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Review

REVIEW

Synergistic effect of immunotherapy and radiotherapy in non-small cell lung cancer: current clinical trials and prospective challenges

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Abstract

Lately, the success of ICIs has drastically changed the landscape of cancer treatment, and several immune checkpoint inhibitors (ICIs) have been approved by the US Food and Drug Administration (FDA) for advanced non-small cell lung cancer (NSCLC). However, numerous patients are resistant to ICIs and require additional procedures for better efficacy results. Thus, combination therapy is urgently needed to strengthen the anti-tumor immunity. A variety of preclinical and clinical studies combining ICIs with radiotherapy (RT) have demonstrated that the combination could induce synergistic effects, as RT overcomes the resistance to ICIs. However, the underlying mechanism of the synergistic effect and the optimal arrangement of the combination therapy are indecisive now. Hence, this review was conducted to provide an update on the current clinical trial results and highlighted the ongoing trials. We also discussed the optimal parameters in clinical trials, including radiation dose, radiation fractionation, radiation target field, and sequencing of combination therapy. In this review, we found that combination therapy showed stronger anti-tumor immunity with tolerable toxicities in clinical trials. However, the best combination mode and potential biomarkers for the target patients in combination therapy are still unclear.

Key words: immunotherapy; radiotherapy; combination; immune checkpoints; non-small cell lung cancer

Introduction

Lung cancer is one of the leading cause of deaths worldwide,[1] with more than 85% of lung cancers being non-small cell lung cancers (NSCLC). Treatment approaches and prognosis of NSCLC patients have been evaluated based on the TNM staging system of the American Joint Committee on Cancer (AJCC).[2] Surgery or stereotactic

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body radiation therapy (SBRT) offers prominent local control of tumors and is considered safe for patients with stage I or II NSCLC.[3] However, patients with early stage NSCLC still have a higher risk of distant metastasis, and patients with resectable stage III NSCLC usually undergo surgery, while unresectable patients receive concurrent chemoradiation (cCRT) treatment. It has been shown that patients with stage III NSCLC have a lower 5-year OS rate of 15–20% after undergoing traditional curative therapies.[4] However, previous studies focusing on increasing radiation dose or involving induction or consolidation chemotherapy failed to show any evidence of extra clinical benefit.[4–6] Thus, improving local control and preventing metastatic spread are still significant challenges in stage III NSCLC patients. It has been reported that oncogene-driven stage IV NSCLC patients are stable within 1–2 years after treatment of tyrosine kinase inhibitors (TKIs),[7] but patients with advanced NSCLC who had not received positive oncogenic drivers demonstrated poor prognosis with conventional therapies and warranted more powerful therapies.

Novel immunotherapeutic approaches aim to enhance the immunity of the host, by gathering together immune cells, and getting these cells to recognize and then eliminate the cancer cells. The US Food and Drug Administration (FDA) has approved various immune checkpoint inhibitors (ICIs) for treatment of advanced NSCLC, including nivolumab, pembrolizumab, atezolizumab, and durvalumab.[8] However, several patients showed no benefit from treatment with ICIs, and are classified as having primary resistance. Ultimately, most primary responders develop acquired resistance after treatment with ICIs, and their conditions progress. Hence, great efforts are being made to improve the efficacy of ICIs. Several studies have reported combinations of ICIs with other therapies, such as chemotherapy, radiotherapy (RT), targeted therapy, or other immunotherapies. RT can augment the benefit of ICIs, providing local control of primary tumors and strengthening immune responses even outside the targeted site, this is known as the "abscopal effect."[9]

In this review, we summarize current clinical trials and discuss the optimal combination modes of ICIs and RT. We also outline challenges and future directions for immunotherapy and RT.

Table 1. Mechanisms of immune resistance.

