractionation, target volume or timing of radiation therapy. As mentioned previously, low-dose radiotherapy may lead to pro-inflammatory and antitumor effects, but both low and high doses of radiotherapy are able to evoke local or abscopal immune responses, presumably through various mechanisms. Abscopal responses to low-dose radiation have been reported in mice [36]. However, (moderately) hypofractionated radiotherapy has been hypothesized to give stronger abscopal responses in various mice/tumor models. Morisada et al. compared the effect of a low-dose fractionated (10x2Gy) to a high-dose hypofractionated regime (2x8Gy) in an oral cavity and a colon carcinoma model. They found suppressed antitumor immunity with low-dose radiotherapy, but high-dose hypofractionated radiation led to preservation of peripheral and tumor-infiltrating effector immune cells, reduction of immunosuppressive immune cells and enhancement of tumor-specific immune responses [44]. Both radiotherapy strategies showed a reduction in MDSCs, but not in Tregs. They also found that addition of anti-PD-1 treatment after high-dose hypofractionated radiotherapy

reversed adaptive immune resistance, but this did not occur after low-dose fractionation. In a lung cancer model, Camphausen et al. had already confirmed that 5x10Gy provided increased tumor growth reduction in a non-radiated tumor lesion compared to low-dose 12x2Gy [45]. Several studies showed that a fractionated dose led to more signs of the abscopal effect compared to a single dose [46-48]. The experiments by Vanpouille-Box et al. also suggested that there might be a maximum dose as a dose of 8-10Gy per fraction induced interferon signaling, whereas higher doses lead to TREX1 upregulation and abrogation of interferon signaling [48]. However, Lee et al. noticed vigorous priming and expansion of effector T cells after a high single dose of 20Gy altering the tumor microenvironment from immune-suppressive to immune-activating in a melanoma mouse model [49].

The ideal timing of radiation with ICIs has been the focus of investigation as well. When fractionated radiotherapy was combined sequentially vs concomitantly with anti-PD-L1 treatment in a variety of syngeneic mouse models of cancer, lead-time of more than one week between radiation and start of immunotherapy rendered T cells anergic [36]. The survival benefit shown in the concomitant regime was lost in the sequential regime suggesting that anti-PD-1 treatment should be started within one week after radiation. The optimal timing may also be dependent on the specific effect of the immunotherapy agent. Applying anti-CTLA-4 treatment before radiation showed longer survival compared to adjuvant treatment *in vivo*. However, anti-OX40, a co-stimulatory agonist, did not provide any antitumor effect when given before radiation, but tumor reduction and improved survival was established when anti-OX40 was given within 24 hours after radiotherapy, suggesting that this compound is effective only in the antigen presentation phase [50]. In melanoma mouse models, Twyman-Saint Victor et al. found a 58% complete response rate after treatment with radiotherapy and doublet immunotherapy: CTLA-4 and PD-L1 inhibition. Again, CTLA-4 antibodies were only effective when given before radiation. Based on tumor and blood analyses, they postulated that CTLA-4 antibodies may downregulate Tregs, that anti-PD-L1 antibodies reinvigorate exhausted CD8 T cells and that radiotherapy broadens the TCR repertoire, illustrating why this proposed 'triple-regime' might even further improve patient outcomes [51].

The number, size and location of the target volume(s) could all possibly influence the immunogenicity of radiotherapy. Since most animal experiments are carried out with an irradiated tumor in one flank and an 'abscopal' unirradiated tumor in the other flank, experiments that irradiate multiple metastases or metastases in various organs are difficult to control and set up in mice.

Also, not just irradiation to various tumor locations, but also irradiation to normal tissues could influence response to (radio-)immunotherapy. As priming of antitumor T cells takes place in tumor draining lymph nodes (TDL), research on the consequences of nodal radiation on the synergistic effect of radiation and ICI seems relevant [52]. Marciscano et al. compared radiation of the tumor only to radiation of the tumor together with the TDL, called elective nodal irradiation (ENI) [53]. It was shown that radiation of the TDL attenuated the influx of antigen-specific intratumoral T cells and adversely affected local tumor control of the radiated tumors in mice. ENI restrained the adaptive immune response and addition of PD-1 antibodies could not restore this lack of T cell trafficking. The addition of CTLA-4 antibodies however, did show improvement of local tumor control and survival after ENI in these mice. Noteworthy, in patients, radiotherapy to the thorax or spine can increase the risk of severe lymphopenia, which was associated with poorer survival in patients treated with ICIs [54].

Thus, besides decreasing tumor load, increasing neoantigen release, presentation and repertoire, increasing APC influx, stimulating T cell recruitment, upregulation of MHC class I molecules and broadening the TCR repertoire, radiotherapy also showed to be able to provide necessary immune stimuli to augment response to immunotherapy (Figure 1). Still, much work needs to be performed concerning optimization of this synergistic effect. Whether these in vitro and *in vivo* successes are reproducible in clinical setting and will eventually lead to relevant clinical benefit, is still under elaborate investigation.

