Systemic Immunostimulatory Effects of Radiation Therapy Improves the Outcomes of Patients With Advanced NSCLC Receiving Immunotherapy

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Abstract: The understanding of localized radiation therapy's immunostimulatory properties combined with its well-known effects on the cell cycle and insights into the immunomodulation mechanisms that occur at the molecular and cellular levels has changed our traditional view of the anticancer effects of ionizing radiation. The potential interactions between the tumor's immune system and radiation therapy have revealed that local radiation has the ability to induce systemic antitumor responses in patients with advanced cancers. The recognition of systemic antitumor effects of radiation therapy has allowed investigators to begin uncovering the integral players in these pathways. Parallel to this, there has been progress in understanding how tumor immunology leads to the development of novel immunotherapies using immune checkpoint blockade therapies in the treatment of advanced cancers. To date there has been limited success in this benefiting only a small fraction of patients. The concept of priming the body's immune system by radiation to make less responsive tumors more responsive to immunotherapy provides an opportunity to explore the use of the combination of radiation therapy and immunotherapy for the treatment of advanced non–small cell lung cancer and other cancers. This article provides an overview of the current state of knowledge of the clinical experience using radiation therapy in combination with immune therapy and discusses the rationale for integrating these 2 modalities in the treatment of advanced non–small cell lung cancer. Available data supports the use of radiation therapy in combination with immunotherapy to achieve improved local and systemic tumor control. Evidence from the early clinical trials has shown that using radiation therapy and immune checkpoint blockade therapies together produces a greater clinical effect than using either modality alone. To maximize the clinical benefit and successful integration of these two modalities as well as optimizing radiation therapy dosing and its schedule, improvement in its field design and the development of reliable predictors of clinical tumor response needs to be established.

Key Words: antitumor effects, immune checkpoint inhibitors, immunogenic cell death, immunotherapeutic agents, immunostimulation, ionizing radiation, metastasis, non–small cell lung cancer, tumor microenvironment

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Since Roentgen's discovery of x-rays over a century ago, radiation has been used in the treatment of a wide variety of malignancies. Currently radiation therapy has become an integral component of the all-anticancer treatments. Over 60% of all newly diagnosed cancer patients will receive radiation therapy with curative intent or in a palliative setting.[1–4] Whether radiation therapy is used in a definitive, neoadjuvant, adjuvant or palliative setting, it is primarily a locoregional treatment, but systemic effects of radiation therapy have been observed as well.[5–8] However, these treatment outcomes still needs to be improved in the various types of malignant tumors especially those with high locoregional failure rates, a high risk for metastatic spread or invasive growth, and a high risk for normal tissue complications, such as lung cancer.

Radiation therapy is effective against local tumor growth and is delivered in multiple small daily fractions to achieve a maximum benefit to destroy the malignant tumor cells while sparing the normal tissues. However, innovations in radiation treatment devices, imaging techniques, and computerized treatment planning systems have allowed the delivery of highly conformal radiation beams with increased precision to the targeted tumor tissue while sparing the adjacent healthy tissue.[4,9,10] In addition, the advent of sophisticated imaging methods together with novel radiation delivery devices has led to the development of 3-dimensional conformal radiation therapy, which uses 3-dimensional based beams to deliver higher doses of radiation. Rapid progress in this field continues to be boosted by advances in radiation techniques such as intensity-modulated radiation therapy (IMRT) and image-guided radiation therapy (IGRT). These developments have transformed the delivery of radiation and broadened its range of clinical applications leading to an improved understanding of the radiobiology of radiation therapy.

The use of radiation as a standard therapeutic modality for malignant tumors is based on its ability to locally damage cellular macromolecules, particularly DNA. Thus, radiation exposure effectively causes growth arrest and cell death in irradiated tumors leading to tumor regression and causes the elimination of the tumor locally. There is accumulating evidence that local radiation-induced tumor cell destruction can also trigger the activation of the immune system through damage-signaling cascades, immunogenic cell death, or both leading to systemic antitumor effects on distant nonirradiated tumor sites. These findings have led to a paradigm shift in the use of radiation therapy for the treatment of various malignant tumors. With the latest emergence of novel immunotherapeutic agents for cancer treatment, there has been a renewed interest in exploring the effectiveness of radiation therapy in combination with immunotherapy. In this article, we provide an overview of the role of radiation therapy used in combination with immunotherapies in the management of advanced non–small cell lung cancer (NSCLC).

DIRECT EFFECT OF RADIATION THERAPY ON TUMOR CELL

The central dogma of traditional radiation biology states that the antitumor effects of radiation therapy are primarily due to the absorption of energy from x-rays or γ-rays, or from charged particles that may be ionized or excited within the cells, initiating a chain of actions that leads to tumor cell

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elimination.[11] In this process, the direct radiation effect occurs to the tumor cells in the pathway of the radiation beam. More specifically, direct radiation action results in the destruction of the double-stranded DNA followed by some form of tumor cell death, including apoptosis, necrosis, autophagy, mitotic catastrophe, or replicative senescence (Fig. 1). Thus, DNA is the main target of radiation within tumor cells. The so-called lethal effect of radiation is caused by the direct action of the radiation beam on the DNA lead to cellular damage.[12] Accordingly, DNA damage and subsequent tumor cell death have been ascribed to 5 basic principles known as the 5 "R's" of radiation biology. These include repair, repopulation, redistribution, reoxygenation, and radiosensitivity, which are critical to achieving the therapeutic goal of tumor control in radiation therapy.[13] In addition, radiation exposure may also act indirectly by interacting with other molecules in the cell, primarily water (H_2O) resulting in radiolysis of water, to produce free radicals (OH^- and H^+) that can tigger a cascade of events with different targets.[14,15] This indirect action of radiation exposure produces sublethal effects leading to immunogenic tumor cell death. Thus, radiation therapy exerts antitumor responses through several biological mechanisms and achieves its therapeutic effects by inducing different types of tumor cell death.[16]