Primary resistance	
Intrinsic factor	Absence of antigen proteins
	Absence of antigen presentation machinery
Extrinsic factor	PD-L1 expression levels
	Insufficient tumor-infiltrating lymphocytes
	Severe exhaustion of T-cells
	Immunosuppressive cells, immunosuppressive cytokines, and inhibitory checkpoints
	Metabolic status
	Alterations of several signaling pathways
Acquired resistance	Loss of functional T-cells
	Escape mutations

Immunotherapy: resistance mechanisms

Several processes are involved in immune system attack on tumor cells, including tumor recognition, antigen presentation, and T-cell activation.[10] However, cancer cells can escape immune surveillance by interaction with immune cells. In attempts to ensure this does not occur, there is current interest in ICIs as therapeutics, in particular the anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitor, anti-programmed death 1 (PD-1) inhibitor, and anti-programmed death ligand 1 (PD-L1) inhibitor. CTLA-4 on T-cells binds to peripheral membrane B7 on antigen presenting cells (APC), and inhibits T-cell activation.[11] Similarly to the CTLA-4/B7 complex, PD-L1 on tumor cells attaches to PD-1 on T-cells. Then, the PD-1/PD-L1 compound releases inhibitory signals and acts in the effector phase of T-cell immune response.[12]

Primary resistance to immunotherapy is related to various tumor intrinsic and extrinsic factors (Table 1).[13] Some tumor cells containing non-synonymous somatic mutations can be recognized by the immune cells as neoantigens. The lack of antigenic proteins is considered to be one of the most significant intrinsic factors.[14] The advancement of next-generation sequencing (NGS) techniques has made possible comprehensive detection of tumor mutation burden (TMB). Moreover, deficient DNA mismatch repair (MMR) can provoke a higher level of genomic instability and tumor-specific mutation.[15] Consequently, NSCLC patients with an absence of antigenic proteins, including proficient DNA MMR, lower TMB, and lower neoantigen load show inferior responses to ICIs and have poor progression-free survival (PFS).[16] Meanwhile, primary resistance is related to the absence of antigen presentation machinery that is caused by downregulation of major histocompatibility complex class 1 (MHC-1).[17,18]

The major extrinsic factors include immunoregulatory factors within the tumor microenvironment (TME), such as low PD-L1 expression levels, insufficient tumor-infiltrating lymphocytes (TILs), and severe exhaustion of T-cells.[19] Besides, the immunosuppressive cells, immunosuppressive cytokines and inhibitory checkpoints involved in resistance to ICIs include regulatory T-cells (Tregs), Th2 cells, myeloid-derived suppressor cells (MDSCs), M2 macrophages, and T-cell immunoglobulin domain and mucin-3 (TIM-3).[20] The metabolic status of the TME is also considered to be a crucial factor, with ICIs more efficacious in patients with higher glycolytic rates.[21] Alterations in several signaling pathways (MAPK, PI3K, WNT, IFN, EGFR, or ALK signaling

pathways) can affect the function of T-cells and response to ICIs.[22] For example, oncogene-driven NSCLC patients had lower levels of TMB, exhibiting insufficient responses to ICIs.[15,23] Although oncogene drivers upregulate expression of PD-L1, such expression levels were reduced after TKI treatment, generating resistance to ICIs.[24,25] On the other hand, the resistance may be correlated with decreased cytotoxic $CD8^+$ T-cells (CTLs) and enhanced T-cell exhaustion induced by the oncogenic signals.[23,24] Several host-related factors are related to ICI resistance, such as age, smoking, background infection or chronic disease, and gut microbiota.[26]

The mechanism of acquired resistance might be correlated with loss of functional T-cells and development of escape mutations, such as mutations of Janus kinase (JAK1/2). JAK1/2 reduces presentation of tumor antigens through the interferon (IFN)-γ signaling pathway.[19]