2.2 Clinical endeavors in NSCLC

2.2.1 Search strategy

We performed a systemic literature search in PubMed to obtain studies reporting on clinical outcomes of the combination of radiotherapy with ICIs in (NSCLC) patients. The search including several terms: “lung cancer”, “NSCLC”, “radiotherapy”, “radiation”, “irradiation”, “SABR”, “SRS”, “SBRT”, “immunotherapy”, “immune checkpoint inhibitors”, “anti-CTLA-4”, “anti-PD-1”, “anti-PD-L1”, “abscopal effect”. The final search was performed in January 2020. After removing duplicates, 513 publications were identified and assessed based on title and abstract. Non-English articles, conference abstracts, editorials, reviews and case reports were excluded. The studies selected for this review comprised of retrospective and prospective series on sequential and/or concurrent ICI-radiation treatment. Series without NSCLC patients and series where all tumor lesions were radiated and therefore no abscopal effect for non-radiated lesions could be established were further excluded, leaving 18 studies eligible for this review (Table 2).

2.2.2 Safety and efficacy data

As radiotherapy induces a locally and systemically inflammatory response, the combination could harbor the risk of increased toxicity. Retrospective series and prospective single arm studies have focused on safety aspects of combining radiotherapy with ICIs. Luke et al. performed a prospective study evaluating the safety of stereotactic body radiation therapy (SBRT) on 2-4 tumor lesions before start of pembrolizumab in 73 patients with solid tumors. They found grade 3 or higher toxicity in 8% (6/73) of patients. Four of these experienced toxicity within the irradiated field: 3 cases of pneumonitis and 1 case of colitis. These numbers are comparable to toxicity from ICI monotherapy and the authors concluded that the combination of radiotherapy with ICI seemed to be well tolerated with acceptable toxicity. Evidence of the abscopal effect was represented by a significant correlation of expression of interferon-gamma-associated genes from post-SBRT tumor biopsies from responding non-radiated tumor lesions [55]. Several retrospective series have been published investigating the possible toxicity risk of ICIs and radiotherapy, but auto-immune toxicities in the radiation field were few and overall well manageable [56-62]. This safety profile was also established for patients with advanced NSCLC with brain metastases who received cranial radiation specifically [63, 64], but there is also concern about an increase in the development of radiation necrosis in ICI-treated patients [65, 66].

Table 2. Clinical results of radiotherapy and ICI combination in metastatic non-small cell lung carcinoma

Author	Study type	Histology	N	IT agent	RT target	RT dose (Gy/fraction)	Treatment sequence	ORR, abscopal	mPFS (months)	mOS (months)	Toxicity Grade ≥3
Luke et al. 2018 [55]	Phase I	Various solid tumors	79	pembro	Various	30-50Gy/3-6 to 2-4 lesions	Sequential	13%	3.1	9.6	10%
Bang et al. 2017 [56]	Retrospective	NSCLC, MEL, RCC	133	aPD-(L)1 and/or aCTLA-4	Various	8-66Gy/1-15	Concurrent or sequential	NR	NR	NR	8%
Mohamad et al. 2018 [62]	Retrospective	Various solid tumors	59	nivo +/- ipi, pembro or atezo	Various	6-54Gy/1-5	Concurrent or sequential	26%	6.5	not reached	20%
v. Reibnitz et al. 2018 [57]	Retrospective	Various solid tumors	79	aPD-(L)1 and/or aCTLA-4	Thoracic	NR	Concurrent or sequential	NR	NR	NR	NR
Hwang et al. 2018 [58]	Retrospective	NSCLC	73	aPD-(L)1	Thoracic	8-54Gy	Concurrent or sequential	NR	NR	12.1	NR
Verma et al. 2018 [59]	Retrospective	Various solid tumors	60	ipi or pembro	Various	45-54Gy/15 50-60Gy/4	Concurrent	NR	NR	NR	25%
Miyamoto et al. 2018 [61]	Prospective	NSCLC	6	nivo	Lung	25-48/3-4	Sequential	75%	NR	NR	17%
Lesueur et al. 2018 [60]	Retrospective	NSCLC	104	nivo	Various	20-36Gy/1-10	Concurrent or sequential	NR	2.7	11.1	10%
Hubbeling et al. 2018 [63]	Retrospective	NSCLC	50	pembro, nivo or atezo	Intracranial	NR	Concurrent or sequential	NR	NR	NR	9%
Chen et al. 2018 [64]	Retrospective	Various solid tumors	79	ipi, pembro or nivo	Intracranial	15-24Gy/1-3 25Gy/5	Concurrent or sequential	NR	2.3	24.7 conc; 14.5 seq	16%
Martin et al. 2018 [66]	Retrospective	NSCLC, MEL, RCC	115	ipi, pembro or nivo	Intracranial	18-20Gy/1 25-30Gy/5	Concurrent or sequential	NR	NR	NR	20%*
Colaco et al. 2016 [65]	Retrospective	Various solid tumors	42	aCD137, aPD-1, aCTLA-4, IL-2	Intracranial	NR	Concurrent or sequential	NR	NR	NR	33%*
Shaverdian et al. 2017 [67]	Retrospective	NSCLC	42	pembro	Various	NR	Sequential	NR	4.4	10.7	2%
Formenti et al. 2018 [68]	Phase II	NSCLC	39	ipi	Various	6Gy/5 or 9Gy/3	Concurrent	18%	NR	7.4	50%
Tang et al. 2017 [69]	Phase I	Various solid tumors	35	ipi	Lung or liver	50Gy/4 60Gy/ 10	Concurrent or sequential	10%	3.2	10.2	34%
Welsh et al. 2019 [70]	Phase II	Various solid tumors	106	ipi	Lung or liver	50Gy/4 60Gy/10	Concurrent or sequential	9%	2.9	not reached	34%
Srivastava & Huang 2017 [71]	Retrospective	NSCLC, MEL	50	pembro or nivo	Intracranial	NR	Concurrent or sequential	NR	NR	NR	NR
Theelen et al. 2019 [72]	Phase II	NSCLC	76	pembro	Various	8Gy/3	Sequential	36%	6.6	15.9	14%