IMMUNOSUPPRESSIVE EFFECT OF RADIATION THERAPY

Ionizing radiation stimulates changes in the tumor's microenvironment and induces the tumor cell death, which in turn can either elicit protective antitumor effects through immunostimulation or activate immunosuppressive immune responses, which can hamper its therapeutic benefits.[17,18] In rare circumstances, radiation therapy can contribute to the anti-immunogenic micro milieu by recruiting various inhibitory immune cells that directly promote tumor growth and contributes to an immunosuppressive environment by making the T-cells dysfunctional.[19] More specifically, radiation-induced immunosuppression is frequently accompanied by the recruitment of immunosuppressive cells such as myeloid-derived suppressor cells, tumor-associated macrophages, T regulatory cells (Tregs), and by the release of immunosuppressive cytokines and chemokines. Treg cells are a subset of $CD4^+$ T cells critical for regulation of inflammation and autoimmunity, which accumulate in tumor microenvironments. They secrete the cytokines transforming growth factor-β and interleukin (IL)-10, suppressing effector T-cell activation and stimulating suppressive myeloid-derived suppressor cell functions.[18,19] In response to localized radiation, the volume of Treg cells increases in tumors, which causes tumor cell resistance to radiation. Furthermore, radiation induces upregulation of the immune checkpoint pathways, particularly an upregulation of programmed death-ligand 1 (PD-L1) expression on tumor cells which in turn blocks the function of activated natural killer T cells against tumor cells leading to cancer immune escape.[18,20] Through the interference of these mechanisms especially the immune checkpoint blockade pathway this therapy can provide a promising strategy to enhance antitumor responses through the activation of natural killer T-cell mediated tumor cell death.

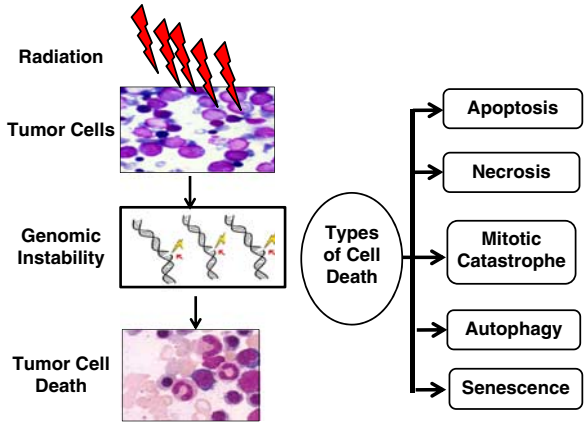

FIGURE 1. Various modes of cancer cell death induced by radiation therapy. full color online

RADIATION THERAPY-INDUCED IMMUNOGENIC CELL DEATH

The traditional view of radiation therapy-induced tumor cell death is limited in the context of the host antitumor immunity and tumor microenvironment. With a new understanding of radiation biology, a newly defined form of cellular demise through the mechanism termed "immunogenic cell death" has been proposed as a contributor to the "in-field" response of the tumor to radiation therapy.[21] Evidence suggests that local radiation exposure not only directly affects tumor cells causing its death but also modulates the complex biological interactions between the tumor and tissue stroma in which they grow, known as the tumor cell microenvironment. It is evident that responses that are triggered within the tumor cell microenvironment are critical in determining the effectiveness of the treatment.[22] Thus, understanding the effects of radiation on the functional status of the tumor cell microenvironment is critical for maximizing the therapeutic effects of radiation therapy.

There is now increasing evidence of the complex interplay between radiation therapy and the immune system that leads to systemic tumor responses through immunogenic cell death. The immunogenic cell death involves the recruitment of the host's immune system as a contributor to the "in-field" response to radiation therapy, thereby resulting in immune memory and advantageous systemic effects.[23] As illustrated in Figure 2, radiation-damaged dying and stressed tumor cells liberate multiple signaling molecules including tumor antigens, chemokines, inflammatory cytokines, and others and the production of damage-associated molecules. The liberation of tumor antigens acts as proinflammatory mediators, stimulating monocyte production of the cytokines such as tumor necrosis factor, IL-1, IL-6, and IL-8. These signaling molecules facilitate the maturation of dendritic cells and modulate the immune system through the activation of cytotoxic T-lymphocytes (T cells) that travel to nonirradiated tumor sites leading to systemic tumor response (Fig. 2). Radiation-induced immunogenic cell death is characterized, in part, by activation of the endoplasmic reticulum stress pathway and tumor cell surface expression of calreticulin, a prophagocytic molecule, facilitating antigen-presenting cell uptake of the tumor antigens.[24] In addition, passive secretions of high mobility group box 1, an evolutionarily conserved nuclear protein, by tumor cells undergoing late apoptosis or necrosis can bind the toll-like receptor-4, the lipopolysaccharide receptor, on dendritic cells and lead to dendritic cells maturation and effective T-cell priming.[25] Moreover, cellular radiation has also been shown to promote effector T-cell recruitment into the tumor through chemokine induction and upregulation of major histocompatibility complex class-1 protein expressions in tumor cells, and even elicit systemic tumor-specific immune responses leading to the regression of

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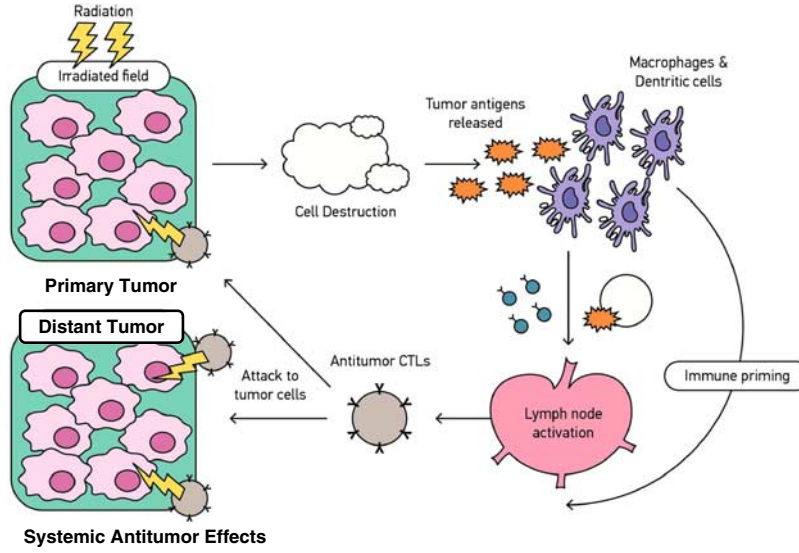

FIGURE 2. Schematic illustration of the mechanisms involving radiation therapy produced systemic antitumor effects after radiation of a malignant tumor including non–small cell lung cancer. Localized radiation causes tumor cell destruction and initiation of the immune process by liberating tumor antigens and producing damage-associated molecules, which lead to the maturation of dendritic cells and the improved priming and activation of effector cytotoxic T lymphocytes. Tumor antigens also act as proinflammatory mediators, stimulating monocyte production of the cytokines such as tumor necrosis factor, interleukin-1 (IL-1), IL-6, and IL-8. These cytokines together with the activated cytotoxic T-lymphocytes facilitate tumor cell elimination by attacking not only the tumor bulk in the irradiated area but also travel to distant nonirradiated tumor sites including any metastatic tumor sites and promote tumor regression or tumor elimination through the systemic antitumor effects of radiation therapy. full color online

nonirradiated tumors.[26,27] Collectively, these immune response pathways triggered by radiation exposure promote tumor cell elimination by primed T cells.