RT: overcoming resistance to immunotherapy

RT is widely used in treatment of lung cancer and can provide great local tumor control. It causes random point mutations and double-stranded breaks in the DNA, increasing TMB and neoantigens.[27] Cell death induced by radiation can occur by apoptosis, necrosis, autophagic death, mitotic catastrophe, or proliferative senescence.[28] Radiation-induced cell death results in release of adenosine triphosphate (ATP), high mobility group box 1 protein (HMGB-1) and calrecticulin (CALR), and a rise in production of IFN-I.[28–30] HMGB-1 binds to toll-like receptor-4 (TLR-4) and the HMGB-1/TLR-4 interaction is involved in tumor antigen progression and presentation.[31] RT also promotes activation and maturation of dendritic cells (DCs) through the above signaling pathways.[32] Hence, the immunogenicity of tumor cells is strengthened by increasing the tumor antigens, activating dendritic cells (DCs), and enhancing antigen cross-presentation to T-cells.[29] The radiation-induced cell death is defined as immunogenic cell death (ICD) as it leads to increased recognition of cancer cells by T-cells, and thus an improved immune response.[28,33] Radiation also increases expression of NK group 2 member D (NKG2D) ligand and first apoptosis signal (FAS) on tumor cells, and promotes recognition and elimination of cancer cells by T-cells and NK cells.[34,35]

On the other hand, the RT could modify the immunosuppressive TME. The DNA damage-induced kinase ABL1 amplifies expression of macrophage colony-stimulating factor 1 (CSF1) in response to radiation, resulting in proliferation of circulating MDSCs.[36] These MDSCs subsequently enhance tumor infiltration.[37] Radiation expands and activates $CD8^+$ T-cells in draining lymph nodes and also improves recruitment of T-cells to tumor sites by upregulating chemokines, such as CXCL10 and CXCL16.[38,39] Meanwhile, RT increases expression of vascular adhesion molecule-1 (VCAM-1) on endothelial cells and permits extravasation of T-cells.[37] RT reprograms macrophages to secrete nitric oxide (NO) and leads to enhanced T-cell infiltration and vascular normalization.[29] MHC-I expression is upregulated on tumor cells and improved tumor recognition.[40] Expression of PD-L1 is also increased on cancer cells after RT and is dependent on IFN-γ produced by CD8+ T-cells.[41,42] Hence, radiation could change tumor metabolism status and enhance immunological response.[27]

RT can overcome resistance to ICIs via complicated mechanisms and can convert non-immunogenic tumors into immunogenic ones, making logical a combination of RT and ICIs (Fig. 1).

Current studies of combination therapy

Previous studies have provided evidence that ICIs can improve survival of patients with advanced NSCLC. Currently, there are various clinical trials investigating combinations of ICIs and RT. Tables 2–4 were generated by searching the ClinicalTrials.gov database using the search terms “radiation,” “immunotherapy,” “NSCLC,” “CTLA-4 inhibitors,” “PD-1 inhibitors,” “PD-L1 inhibitors,” and several variations. The results were then manually filtered for inclusion of studies that were conducted on ICIs and RT. The clinical trial results highlighted the advantages of combinations in patients at different stages.

Surgery or SBRT demonstrated outstanding local control in patients with early stage NSCLC, with a 5-year local control rate of 92% in stage IA NSCLC patients and of 73% in stage IB and IIA NSCLC patients.[43] However, there is also a probability of relapse or metastasis in early stage NSCLC patients and hence improved treatments are required. Currently, there are six ongoing clinical trials on the combination of SBRT with ICIs, as presented in Table 2.[44–49] The dose of SBRT ranges from 48 to 70 Grey (Gy) [3–10 fx (fractions)], and there is no consensus on administration with some trials administering SBRT before ICI administration and some after, or even concurrently with ICIs. We defined combination modes as 1. sequential therapy, where the patients received ICI after the final fraction of SBRT; 2. induction therapy, where the patients received SBRT after a few cycles of ICI; and 3. concurrent therapy, where the patients received SBRT and ICIs concurrently. The ICIs used in the clinical trials included nivolumab, durvalumab, atezolizumab, and avelumab, and patients received consolidation ICI after immunoradiation. All the trials are in phase I or II and are still recruiting patients without any published results (Table 2 and Fig. 2).