IT agent = immunotherapy agent, RT = radiotherapy, ORR = overall response rate, PFS = progression free survival, OS = overall survival, NSCLC = non-small cell lung cancer, MEL = melanoma, RCC = renal cell carcinoma, aPD-1 = anti-programmed cell death protein-1; aPD-L1 = anti-programmed death-ligand, aCTLA-4 = anti-cytotoxic T-lymphocyte-associated antigen 4, pembro = pembrolizumab, nivo = nivolumab; atezo = atezolizumab; ipi = ipilimumab, IFN = interferon, IL-2 = interleukin-2, Concurrent = radiotherapy was applied during ongoing ICI treatment, sequential = ICI treatment was started >1 day after radiotherapy was applied, NR = not reported. * symptomatic radiation necrosis

Several trials have tried to improve insights in the efficacy of a possible radiotherapy augmentation on ICI responses. A retrospective analysis of NSCLC patients in the phase I KEYNOTE-001 evaluated the effect of previous radiotherapy given anywhere during the disease period on clinical outcomes. Patients that had received previous radiotherapy showed a significant PFS and OS benefit over patients that were never irradiated. Toxicity data of the radiation group was comparable to treatment with ICI monotherapy [67]. Another trial investigated the combination of ipilimumab with radiation in advanced NSCLC patients. In this heavily pre-treated cohort, ORR measured in non-radiated lesions only was relatively high with 18% (7/39). Functional analysis in one responding patient showed the rapid *in vivo* expansion of CD8 T cells recognizing a neoantigen encoded in a gene upregulated by radiation, supporting the hypothesis that part of the explanation for the abscopal response is radiation-induced exposure of immunogenic mutations to the immune system [30].

In a phase I trial comparing several SBRT regimes -concurrently vs sequentially in two different fractionated schemes and lung vs liver lesions- together with ipilimumab in solid tumors of which 8/36 (23%) NSCLC patients, showed clinical benefit in 23% of patients, which was associated with an increase in peripheral CD8 T cells count [68]. T-cell activation measured by the expression of stimulatory signals -ICOS, GITR, and 4-1BB- by peripheral T cells was more pronounced after radiation of a liver lesion compared to lung lesions, leading the authors to the suggestion that the site of SBRT may be of relevance. However, their phase II trial including 30/106 (28%) NSCLC patients showed considerably higher disease control rate at 6 months with ipilimumab and sequential radiotherapy on a lung lesion (42%) compared to concurrent radiation on a liver lesion (5%) [69]. Therefore, we should be aware of a possible bias in selection of radiation-site due to metastatic pattern in these trials.

Besides location of SBRT, timing should be carefully addressed as well. Unfortunately, not much clinical data regarding this aspect has yet been generated. In a retrospective series of patients that received stereotactic radiosurgery (SRS) for brain metastases of several solitary tumors, mostly NSCLC or melanoma, local control and distant brain control were better when immunotherapy was started within 3 weeks of SRS [70].