The relative importance of radiation therapy-induced alterations in the tumor cells microenvironment is still unclear, however, it is believed that the immune system senses this damage and triggers immune response pathways resulting in several areas of systemic stimulation. Evidence shows that radiation therapy can produce epitope spreading which can boost the diversity of the T-cell receptors repertoire of intratumoral T cells leading to widespread systemic antitumor effects. However, such systemic antitumor responses to radiation therapy alone have only occasionally been observed in cancer patients, presumably because the tumor microenvironment efficiently shapes tumor immune escape at multiple levels and thus hampers beneficial radiation therapy-induced immune stimulation.[28] Because of the limited success of conventional therapies in patients with resistant and metastatic tumors, current clinical studies have focused on combining radiation therapy with immunotherapy, particularly immune checkpoint inhibition, to overcome these limitations and harness the combined therapeutic potential of both therapies. There is a growing consensus from many studies that indicate combining radiation therapy with immunotherapy provides an opportunity to boost the systemic antitumor response rates.

RATIONAL FOR INTEGRATION OF RADIATION THERAPY AND IMMUNOTHERAPY

The advent of immunotherapy has revolutionized cancer treatment. Significant progress has been witnessed recently in the field of immuno-oncology with the invention of immune checkpoint inhibitors that regulate key immunosuppressive pathways of tumor cells. The incorporation of immunotherapies into the cancer treatment armamentarium has resulted in significant improvements in survival outcomes of patients with advanced cancer. Current targets of checkpoint blockades are cytotoxic T-lymphocyte associated protein-4 (CTLA-4) and programmed cell death-1 (PD-1) molecules crucial for the peripheral T-cell tolerance induced by antigen-presenting cells.

CTLA-4 is a transmembrane protein receptor expressed in T cells. James P. Allison[29] received the 2018 Nobel Prize in Medicine for discovering CTLA-4, the first coinhibitory receptor on T cells. CTLA-4 is expressed on T helper and Tregs.[30] It competes for the binding of the ligands (CD80 and CD86) that provide a costimulatory signal when bound to CD28 expressed on T cells. CTLA-4 acts as an immune checkpoint and down-regulates the immune responses by affecting the initial priming phase of the naive T-cell activation. It is transported to the surface when the T-cell receptor recognizes an antigenic peptide in association with the major histocompatibility complex class-1 of the antigen-presenting cells. The higher affinity of CTLA-4 inhibits T-cell proliferation by outcompeting its receptors for ligand binding. T-cell immune tolerance mediated by CTLA-4 can also be achieved with the production of cytokines such as transforming growth factor-β in Tregs.[31] By blocking the CTLA-4 axis T cell responses can be amplified directly and indirectly. Thus, CTLA-4 plays a very dominant function in the stoppage of T cells that are potentially autoreactive at the initial stages of T-cell activation.

The other key immune checkpoint inhibitory receptor is PD-1, which was originally discovered by a Japanese immunologist, Tasuku Honjo who received the Nobel Prize jointly with James P. Allison in 2018. PD-1 is expressed on the surface of T cells and B cells and binds to PD-L1. There are 2 ligands known as PD-L1 and PD-L2 that are abundantly expressed on hematopoietic and non-hematopoietic cells. The principal role of the PD-1/PD-L1 axis is to limit the response of effector T cells and also the immune-mediated tissue damage. PD-1 signaling in tumor-infiltrating lymphocytes

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contributes to T-cell exhaustion and tumors are known to upregulate the PD-1 ligand PD-L1 to exploit this pathway.[32] Since the PD-1 immune checkpoint signaling pathway directly plays a role in regulating immune responses of tumor-infiltrating lymphocytes in the tumor microenvironment, it is an ideal target for immune checkpoint inhibitors.

The development of Immune checkpoint inhibitors is a revolutionary milestone in the field of immuno-oncology. In fact, anti-CTLA-4 and anti-PD-1/PD-L1 interventions have paved the way for immuno-oncology as a field. Immune checkpoint inhibitors reinvigorate antitumor immune responses by interrupting coinhibitory signaling pathways and promote immune-mediated elimination of these tumor cells. In the clinical setting, the checkpoint blockade therapies using monoclonal antibodies targeting CTLA-4 and PD-1 pathways resulted in improved survival outcomes in patients with advanced solid tumors including NSCLC. To date, in the United States, nivolumab, pembrolizumab, atezolizumab, and durvalumab have been approved by the Food and Drug Administration as a treatment option for pretreated patients with advanced NSCLC and other solid tumors. Pembrolizumab is also approved as a first-line treatment for advanced NSCLC patients with $>50\%$ PD-L1 expression.[33] Thus, immunotherapy has represented a paradigm shift in the treatment of patients with advanced NSCLC.

Despite significant advances in the development of anti-CTLA-4 and anti-PD-1/PD-L1 therapies as standalone therapeutic interventions, the improvement in survival outcomes in advanced NSCLC was found in only a small fraction ($<20\%$) of patients, even when selection strategies aimed at enriching for potential responders were employed.[34] In fact, immunotherapy as monotherapy was expected to result in significant benefits for only a subset of patients, especially those with high tumor mutational burden.[35] Thus, various approaches have been explored to improve the efficacy of immunotherapy with immune checkpoint blockers in patients with advanced NSCLC, including the development of combinatorial regimens involving radiation therapy.[36]

There is a strong rationale for combining radiation therapy and immune checkpoint blockade therapies. Radiation therapy-induced cancer cell damage releases tumor-specific antigens that make them visible to the immune surveillance and promotes the priming and activation of cytotoxic T cells. Radiation therapy-induced modulation of the tumor microenvironment may also facilitate the recruitment and infiltration of immune cells. However, Tregs are more radioresistant than other T cells, consequently increased after radiation therapy.[37] Naturally, an important role of Tregs is to maintain immune tolerance, even in tumorous condition. CTLA-4 is reported to be a key target to control the suppressive function of Tregs.[38] This forms the rationale for combining radiation therapy with immune checkpoint blockade therapies to overcome tumor immunity. When administered according to precise dose and fractionation schedules, radiation therapy has the ability to produce robust immunostimulatory effects that may be harnessed to boost the efficacy of Immune checkpoint blockade therapies and other forms of immunotherapy.[39,40] Clinical evidence is accumulating demonstrating the synergistic effects of radiation therapy when combined with immune checkpoint blockade therapies in patients with advanced solid tumors including lung cancer.