In the phase I clinical trial KEYNOTE-001 (NCT01295827), 38 (39%) of 97 patients with locally advanced or metastatic NSCLC received extracranial RT, and 24 (25%) patients among them received thoracic RT before the first cycle of pembrolizumab. A secondary analysis of this trial compared the PFS and OS of patients with or without prior RT before the first cycle of pembrolizumab.[50] Patients who had previously received extracranial RT had a significantly longer median PFS [6.3 vs. 2.0 months; hazard ratio (HR): 0.50 (95% CI: 0.30–0.84), $P = 0.0084$] and OS [11.6 vs. 5.3 months; HR: 0.59 (95% CI: 0.36–0.96), $P = 0.034$]. The study also noted

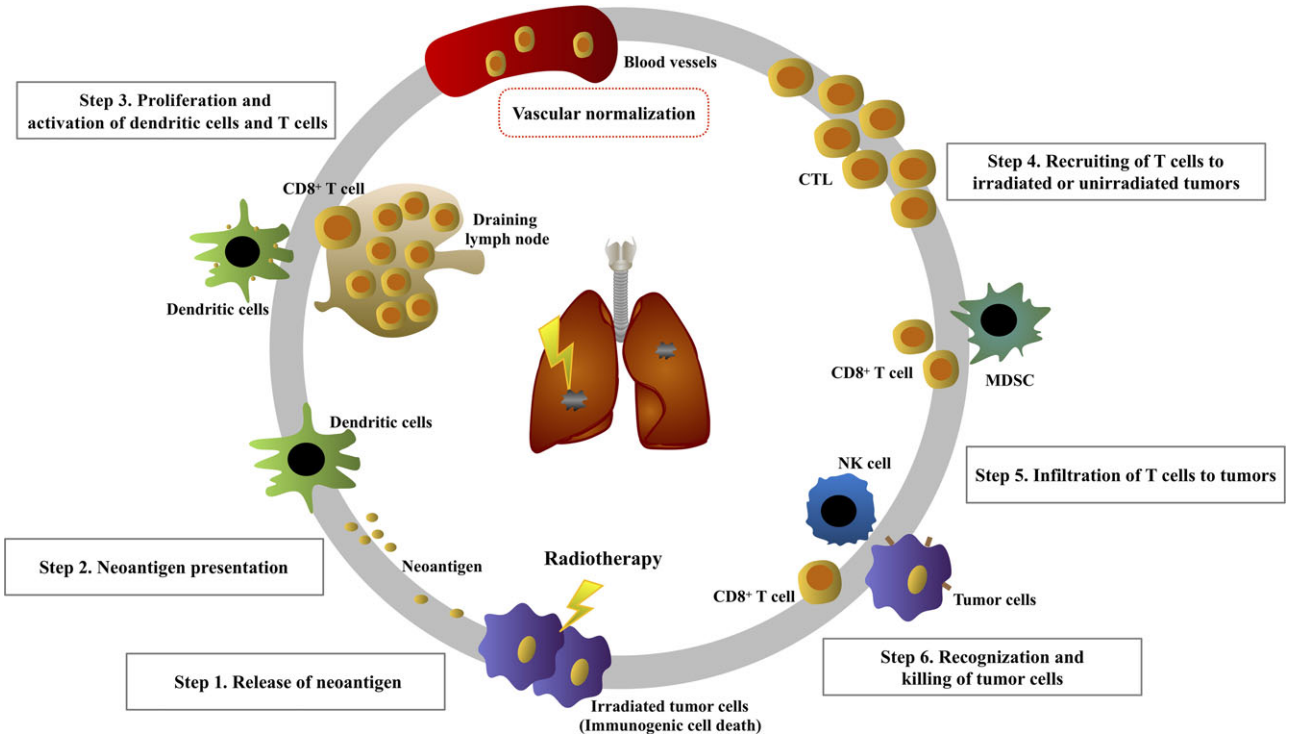

Figure 1. Radiation activates the host immune system. Step 1. Release of neoantigen: Neoantigen increasing; Step 2. Neoantigen presentation: ATP/HMGB-1/CALR/IFN-I from tumor cells; Step 3. Proliferation and activation of dendritic cells and T cells: ATP/HMGB-1/CALR/IFN-I and CXCL10/CXCL 16 from tumor cells; Step 4. Recruiting of T cells to irradiated or unirradiated tumors: CXCL10/CXCL16 from tumor cells, increasing of VCAM-1 on endothelial cells; Step 5. Infiltration of T cells to tumors: CSF-1 from tumor cells, MDSC increasing; Step 6. Recognization and killing of tumor cells: PD-L1/MHC-1/NKG2D/FAS increasing on tumor cells. Vascular normalization: NO from macrophages.