Our group recently published the results from the PEMBRO-RT study, a hypothesis-generating phase II trial, where patients in second-line, metastatic NSCLC setting were randomized to either pembrolizumab alone vs pembrolizumab within one week of SBRT (3x8Gy) to a single tumor lesion [71]. The ORR at 12 weeks doubled in the SBRT arm and this led to an increase of PFS and OS without increase in treatment related toxicity. Although these improvements did not meet the predefined clinical endpoints and randomization was not stratified based on PD-L1 expression, subgroup analyses showed a significant benefit in PFS and OS from the addition of radiotherapy in patients with PD-L1 negative tumors (<1%). Translational research on collected blood samples, baseline and on-treatment biopsies is still ongoing to further explore in more detail as to what extend SBRT has improved patient outcomes in this trial.

2.2.3 Adjuvant ICIs after ablative radiotherapy

A subgroup of NSCLC patients can be defined as having oligometastatic disease. Treating these patients with locally ablative therapy to all tumor sites as adjuvant to platinum-doublet chemotherapy

has been associated with improved PFS and OS [72, 73]. Bauml et al. reported superior outcomes of patients that had received LAT after chemotherapy for oligometastatic disease (≤4 metastasis) and treated with one year of adjuvant pembrolizumab compared to historic controls [74]. This strategy was deemed safe.

As already mentioned, the PACIFIC-trial investigated the role of adjuvant ICI after CRT in curable stage III NSCLC [16]. Besides improved patient outcomes, PACIFIC showed a slight increase in toxic effects in the durvalumab group compared to the placebo arm, but the rates of severe immune-related adverse events, and of pneumonitis in particular, were not significantly different. Also, patients showed lower recurrence of disease when durvalumab was initiated within ≤2 weeks of last radiation dose rather than >2 weeks after radiation. This may suggest that a short window between radiotherapy and start of ICI should be pursued, but it cannot be excluded that this is a selection bias where the 'best' patients are the ones that are able to start sooner with adjuvant treatment [75]. The single arm LUN 14-179 study investigating adjuvant pembrolizumab after CRT showed improvement of recurrence rate and PFS compared with historical controls with no significant immune-related toxicity increase [76].

However, in adjuvant setting where all tumor localizations are treated with local radical radiotherapy for oligometastatic or stage III disease, proving an abscopal effect will be difficult. As there is no residual disease for response measurement, time to disease recurrence or OS are the only assessable clinical endpoints. But the synergistic effect between radiation and ICIs, the 'true' abscopal effect, is indistinguishable from a lack of residual disease -cure by CRT alone- or earlier onset of successful systemic treatment for occult metastatic disease irrespective of radiotherapy synergy. We will need dedicated translational research to help us bring better insights in these non-overlapping patient categories as this might warrant a more individualized approach.

2.3 Future perspectives

Many NSCLC trials are currently ongoing to further assess the safety and efficacy of the combination of radiation and immunotherapy in metastatic setting. Unfortunately, most of these trials do not focus on comparison of different radiation regimes, timing, anatomical site or number of radiated lesions, so the question as how to optimize this synergistic opportunity might remain unsolved for some time to come. A multi-arm optimal dose/fractionation-finding study based on clinical outcomes and translational endpoints would be a reasonable next step. Besides anti-PD-(L)1 treatment, the effect of immunotherapy combinations could be explored in such a setting as well. And with new radiotherapy modalities becoming available, it would be interesting to compare FLASH radiotherapy to heavy ion or photon beam radiotherapy in regards to the immunomodulation. Brooks et al. claimed that maybe more than one lesion should be radiated to obtain a maximum abscopal effect as this could help tackle tumor heterogeneity and clonality of metastases, differences in immunogenicity of lesions or local immune suppressive effects and a decrease in tumor load [77]. McGee et al. compared immune response generated by SBRT on metastatic lesion of solid tumors in different organs in a small prospective patient series. They found that irradiation to lung and liver metastases showed an induction of immune response, whereas this was not observed for radiotherapy to bone or brain metastases, endorsing further development of these comparative trial setups [78].

Retrospective evidence of re-invigoration of ICI responses through the addition of radiotherapy after development of secondary resistance has recently been described [79, 80]. It would be interesting to investigate this ability of radiotherapy in a prospective manner.

Currently, the role of ICIs in locally advanced stage III NSCLC is being investigated beyond adjuvant durvalumab. The first safety data shows that concomitant addition of immunotherapy, nivolumab and atezolizumab, to concurrent CRT is safe and tolerable [81, 82]. A randomized phase III study of CRT with concomitant durvalumab, the PACIFIC-2 study [ClinicalTrials.gov identifier: NCT03519971], is currently ongoing. The phase II BTCRC-LUN16-081 trial [NCT03285321] is including patients to investigate the safety and efficacy of a one-year adjuvant ICI-combination nivolumab and ipilimumab after CRT. Based partly on pre-clinical evidence that addition of anti-CTLA-4 treatment may have a better abscopal effect before the application of radiation [50, 51], our group is now recruiting patients for a phase I trial, evaluating safety of neo-adjuvant durvalumab and tremelimumab before CRT, the Induction-1 study [NCT04287894]. Again, as all known disease locations in these settings will be radically treated, it will be difficult to differentiate the abscopal and therefore synergistic effect from the advantage of moving a possible beneficial effect of immunotherapy to an earlier time point in disease treatment. Nevertheless, many new insights will be gained from these trials.