BOOSTING THE IMMUNOSTIMULATORY EFFECTS IN LUNG CANCER

Lung cancer is the second most common cancer in the United States as well as in the world and is the leading cause of cancer-related deaths.[41,42] Despite significant advances, no significant changes in 5-year survival rates have been achieved during the last 3 decades. NSCLC accounts for up to 85% of all lung cancer cases,[43,44] and roughly $>70\%$ of patients present with locally advanced or disseminated disease at the time of diagnosis and are not appropriate candidates for surgery.[45] Most NSCLC patients present with locally advanced inoperable or metastatic disease, which in the past made their cancer incurable, and almost all of these patients died from their disease. The management of patients with metastatic NSCLC remains challenging, as long-term clinical outcomes are generally poor. Radiation therapy remains an important and potentially curative treatment for localized and locally advanced NSCLC patients who are not amenable to surgery. Over the past few years, results from multiple clinical trials demonstrated that immunotherapy with immune checkpoint blockade therapies administered with radiation therapy constitutes a promising approach for advanced NSCLC (Table 1).

Traditionally radiation therapy has been combined with chemotherapy to improve the local antitumor effects. Immunotherapies could be an alternative and more effective systemic anticancer treatment. Given the immunostimulatory effects of radiation therapy, the survival outcomes of cancer patients can be further improved when radiation is combined with immunotherapy. There is growing recognition of the increasingly complex interplay between radiation therapy and the immune system. Therefore, it is crucial to exploit the potential effects of the combination of radiation therapy with immune checkpoint inhibitors in patients with advanced solid tumors including NSCLC. The current treatment approaches using chemoradiation therapies provide only a modest improvement in the survival endpoints including freedom from progression, recurrence, and/or metastasis. By having a synergistic approach of radiation therapy combined with immunotherapy, there is the potential to improve the overall survival outcomes in advanced NSCLC patients.

CLINICAL EXPERIENCE WITH THE COMBINED THERAPY IN ADVANCED NSCLC

A growing body of evidence is emerging from ongoing clinical studies supporting the combination of radiation therapy with immunotherapy for the treatment of advanced NSCLC. These emerging clinical studies have been utilizing either concurrent or sequential radiation therapy in combination with immune checkpoint blockade therapies in patients with advanced NSCLC (Table 1).

Recently, Formenti et al[46] have reported on the outcomes of a phase II clinical trial evaluating the combination of local radiation therapy and ipilimumab, an immune checkpoint blocker targeting CTLA-4, in patients with metastatic NSCLC, a setting in which ipilimumab alone had limited efficacy. The study included 39 patients aged 18 years or older who had at least 2 distinct measurable metastatic sites. The patients were required to have an Eastern Cooperative Oncology Group performance status of ≤ 2 and a life expectancy of >3 months with adequate organ and marrow function. The primary endpoint of the study was the induction of immune-mediated systemic tumor response as evidenced by the synergistic effects of radiation therapy with Ipilimumab. The radiation therapy regimen consisted of 6 Gy delivered in 5 daily fractions in the phase I portion of the study, and 9.5 Gy delivered in 3 daily fractions in the phase II portion of the study. Radiation therapy was delivered by external-beam with a linear accelerator with IGRT and IMRT techniques. Ipilimumab was given intravenously after the first radiation treatment, at a dose of 3 mg/kg and repeated every 3 weeks for 4 cycles.

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TABLE 1. Summary of Clinical Studies of Radiation Therapy in Combination With Immunotherapy in Advanced NSCLC

Study Type	Patients (N)	Treatment Approach	RT Target	RT (Gy/ Fraction)	IT Agent	IT Dose	LCR (CR+PR+SD)	DCR Rate CR+PR +SD)	Median PFS (mo)	Median OS (mo)	References
Phase I to II	39	Concurrent	NR	28.5 to 30/ 3 to 5	IPI	3 mg/kg/3 wk	NR	31% (abscopal)	3.8	13	Formenti et al[46]
Phase II	74	Sequential	NR	24/3	PEM	200 mg q3w	NR	41%	6.4	NR	Theelen et al[47]
Phase I	79 Lung: 7	Sequential	Lung, liver, bone, abdomen	30.50/3.5	PEM	200 mg q3w	NR	Distant: 26.9% Systemic: 13%	3.1	9.6	Luke et al[48]
Phase I	35 Lung: 14	Concurrent sequential	Lung, liver	50/4	IPI	3 mg/kg/3 wk	90%	42%	3.2	10.2	Tang et al[49]
Phase II	100	Concurrent sequential	Lung, liver	50/4	IPI	3 mg/kg/3 wk	NR	67%	5	12	Welsh et al[50]
Phase II	12	Concurrent	Lung	30/5	IPI	3 mg/kg/3 wk	NR	44.4%	NR	NR	Golden et al[51]
Phase I to II	24 Lung: 19	Concurrent	NR	30/3	PEM	200 mg q3w	Ongoing with continued response	NR	NR	NR	Campbell et al[52]
Phase Ib	13	Sequential	Various	20/5	IL-2	Escalating doses	NR	NR	2.9	8.6	Van den Heuvel et al[53]
RS	1736	NR	NR	NR	NR	NR	NR	NR	4.6	12.4	Chicas-Sett et al[54]
RS	2084	NR	Intracranial	18.50/1.5	NR	NR	NR	NR	NR	18.2	Foster et al[55]
RS	37	Concurrent sequential	Lung, brain	NR	PEM NIV ATE	NR	Concurrent 100% Sequential 72.3%	Concurrent 38.5% Sequential 65.8%	NR	NR	Schapira et al[56]
RS	42	Sequential	Thoracic	NR	PEM	2 or 10 mg/kg q3w; 10 mg/kg q2w	NR	NR	4.4	10.7	Shaverdian et al[57]
RS	20 Lung: 17	Concurrent	Intracranial	5.10 SRS: 18.40/1.5	NIV	NR	NR	50%	7	12.5	Desideri et al[58]
RS	260; Lung 157	Concurrent (n = 28) sequential (n = 51)	Intracranial	15.25/1.5	Anti-CTLA-4 anti-PD-1	NR	Concurrent: 1 y-LCR: 88% Sequential: 1 y-LCR: 79%	NR	2.3	Concurrent 24.7 Sequential 14.5	Chen et al[59]
RS	104	Various	Various	NR	NIV	NR	NR	NR	2.7	11.1	Lesueur et al[60]