Table 2. Active clinical trials involving the use of both SBRT and immunotherapy in early stage NSCLC.

NCT number	Reference	Radiation	Sequencing	Radiation dose	Immunotherapy	Stage	Phases	Enrollment
NCT03383302	45	SBRT	Sequential	54 Gy/3 fx, 55 Gy//5 fx	Nivolumab	I–II	1/2	31
NCT03110978	46	SBRT	Concurrent	50 Gy/4 fx, 70 Gy/10 fx	Nivolumab	I/IIA (T2N0M0)	2	140
NCT03148327	47	SBRT	Concurrent	54 Gy/3 fx, 50 Gy/4 fx, 65 y/10 fx	Durvalumab	I/IIA (T2bN0M0)	1/2	105
NCT03446547	48	SBRT	Sequential	3–4 fx (dose NM)	Durvalumab	I	2	216
NCT02599454	49	SBRT	Induction	50 Gy/4 fx, 50 Gy/5 fx	Atezolizumab	I	1	33
NCT03050554	50	SBRT	Concurrent	48 Gy/4 fx, 50 Gy/5 fx	Avelumab	I	1/2	56

Fx, fractions; Gy, gray; NM, not mentioned; NSCLC, non-small cell lung cancer; SBRT, stereotactic body radiation therapy.
Note: all trials used consolidation immunotherapy after the combination therapy which is not shown in this table.

that patients with prior thoracic RT had a higher risk of treatment-related pulmonary toxicity [13% (3/24) vs. (1/73) 1%], but had similar incidence of grade 3 or worse pulmonary toxicities (one patient in each group). The tolerable safety profile and promising efficacy in this trial highlighted the potential of combination therapy and the requirement for carefully monitoring the toxicity in patients.

Resectable locally advanced patients, typically stage IIIA (AJCC), normally undergo initial surgery before or after chemotherapy and RT. The latest clinical trial NCT02259621 reported use of neoadjuvant nivolumab (anti-PD-1inhibitor) in resectable NSCLC patients (stages I, II and III).[51] The first dose of nivolumab was given to the patients about 4 weeks before the planned surgery.

Table 3. Combination of radiotherapy and immunotherapy in stage III NSCLC.

NCT number	Reference	Radiation	Sequencing	Radiation dose	Immunotherapy	Stage	Phases	Enrollment
Resectable stage III NSCLC								
NCT03217071	53	SBRT	Induction	12 Gy/1 fx	Pembrolizumab	I–IIIA	2	40
NCT02987998	54	cCRT	Concurrent	45 Gy/25 fx	Pembrolizumab	IIIA	1	20
NCT03053856	56	cCRT	Adjuvant	44 Gy//22 fx	Pembrolizumab	IIIA	2	37
NCT03237377	55	TRT	Concurrent	45–50 Gy/25 fx	Durvalumab (+tremelimumab)	IIIA	2	32
Unresectable stage III NSCLC								
NCT02768558	60	cCRT	Sequential	60 Gy	Nivolumab	III	3	13
NCT03285321	61	cCRT	Sequential	59.4–66.6 Gy	Nivolumab (+ipilimumab)	III	2	108
NCT02434081[a]	62	cCRT	Concurrent	NM	Nivolumab	III	2	78
NCT02525757[a]	58	cCRT	Sequential/ concurrent	60–66 Gy/30–32 fx	Atezolizumab	III	2	52
NCT03102242	63	cCRT	Induction	60 Gy/30 fx	Atezolizumab	III	2	63
NCT02125461[a]	57	cCRT	Sequential	54–66 Gy	Durvalumab	III	3	713
NCT03509012	64	cCRT	Concurrent	NM	Durvalumab	III	1	300
NCT02343952[a]	59	cCRT	Concurrent	59.4–66.6 Gy	Pembrolizumab	III	2	93
NCT02621398	65	cCRT	Concurrent	30 fx (dose NM)	Pembrolizumab	II–IIIB	1	30

cCRT, concurrent chemoradiotherapy; Fx, fractions; Gy, gray; NM, not mentioned; NSCLC, non-small cell lung cancer; RT, radiation therapy; SBRT, stereotactic body radiation therapy; TRT, thoracic radiotherapy.