3. Conclusion

Pre-clinical research has provided convincing evidence that radiotherapy has immunomodulatory qualities leading to synergistic effects when combined with ICIs. Still, much remains unclear regarding optimal dose, fractionation, target volume or timing of radiation therapy. Patients/tumors with different mechanisms for treatment failure, might benefit from different immune-stimulating radiotherapy regimens. Pre-clinical and translational experiments that focus on one aspect of the radiation regime could hopefully answer some of these specific questions. Also, establishing assessable biomarkers for immunogenic cell death or radiotherapy-induced anti-tumor immune responses will help to bring research in this field forward. Although biomarker research for response to immunotherapy is a fast-evolving field with new pieces of the puzzle generated almost daily, the many elements of the immune system involved together with a diverse interaction with tumor cells makes this a difficult enterprise. Future clinical research will need to focus on implementing data from pre-clinical and translational findings in the development of new clinical trials in order to proof reproducibility in a patient setting and to help optimizing the abscopal potential.

References

1. Freeman, G.J., et al., *Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation.* J Exp Med, 2000. **192**(7): p. 1027-34.
2. Keir, M.E., et al., *PD-1 and its ligands in tolerance and immunity.* Annu Rev Immunol, 2008. **26**: p. 677-704.
3. Herbst, R.S., et al., *Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients.* Nature, 2014. **515**(7528): p. 563-7.

4. Pardoll, D.M., *The blockade of immune checkpoints in cancer immunotherapy.* Nat Rev Cancer, 2012. **12**(4): p. 252-64.
5. Borghaei, H., et al., *Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer.* N Engl J Med, 2015. **373**(17): p. 1627-39.
6. Brahmer, J., et al., *Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer.* N Engl J Med, 2015. **373**(2): p. 123-35.
7. Herbst, R.S., et al., *Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial.* Lancet, 2015.
8. Rittmeyer, A., et al., *Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial.* Lancet, 2016.
9. Reck, M., et al., *Updated Analysis of KEYNOTE-024: Pembrolizumab Versus Platinum-Based Chemotherapy for Advanced Non-Small-Cell Lung Cancer With PD-L1 Tumor Proportion Score of 50% or Greater.* J Clin Oncol, 2019. **37**(7): p. 537-546.
10. Gandhi, L., et al., *Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer.* N Engl J Med, 2018.
11. Paz-Ares, L., et al., *Pembrolizumab plus Chemotherapy for Squamous Non-Small-Cell Lung Cancer.* N Engl J Med, 2018. **379**(21): p. 2040-2051.
12. Socinski, M.A., et al., *Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC.* N Engl J Med, 2018. **378**(24): p. 2288-2301.
13. West, H., et al., *Atezolizumab in combination with carboplatin plus nab-paclitaxel chemotherapy compared with chemotherapy alone as first-line treatment for metastatic non-squamous non-small-cell lung cancer (IMpower130): a multicentre, randomised, open-label, phase 3 trial.* Lancet Oncol, 2019. **20**(7): p. 924-937.
14. Hellmann, M.D., et al., *Nivolumab plus Ipilimumab in Advanced Non-Small-Cell Lung Cancer.* N Engl J Med, 2019. **381**(21): p. 2020-2031.
15. Rizvi, N.A., et al., *LBA6Durvalumab with or without tremelimumab vs platinum-based chemotherapy as first-line treatment for metastatic non-small cell lung cancer: MYSTIC.* Annals of Oncology, 2018. **29**(suppl_10).
16. Antonia, S.J., et al., *Overall Survival with Durvalumab after Chemoradiotherapy in Stage III NSCLC.* N Engl J Med, 2018. **379**(24): p. 2342-2350.
17. Gettinger, S.N., et al., *Overall Survival and Long-Term Safety of Nivolumab (Anti-Programmed Death 1 Antibody, BMS-936558, ONO-4538) in Patients With Previously Treated Advanced Non-Small-Cell Lung Cancer.* J Clin Oncol, 2015. **33**(18): p. 2004-12.
18. Garon, E.B., et al., *Five-Year Overall Survival for Patients With Advanced NonSmall-Cell Lung Cancer Treated With Pembrolizumab: Results From the Phase I KEYNOTE-001 Study.* J Clin Oncol, 2019: p. Jco1900934.
19. Hanahan, D. and R.A. Weinberg, *Hallmarks of cancer: the next generation.* Cell, 2011. **144**(5): p. 646-74.
20. Chen, D.S. and I. Mellman, *Elements of cancer immunity and the cancer-immune set point.* Nature, 2017. **541**(7637): p. 321-330.
21. O'Donnell, J.S., M.W.L. Teng, and M.J. Smyth, *Cancer immunoediting and resistance to T cell-based immunotherapy.* Nat Rev Clin Oncol, 2019. **16**(3): p. 151-167.
22. Schaue, D. and W.H. McBride, *T lymphocytes and normal tissue responses to radiation.* Front Oncol, 2012. **2**: p. 119.
23. Zitvogel, L. and G. Kroemer, *Subversion of anticancer immunosurveillance by radiotherapy.* Nat Immunol, 2015. **16**(10): p. 1005-7.
24. Sharabi, A.B., et al., *Radiation and checkpoint blockade immunotherapy: radiosensitisation and potential mechanisms of synergy.* Lancet Oncol, 2015. **16**(13): p. e498-509.
25. Galluzzi, L., L. Zitvogel, and G. Kroemer, *Immunological Mechanisms Underneath the Efficacy of Cancer Therapy.* Cancer Immunol Res, 2016. **4**(11): p. 895-902.
26. Reits, E.A., et al., *Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy.* J Exp Med, 2006. **203**(5): p. 1259-71.
27. Demaria, S. and S.C. Formenti, *Radiation as an immunological adjuvant: current evidence on dose and fractionation.* Front Oncol, 2012. **2**: p. 153.
28. Deng, L., et al., *Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice.* J Clin Invest, 2014. **124**(2): p. 687-95.