ATE indicates atezolizumab; CR, complete response; CTLA-4, cytotoxic T-lymphocyte associated antigen 4; DCR, distant control rate; IL-2, interleukin-2; IPI, ipilimumab; IT, immunotherapy; LCR, local control rate; NIV, nivolumab; NR, not reported; NSCLC, non–small cell lung cancer; OS, overall survival; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand; PEM, pembrolizumab; PFS, progression-free survival; PR, partial response; RS, retrospective; RT, radiation therapy; SD, stable disease.

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Of the 39 patients who had progressed after at least 1 line of prior systemic therapy, 2 (5%) achieved a radiographic complete response, 5 (13%), a partial response, and 5 (13%) had stable disease. Thus, a radiologic overall response rate was seen in 18% of the patients and 31% of all patients experienced disease control. The magnitude of the response rate was significantly associated with survival endpoints: median overall survival was 20.4 months in the responding patients and only 3.5 months in patients who had progressive disease. Similarly, a progression-free survival of 7.1 months was seen in the responding patients and only 3.0 months in the patients who had continued progressive disease.[46] These clinical outcomes are superior to the historically reported outcomes using ipilimumab alone and suggested that the addition of radiation therapy may trigger systemic immune responses in patients with advanced NSCLC.

The PEMBRO-RT phase II randomized trial by Theelen et al[47] evaluated the clinical efficacy of pembrolizumab after stereotactic body radiation therapy (SBRT) versus pembrolizumab alone in patients with advanced NSCLC. Of the 72 randomized patients, 64 patients received at least 1 cycle of pembrolizumab at the time of the interim analysis. The overall response rate was significantly higher in patients receiving radiation therapy plus pembrolizumab compared with those who received pembrolizumab alone (41% vs. 19%). Similarly, the median progression-free survival was also significantly higher in patients receiving radiation therapy plus pembrolizumab compared with those receiving pembrolizumab alone (6.4 vs. 1.8 mo). Overall, these findings indicate that the combination of radiation therapy and immunotherapy is superior to that of pembrolizumab alone in achieving antitumor responses in patients with advanced NSCLC.

A phase I study by Luke et al[48] evaluated the clinical activity of multisite SBRT followed by pembrolizumab in patients with metastatic solid tumors including lung cancer. A total of 73 heavily pretreated patients were included in the study. Two to 4 metastasis lesions were treated with SBRT to 30 to 50 Gy delivered in 3 to 5 fractions, and pembrolizumab 200 mg every 3 weeks was initiated 7 days after completion of SBRT. Of the 151 metastasis lesions irradiated, 68 were in the lung, 24 in the liver, 28 in other abdomen/pelvis sites, 16 in the bone, and 15 near the spine. The response rate in any single nonirradiated target metastasis lesion was 26.9%. These findings support that radiation therapy combination with pembrolizumab has synergistic effects in patients with advanced cancers.

In a phase I/II prospective study, Tang et al[49] assessed concurrent treatment versus sequential ipilimumab in combination with SBRT in patients with advanced lung and liver cancers. In the phase I portion of the study, 7 of the 31 (23%) patients saw a clinical benefit, either partial response (n = 3) or stable disease (n = 4) outside the irradiated field, indicating the systemic effects. In the updated phase II study at the 2017 American Society for Radiation Oncology (ASTRO) annual meeting,[50] 100 patients had been enrolled. Of the 100 patients, 67 with advanced NSCLC achieved clinical benefits as defined as stable disease or complete/partial response. Stable disease was seen in 60% and 45% of the patients who had sequential and concurrent ipilimumab, respectively. The findings of these phases I and II studies demonstrated that both concurrent and sequential ipilimumab in combination with radiation therapy is effective in patients with advanced NSCLC.

In another phase II study, patients with chemorefractory metastatic NSCLC received anti-CTLA-4 ipilimumab within 24 hours of starting palliative radiation therapy to at least 1 metastatic lesion (30 Gy in 5 fractions), with ipilimumab repeated every 3 weeks for 4 cycles.[51] Of the 27 evaluable patients, 12 achieved a clinical benefit as defined as stable disease or a complete/partial response with overall response rates of 44.4%. Complete response was seen in 3 patients and 4 had a partial response, and 5 experienced stable disease indicating the synergistic effect of the combination therapy. Systemic immune responses were seen in all responding patients. The trial continues its planned accrual of 39 patients.

In a phase I prospective study, Campbell et al[52] evaluated the combination of SBRT with concurrent pembrolizumab in patients with advanced NSCLC and melanoma. A total 24 patients with advanced NSCLC (n = 19) and melanoma (n = 5) were included. Of the 24 patients, 9 had received prior anti-PD-1 therapy and therefore were treated with SBRT. The remaining immunotherapy naive patients (n = 15) received the combination of SBRT with concurrent pembrolizumab. The preliminary efficacy data indicate that the addition of SBRT to a single site of disease resulted in a mean of 19.8 weeks of continued response indicating the synergistic potential between the radiation therapy and the immunotherapy.

In a phase Ib study, Van den Heuvel et al[53] assessed the effects of radiation therapy delivered in combination with immunotherapy using a nonimmune checkpoint inhibitor NHS-IL2 in patients with advanced NSCLC. NHS-IL2 (selectikine) is a novel immunocytokine which consists of a human tumor necrosis-targeting antibody (NHS76) that binds to exposed DNA in necrotic regions of tumors and is fused to the genetically modified IL-2 designed to decrease vascular toxicity by signaling through the high-affinity IL-2 receptor. A total of 13 patients with NSCLC stage IV disease received radiation therapy (20 Gy delivered in 5 daily fractions) followed by escalating doses of NHS-IL2 over 3 consecutive days in a 21-day treatment cycle repeated until progression of the disease or unacceptable toxicity was seen. A median progression-free survival of 2.9 months and median overall survival of 8.6 months was achieved indicating radiation therapy in combination with NHS-IL2 therapy has the potential to induce systemic immune responses in patients with advanced NSCLC.

Recently, Chicas-Sett et al[54] using pooled data from published studies evaluated the combination of SBRT and immunotherapy in patients within advanced NSCLC. This pooled analysis included 1736 advanced NSCLC patients who were treated with the combination of SBRT and immune checkpoint inhibitors. The mean control of local and distant disease response rates were 71% and 41%, respectively. The mean progression free and overall survivals were 4.6 and 12.4 months, respectively. These findings show that SBRT in combination with immune checkpoint inhibitors achieves high rates of local control and provide a greater chance of obtaining distant responses indicating the synergistic effects of these modalities in producing systemic immune responses in patients with advanced NSCLC.

More recently, Foster et al[55] retrospectively analyzed a National Cancer Database that included stage IV NSCLC patients who received combined radiation therapy and immunotherapy and compared the outcomes with those treated with immunotherapy or chemotherapy alone. The study included patients who were treated with radiation therapy to their intracranial and/or extracranial sites. Of the 44,498 patients, 5807 (13%) had immunotherapy, and 2084 (4.7%) stereotactic radiation therapy (SRT), and the rest 20,821 (46.8%) had external-beam radiation therapy. Multivariate analysis indicated that patients receiving SRT had a longer median overall survival compared with those receiving immunotherapy (18.2 vs. 14.5 mo, $P < 0.0001$). However, patients who received external-beam radiation therapy had a shorter median overall survival

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than those receiving immunotherapy (10.9 vs. 14.5 mo, $P < 0.0001$) indicating SRT combination is associated with improved overall survival for patients with metastatic NSCLC. In subset of patients receiving SRT, the median overall survival for the addition of immunotherapy and chemotherapy was 18.2 and 14.3 months, respectively ($P = 0.004$), with immunotherapy strongly associated with an increase in overall survival (hazard ratio [HR]: 0.82, 95% confidence interval: 0.69-0.98). SRT with biologically effective dose of > 60 Gy was also independently associated with improved overall survival. The higher survival rates for patients receiving SRT plus immunotherapy suggest that higher doses of radiation therapy in combination with immunotherapy augments tumor response rates and prolongs overall survival in patients with advanced NSCLC.

Recently, a retrospective study by Schapira et al[56] assessed treatment outcomes of 37 metastatic lung cancer patients on immunotherapy (nivolumab 83.8%, atezolizumab 10.8%, pembrolizumab 5.4%) with brain metastases receiving stereotactic radiosurgery (SRS) to a total of 85 lesions. The findings demonstrate that patients treated with concurrent SRS and immune checkpoint blockers had longer overall survivals and a reduced rates of distant brain failure than those who received SRS either before or after starting immunotherapy (1 y overall survival, 87.3% vs. 70.0% vs. 0%, $P = 0.008$; 1 y distant brain failure, 38.5% vs. 65.8% vs. 100%, $P = 0.042$). In addition, the local control rates were significantly improved with the combination therapy at 1 year (100% vs. 72.3%, $P = 0.016$).

In a phase I KEYNOTE-001 (NCT01295827) study of pembrolizumab, a secondary analysis by Shaverdian et al[57] showed a synergistic effect of radiation therapy in advanced NSCLC patients. The study objective was to evaluate and establish a safety profile and the antitumor activity of radiation therapy combined with pembrolizumab. Of the 98 enrolled patients presenting with metastatic NSCLC, 42 (43%) received radiation therapy before their first cycle of pembrolizumab. These patients experienced significantly longer median progression-free survival as compared with the patients who had not received radiation therapy prior to the administration of pembrolizumab (4.4 vs. 2.1 mo, $P = 0.019$). The 6-month progression-free survival rate was also higher in those patients who had received radiation therapy prior to pembrolizumab than those who did not (49% vs. 23%). In addition, those patients who received both radiation therapy and pembrolizumab experienced an improved overall survival compared with those who did not receive prior radiation therapy (10.7 vs. 5.3 mo, $P = 0.026$). Similarly, the 6-month overall survival rate was also higher in those patients who had received radiation therapy before pembrolizumab than those who did not receive it (73% vs. 45%). These findings indicate how the synergistic effects of radiation therapy and immunotherapy improves both the local and distant tumor control in patients with advanced NSCLC.

Desideri et al[58] recently reported findings of a retrospective analysis that included 20 patients with advanced NSCLC (n = 17) and renal cell carcinoma (n = 3) who received concomitant radiation therapy and nivolumab. Nivolumab, a fully human immunoglobulin G4 PD-1 immune checkpoint inhibitor antibody, blocks PD-1 and promotes antitumor immunity. Radiation therapy was administered either as an ablative therapy in the oligometastatic/oligoprogressive setting or as a palliative-only treatment for symptomatic patients. The median progression-free survival and overall survival times were 7.0 and 12.5 months in the entire group, respectively. Oligoprogressive patients treated with ablative intent, compared with patients undergoing radiation therapy with palliative-only intent, had a statistically longer overall survival (17.9 vs. 10.3 mo, HR: 0.41, 95% confidence interval: 0.16 to –1.02, $P = 0.04$). These findings suggest that higher doses of radiation therapy in combination with nivolumab produce synergistic effects in patients with advanced NSCLC.

Chen et al[59] retrospectively assessed the effect of concurrent SRS-SRT and immune checkpoint inhibitors on patients' outcomes and its safety in NSCLC/melanoma/kidney cancer patients with brain metastases. Of the 260 total patients who received SRS-SRT, 157 had advanced NSCLC with brain metastases. Of the 260 patients, 181 were treated with SRS-SRT alone, and 79 received SRS-SRT in combination with immune checkpoint inhibitors, 35% were treated with concurrent SRS-SRT and immune checkpoint inhibitors. The median overall survival for patients receiving SRS-SRT, SRS-SRT with nonconcurrent immune checkpoint inhibitors, and SRS-SRT with concurrent immune checkpoint inhibitors were 12.9, 14.5, and 24.7 months, respectively. Multivariate analysis showed that SRS-SRT in combination with concurrent immune checkpoint inhibitors was associated with improved overall survival compared with SRS-SRT alone ($P = 0.002$; HR: 2.69) and compared with nonconcurrent SRS-SRT and immune checkpoint inhibitors ($P = 0.006$; HR: 2.40). The overall survival benefit of concurrent SRS-SRT and immune checkpoint inhibitors was significantly improved in comparison with those patients treated with SRS-SRT before immune checkpoint inhibitors ($P = 0.002$; HR: 3.82) or after immune checkpoint inhibitors ($P = 0.021$; HR: 2.64). Overall, these findings suggest that delivering SRS-SRT in combination with concurrent immune checkpoint inhibitors is associated with a decreased incidence of new metastasis lesions and improved survival outcomes.