29. Deng, L., et al., *STING-Dependent Cytosolic DNA Sensing Promotes Radiation-Induced Type I Interferon-Dependent Antitumor Immunity in Immunogenic Tumors.* Immunity, 2014. **41**(5): p. 843-52.
30. Rudqvist, N.P., et al., *Radiotherapy and CTLA-4 Blockade Shape the TCR Repertoire of Tumor-Infiltrating T Cells.* Cancer Immunol Res, 2018. **6**(2): p. 139-150.
31. Rodriguez-Ruiz, M.E., et al., *Abscopal Effects of Radiotherapy Are Enhanced by Combined Immunostimulatory mAbs and Are Dependent on CD8 T Cells and Crosspriming.* Cancer Res, 2016. **76**(20): p. 5994-6005.
32. Wu, Q., et al., *Macrophage biology plays a central role during ionizing radiation-elicited tumor response.* Biomed J, 2017. **40**(4): p. 200-211.
33. Geara, F.B., et al., *Intrinsic radiosensitivity of normal human fibroblasts and lymphocytes after high- and low-dose-rate irradiation.* Cancer Res, 1992. **52**(22): p. 6348-52.
34. Cervelli, T., et al., *Effects of single and fractionated low-dose irradiation on vascular endothelial cells.* Atherosclerosis, 2014. **235**(2): p. 510-8.
35. Klug, F., et al., *Low-dose irradiation programs macrophage differentiation to an iNOS(+)/M1 phenotype that orchestrates effective T cell immunotherapy.* Cancer Cell, 2013. **24**(5): p. 589-602.
36. Dovedi, S.J., et al., *Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade.* Cancer Res, 2014. **74**(19): p. 5458-68.
37. Gong, X., et al., *Combined Radiotherapy and Anti-PD-L1 Antibody Synergistically Enhances Antitumor Effect in Non-Small Cell Lung Cancer.* J Thorac Oncol, 2017. **12**(7): p. 1085-1097.
38. Li, A., et al., *IDO1 Inhibition Overcomes Radiation-Induced "Rebound Immune Suppression" by Reducing Numbers of IDO1-Expressing Myeloid-Derived Suppressor Cells in the Tumor Microenvironment.* Int J Radiat Oncol Biol Phys, 2019. **104**(4): p. 903-912.
39. Rees, G.J. and C.M. Ross, *Abscopal regression following radiotherapy for adenocarcinoma.* Br J Radiol, 1983. **56**(661): p. 63-6.
40. Siva, S., et al., *Abscopal [corrected] effects after conventional and stereotactic lung irradiation of non-small-cell lung cancer.* J Thorac Oncol, 2013. **8**(8): p. e71-2.
41. Zeng, J., et al., *Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas.* Int J Radiat Oncol Biol Phys, 2013. **86**(2): p. 343-9.
42. Kroon, P., et al., *Concomitant targeting of programmed death-1 (PD-1) and CD137 improves the efficacy of radiotherapy in a mouse model of human BRAFV600-mutant melanoma.* Cancer Immunol Immunother, 2016. **65**(6): p. 753-63.
43. Dovedi, S.J., et al., *Fractionated Radiation Therapy Stimulates Antitumor Immunity Mediated by Both Resident and Infiltrating Polyclonal T-cell Populations when Combined with PD-1 Blockade.* Clin Cancer Res, 2017. **23**(18): p. 5514-5526.
44. Morisada, M., et al., *PD-1 blockade reverses adaptive immune resistance induced by high-dose hypofractionated but not low-dose daily fractionated radiation.* Oncoimmunology, 2018. **7**(3): p. e1395996.
45. Camphausen, K., et al., *Radiation abscopal antitumor effect is mediated through p53.* Cancer Res, 2003. **63**(8): p. 1990-3.
46. Dewan, M.Z., et al., *Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody.* Clin Cancer Res, 2009. **15**(17): p. 5379-88.
47. Schaue, D., et al., *Maximizing tumor immunity with fractionated radiation.* Int J Radiat Oncol Biol Phys, 2012. **83**(4): p. 1306-10.
48. Vanpouille-Box, C., et al., *DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity.* Nat Commun, 2017. **8**: p. 15618.
49. Lee, Y., et al., *Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment.* Blood, 2009. **114**(3): p. 589-95.
50. Young, K.H., et al., *Optimizing Timing of Immunotherapy Improves Control of Tumors by Hypofractionated Radiation Therapy.* PLoS One, 2016. **11**(6): p. e0157164.
51. Twyman-Saint Victor, C., et al., *Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer.* Nature, 2015. **520**(7547): p. 373-7.
52. Fuertes, M.B., et al., *Host type I IFN signals are required for antitumor CD8+ T cell responses through CD8{alpha}+ dendritic cells.* J Exp Med, 2011. **208**(10): p. 2005-16.
53. Marciscano, A.E., et al., *Elective Nodal Irradiation Attenuates the Combinatorial Efficacy of Stereotactic Radiation Therapy and Immunotherapy.* Clin Cancer Res, 2018. **24**(20): p. 5058-5071.