In phase I prospective study, Campbell et al[52] evaluated the combination of SBRT with concurrent pembrolizumab in patients with advanced NSCLC and melanoma. A total 24 patients with advanced NSCLC (n = 19) and melanoma (n = 5). Of the 24 patients, 9 had received prior anti-PD-1 therapy and therefore were treated with SBRT. The remaining immunotherapy naive patients (n = 15) received the combination of SBRT with concurrent pembrolizumab. The preliminary efficacy data indicate that the addition of SBRT to a single site of disease resulted in a mean of 19.8 weeks of continued response indicating the synergistic potential between the radiation therapy and the immunotherapy.

Lesueur et al[60] reported on the outcomes of a retrospectively study involving a large series of advanced NSCLC patients who received radiation therapy during the 6 months preceding concomitant, or 3 months after nivolumab administration. A total of 104 patients with advanced NSCLC were included in the study. The overall survival rates at 1 and 2 years were 48.8% and 29.1%, respectively. Similarly, the progression-free survival rates at 1 and 2 years were 20.9% and 10.1%, respectively. The median overall survival and progression-free survival was 11.1 and 2.7 months, respectively. However, these investigators concluded that delivering radiation before or during or after nivolumab administration was not associated with improved survival.

ONGOING CLINICAL TRIALS OF THE COMBINATION THERAPY IN ADVANCED NSCLC

Promising clinical and preclinical data from the combined approach using radiation therapy and immunotherapy has led to a number of phases I to II clinical trials in patients with advanced NSCLC. Currently, a number of ongoing or planned clinical trials are investigating radiation therapy combined with immune checkpoint blockade therapies (Table 2). Different approaches using a combination of induction, sequential, or concurrent therapies are being evaluated in these trials (Fig. 3). These clinical trials have also adopted various radiation

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TABLE 2. Ongoing Clinical Trials of Radiation Therapy in Combination With Immunotherapy in Advanced NSCLC

NCT ID	Phase	Patients	Radiation	Approach	Radiation Dose	IT Agent	Location/Country
NCT03391869	III	270	RT	Induction	NP	Ipilimumab plus nivolumab	MD Anderson Cancer Center, USA
NCT03313804	II	57	SBRT	Concurrent	SBRT: BED > 100 Gy, 3D-CRT: 30 Gy	Nivolumab/pembrolizumab/ atezolizumab	University of Kentucky, USA
NCT03004183	II	57	SBRT	Sequential	30 Gy/5 fx	Pembrolizumab	Houston Methodist Cancer Center, USA
NCT02658097	II	48	RT	Concurrent	8 Gy/1 fx	Pembrolizumab	Case Comprehensive Cancer Center, USA
NCT03644823	II	30	RT	Concurrent	18 Gy/3 fx	Atezolizumab	Oslo University, Norway
NCT02839265	II	29	SBRT	Concurrent	NP	FLT3 ligand	Albert Einstein College of Medicine, USA
NCT02492568	II	92	SBRT	Sequential	24 Gy/3 fx	Pembrolizumab	The Netherlands Cancer Institute, The Netherlands
NCT02831933	II	25	SBRT	Sequential	30 Gy/5 fx	Nivolumab	Houston Methodist Cancer Center, USA
NCT02221739	II	27	RT	Concurrent	30 Gy/5 fx, 28.5 Gy/3 fx	Ipilimumab	New York University, USA
NCT03044626	II	130	RT	Concurrent	20 Gy/5 fx	Nivolumab	Thoraxklinik at Heidelberg University Hospital, Germany
NCT03511391	II	97	SBRT	Induction	24 Gy/3 fx	Nivolumab/pembrolizumab	Ghent University, Belgium
NCT02888743	II	180	RT	Induction	NP	Durvalumab plus tremelimumab	Mayo Clinic, USA
NCT03050060	II	120	HIGRT	Induction	NP	Atezolizumab	University of Washington, USA
NCT03176173	II	85	IGRT	Concurrent	10 fx (dose NM)	Nivolumab/pembrolizumab/ atezolizumab	Stanford University, USA
NCT02239900	I/II	120	SBRT	Sequential/concurrent	50 Gy/4 fx, 60 Gy/10 fx, 20 Gy/5 fx	Ipilimumab	MD Anderson Cancer Center, USA
NCT03168464	I/II	45	RT	Concurrent	30 Gy/5 fx	Ipilimumab plus nivolumab	Cornell University, USA
NCT03212469	I/II	40	SBRT	Induction	NP	Durvalumab plus tremelimumab	Gustave Roussy Villejuif, Val De Marne, France
NCT02444741	I/II	104	SBRT/HFRT	Concurrent/induction	50 Gy/4 fx, 45 Gy/15 fx	Pembrolizumab	MD Anderson Cancer Center, USA
NCT02608385	I	35	SBRT	Sequential	3 to 5 fx (dose NM)	Pembrolizumab	University of Chicago, USA
NCT02318771	I	40	RT	Sequential/concurrent	8 Gy/1 fx, 20 Gy/5 fx	Pembrolizumab	Thomas Jefferson University, USA
NCT03307759	I	32	SBRT	Sequential/induction	NP	Pembrolizumab	Peter MacCallum Cancer Centre, Australia
NCT03245177	I	25	TRT	Induction	60 to 66 Gy/30 to 32 fx	Pembrolizumab	University of Leeds, UK
NCT03436056	I	24	SBRT	Induction	30 Gy/3 fx, 54 Gy/3 fx	Pembrolizumab	Royal Marsden NHS Foundation Trust, UK
NCT02587455	I	48	RT	Concurrent	NP	Pembrolizumab	Royal Marsden NHS Foundation Trust, UK
NCT03223155	I	80	TRT	Sequential/concurrent	3 to 5 fx (dose NM)	Nivolumab/ipilimumab	University of Chicago, USA
NCT03224871	I	30	HFRT	Concurrent	24 Gy3 fx	IL-2/nivolumab/pembrolizumab	University of California, USA
NCT03035890	NP	33	HFRT	Concurrent	24 to 45 Gy/3 fx, 30 to 50 Gy/5 fx	Nivolumab/pembrolizumab/ atezolizumab	West Virginia University, USA
NCT03431948	I	60	SBRT	Concurrent	30 to 50 Gy	Nivolumab plus urelumab/ cabiralizumab	University of Chicago, USA
NCT03509584	I	24	HFRT	Concurrent	24 Gy/3 fx	Nivolumab plus ipilimumab	Assistance Publique Hôpitaux de Marseille, France
NCT02400814	I	45	SBRT	Sequential/induction/ concurrent	5 fx (dose NM)	Atezolizumab	University of California, USA
NCT02463994	I	12	HIGRT	Sequential	NP	Atezolizumab	University of Michigan, USA
NCT03275597	Ib	180	SBRT	Sequential	30 to 50 Gy/5 fx	Durvalumab plus tremelimumab	University of Wisconsin, USA
NCT02639026	I	30	HFRT	Concurrent	24 Gy/3 fx, 17 Gy/1 fx	Durvalumab plus tremelimumab	University of Pennsylvania, USA