54. Pike, L.R.G., et al., *The Impact of Radiation Therapy on Lymphocyte Count and Survival in Metastatic Cancer Patients Receiving PD-1 Immune Checkpoint Inhibitors.* Int J Radiat Oncol Biol Phys, 2019. **103**(1): p. 142-151.
55. Luke, J.J., et al., *Safety and Clinical Activity of Pembrolizumab and Multisite Stereotactic Body Radiotherapy in Patients With Advanced Solid Tumors.* J Clin Oncol, 2018: p. Jco2017762229.
56. Bang, A., et al., *Multicenter Evaluation of the Tolerability of Combined Treatment With PD-1 and CTLA-4 Immune Checkpoint Inhibitors and Palliative Radiation Therapy.* Int J Radiat Oncol Biol Phys, 2017. **98**(2): p. 344-351.
57. von Reibnitz, D., et al., *Safety of combining thoracic radiation therapy with concurrent versus sequential immune checkpoint inhibition.* Adv Radiat Oncol, 2018. **3**(3): p. 391-398.
58. Hwang, W.L., et al., *Clinical Outcomes in Patients With Metastatic Lung Cancer Treated With PD-1/PD-L1 Inhibitors and Thoracic Radiotherapy.* JAMA Oncol, 2018. **4**(2): p. 253-255.
59. Verma, V., et al., *Safety of Combined Immunotherapy and Thoracic Radiation Therapy: Analysis of 3 Single-Institutional Phase I/II Trials.* Int J Radiat Oncol Biol Phys, 2018. **101**(5): p. 1141-1148.
60. Lesueur, P., et al., *Safety of combined PD-1 pathway inhibition and radiation therapy for non-small-cell lung cancer: A multicentric retrospective study from the GFPC.* Cancer Med, 2018. **7**(11): p. 5505-5513.
61. Miyamoto, S., et al., *Nivolumab and stereotactic radiation therapy for the treatment of patients with Stage IV non-small-cell lung cancer.* Jpn J Clin Oncol, 2019. **49**(2): p. 160-164.
62. Mohamad, O., et al., *Safety and efficacy of concurrent immune checkpoint inhibitors and hypofractionated body radiotherapy.* Oncoimmunology, 2018. **7**(7): p. e1440168.
63. Hubbeling, H.G., et al., *Safety of Combined PD-1 Pathway Inhibition and Intracranial Radiation Therapy in Non-Small Cell Lung Cancer.* J Thorac Oncol, 2018. **13**(4): p. 550-558.
64. Chen, L., et al., *Concurrent Immune Checkpoint Inhibitors and Stereotactic Radiosurgery for Brain Metastases in Non-Small Cell Lung Cancer, Melanoma, and Renal Cell Carcinoma.* Int J Radiat Oncol Biol Phys, 2018. **100**(4): p. 916-925.
65. Colaco, R.J., et al., *Does immunotherapy increase the rate of radiation necrosis after radiosurgical treatment of brain metastases?* J Neurosurg, 2016. **125**(1): p. 17-23.
66. Martin, A.M., et al., *Immunotherapy and Symptomatic Radiation Necrosis in Patients With Brain Metastases Treated With Stereotactic Radiation.* JAMA Oncol, 2018. **4**(8): p. 1123-1124.
67. Shaverdian, N., et al., *Previous radiotherapy and the clinical activity and toxicity of pembrolizumab in the treatment of non-small-cell lung cancer: a secondary analysis of the KEYNOTE-001 phase 1 trial.* Lancet Oncol, 2017. **18**(7): p. 895-903.
68. Tang, C., et al., *Ipilimumab with Stereotactic Ablative Radiation Therapy: Phase I Results and Immunologic Correlates from Peripheral T Cells.* Clin Cancer Res, 2017. **23**(6): p. 1388-1396.
69. Welsh, J.W., et al., *Phase II Trial of Ipilimumab with Stereotactic Radiation Therapy for Metastatic Disease: Outcomes, Toxicities, and Low-Dose Radiation-Related Abscopal Responses.* Cancer Immunol Res, 2019. **7**(12): p. 1903-1909.
70. Srivastava, A. and J. Huang, *The Impact of the Timing of PD-1 Inhibition on Disease Control for Brain Metastases Treated with Stereotactic Radiosurgery.* International Journal of Radiation Oncology • Biology • Physics, 2017. **99**(2): p. E111.
71. Theelen, W., et al., *Effect of Pembrolizumab After Stereotactic Body Radiotherapy vs Pembrolizumab Alone on Tumor Response in Patients With Advanced Non-Small Cell Lung Cancer: Results of the PEMBRO-RT Phase 2 Randomized Clinical Trial.* JAMA Oncol, 2019.
72. Gomez, D.R., et al., *Local Consolidative Therapy Vs. Maintenance Therapy or Observation for Patients With Oligometastatic Non-Small-Cell Lung Cancer: Long-Term Results of a Multi-Institutional, Phase II, Randomized Study.* J Clin Oncol, 2019. **37**(18): p. 1558-1565.
73. Iyengar, P., et al., *Consolidative Radiotherapy for Limited Metastatic Non-Small-Cell Lung Cancer: A Phase 2 Randomized Clinical Trial.* JAMA Oncol, 2018. **4**(1): p. e173501.
74. Bauml, J.M., et al., *Pembrolizumab After Completion of Locally Ablative Therapy for Oligometastatic Non-Small Cell Lung Cancer: A Phase 2 Trial.* JAMA Oncol, 2019.
75. McCall, N.S., A.P. Dicker, and B. Lu, *Beyond Concurrent Chemoradiation: The Emerging Role of PD-1/PD-L1 Inhibitors in Stage III Lung Cancer.* Clin Cancer Res, 2018.
76. Durm, G.A., et al., *Phase II trial of concurrent chemoradiation with consolidation pembrolizumab in patients with unresectable stage III non-small cell lung cancer: Hoosier Cancer Research Network LUN 14-179.* Journal of Clinical Oncology, 2018. **36**(15_suppl): p. 8500-8500.
77. Brooks, E.D. and J.Y. Chang, *Time to abandon single-site irradiation for inducing abscopal effects.* Nat Rev Clin Oncol, 2019. **16**(2): p. 123-135.

78. McGee, H.M., et al., *Stereotactic Ablative Radiation Therapy Induces Systemic Differences in Peripheral Blood Immunophenotype Dependent on Irradiated Site.* Int J Radiat Oncol Biol Phys, 2018. **101**(5): p. 1259-1270.
79. Trommer, M., et al., *Abscopal Effects in Radio-Immunotherapy-Response Analysis of Metastatic Cancer Patients With Progressive Disease Under Anti-PD-1 Immune Checkpoint Inhibition.* Front Pharmacol, 2019. **10**: p. 511.
80. Garelli, E., et al., *Abscopal effect in lung cancer: three case reports and a concise review.* Immunotherapy, 2019. **11**(17): p. 1445-1461.
81. Peters, S., et al., *Safety evaluation of nivolumab added concurrently to radiotherapy in a standard first line chemo-radiotherapy regimen in stage III non-small cell lung cancer-The ETOP NICOLAS trial.* Lung Cancer, 2019. **133**: p. 83-87.
82. Lin, S.H., et al., *Phase II Trial of Concurrent Atezolizumab With Chemoradiation for Unresectable NSCLC.* J Thorac Oncol, 2019.