3D-CRT indicates 3-dimensional conformal radiation therapy; Fx, fractions; HFRT, hypofractionated radiation therapy; HIGRT, hypofractionated image-guided radiation therapy; IGRT, image-guided radiation therapy; NCT ID: national clinical trial identification number; NP, not provided; NSCLC, non–small lung cancer; RT, radiation therapy; SBRT, stereotactic body radiation therapy; TRT, thoracic radiation therapy; WFRT, wide-field radiation therapy.

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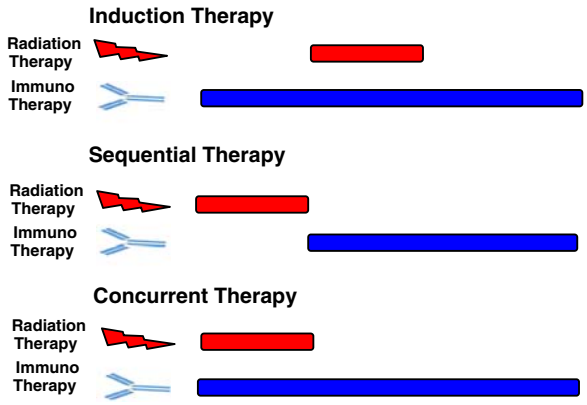

FIGURE 3. Illustration depicting various modes of radiation therapy in combination with immunotherapy for the treatment of malignant tumors including non–small cell lung cancer. full color online

delivery techniques such as IGRT, IMRT, SBRT, WFRT, and hypofractionated radiation therapy. Several immune checkpoint blockers including ipilimumab, pembrolizumab, nivolumab, atezolizumab, and durvalumab are being investigated in combination with these various radiation delivery techniques. Thus, the variety of these immune checkpoint blockers together with the different radiation technologies, doses, and schedules has produced many possible combinations to be evaluated in clinical trials. These trials represent the initial exploration and the beginning of a new era of assessing the synergistic effects of radiation therapy in combination with immune checkpoint inhibitors in patients with advanced NSCLC.

CONCLUSION AND FUTURE PERSPECTIVES

Radiation therapy has long been recognized as a potent local cytotoxic therapy for the management of cancer including NSCLC. It is considered an integral part of the treatment of a wide variety of cancers. Cellular DNA remains the principal target for ionizing radiation damage leading to cell death. However, more insight into the molecularly controlled immune mechanisms at the cellular level and the interactions between radiation and the tumor's immune system has revealed that local radiation is capable of inducing systemic antitumor responses in patients with advanced malignancies. The understanding of the immune-activating properties of radiation therapy combined with its well-known effects on the cell cycle gives us a glimpse into the complexity of the cellular immune interactions and the signaling pathways that are involved in controlling tumor growth and improved clinical outcomes.

Nevertheless, the progress that has been made in understanding tumor immunology has led to the development of novel immunotherapies using immune checkpoint inhibitors to treat advanced cancers including NSCLC. These therapies use monoclonal antibodies targeting CTLA-4 and PD-1 signaling pathways that play a crucial role in tumor growth and progression and its metastasis. Currently, nivolumab, pembrolizumab, atezolizumab, and durvalumab have been approved for the treatment of advanced tumors. For patients with advanced NSCLC, recent clinical studies incorporating these novel immune checkpoint blockade therapies have demonstrated dramatic improvements in survival outcomes compared with conventional chemotherapy regimens. However, these novel immunotherapies have thus far benefited only a small fraction (< 20%) of patients when used as standalone therapeutic interventions. The success of immune checkpoint blockade therapies in advanced NSCLC is those that have been combined and have primed the immune system with radiation to make less responsive tumors more responsive to immunotherapy.

Combining radiation therapy and immunotherapy can increase cellular immune response. Currently, a number of clinical studies that support the use of radiation therapy in combination with immunotherapy to achieve better local and systemic tumor control in advanced NSCLC and other cancers are underway. These clinical trials have demonstrated the potential synergistic relationship of these combinations. However, current data are limited to trials of small sample size, short follow-up periods, or the trials lack a randomized comparison arm, or some trials use diverse immunotherapies, various radiation doses, or fractionations. Nonetheless, the findings from these early clinical trials show that combining radiation therapy and immune checkpoint blockade therapies produced a greater clinical effect than either therapy alone in patients with advanced NSCLC. The mechanisms inducing the synergistic effect of radiation therapy and immunotherapy are not completely understood at this time. In addition, the ideal dose or fractionation of radiation therapy and its treatment schedule for the optimal priming for immunotherapy remains to be defined. The successful integration of immunotherapy with definitive radiation therapy regimens may require significant alterations in radiation therapy dosing and field designs to maximize any benefits. Moreover, it is important to consider the patients' baseline immune function and the impact of either short-term or long-term immunosuppressive medications. Furthermore, identification of clinical potential biomarkers and the development of reliable predictors of tumor response may further enhance the clinical effectiveness of this unique combination therapy in patients with advanced NSCLC and other cancers. Prospective randomized controlled trials of large patients cohorts designed based on preliminary data from these early studies are needed to establish the optimal radiation doses, schedules, and delivery methods and to confirm that the synergistic effects of the combination therapy of radiation and immunotherapy produce better survival rates, compared with conventional treatments in patients with advanced NSCLC and other cancers.

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