Note: trials of resectable stage III NSCLC received surgery after combination therapy and trials of unresectable stage III NSCLC used consolidation immunotherapy after the combination therapy which is not shown in this table.

[a] Trials with reported results.

Patients were given conventional adjuvant chemotherapy or RT if necessary. Neoadjuvant nivolumab showed tolerable adverse effects (AEs), with only one grade 3 pneumonia and no grade 4 or 5 AEs among 21 patients, and there was no delays to any surgeries. The median interval between the second dose of nivolumab and surgery was 18 days (range 11-29 days). After neoadjuvant nivolumab, pathological down-staging occurred in eight patients (40%), and shrinkage of the tumors made the surgeries safer. The major pathologic response (MPR), defined as ≤10% residual viable tumor cells in surgical specimens viewed with hematoxylin and eosin staining, was seen in nine patients (45%). There are three other clinical trials regarding combination of ICIs and RT or chemoradiotherapy (CRT) before undergoing the surgery in resectable stage III NSCLC patients[52–54] (Table 3). Another trial involves administration of adjuvant pembrolizumab after neoadjuvant concurrent chemoradiotherapy (cCRT) and surgery[55] (Table 3). The adjuvant usage of ICIs after surgery is controversial, and further clinical trials are required to provide sufficient evidence for clinical decision-making.

Conventional treatment for patients with unresectable locally advanced NSCLC is curative CRT. In the PACIFIC study (NCT02125461), a phase III study for patients with stage III unresectable lung cancer, patients received consolidation durvalumab (anti-PD-L1 inhibitor) until disease progression (up to 12 months) after initial cCRT.[56] Durvalumab was given for no more than 6 weeks after CRT, with results showing an improvement of 11.6 months in median PFS when compared with the control group (17.2 vs. 5.6 months). These results accelerated approval of durvalumab by the FDA as consolidation therapy in stage III unresectable lung cancer patients after cCRT. The latest report even showed that the durvalumab group had a better OS (2-year OS rate: 66.3% vs. 55.6%).[56] The phase II trial DETERRED (NCT02525757) on another anti-PD-L1inhibitor, atezolizumab, is an ongoing trial conducted in unresectable stage III NSCLC patients.[57] After a resting period of 3-4 weeks followed by definitive cCRT, patients received atezolizumab in addition to chemotherapy for two cycles. Later, the patients received extra consolidation treatment with atezolizumab for 12 months. Of the seven patients, two showed progression after six and eight doses of atezolizumab. The LUN 14-179 (NCT02343952) study examined use of pembrolizumab in unresectable stage III NSCLC patients, reporting a similar median PFS of 15.4 months with a resting period between pembrolizumab and CRT of 4-8 weeks.[58] The 1-year OS rate was 80.5% and the 2-year OS rate was 68.7%. A comparable trial, RTOG 3505 (NCT02768558), on nivolumab in unresectable stage III NSCLC patients is ongoing, but no results have yet been reported.[59] The combination modes of CRT and ICIs are summarized as follows: 1. sequential therapy: patients receiving ICI after CRT; 2. induction therapy: patients receiving CRT after a few cycles of ICI; and 3. concurrent therapy: patients receiving CRT and ICI concurrently[60–64] (Table 3 and Fig. 2).

In patients with advanced NSCLC, usage of combinations of ICIs and RT is more frequent and more complicated. There are several ongoing clinical trials using various ICIs, such as ipilimumab, tremelimumab, pembrolizumab,

Table 4. Clinical trials involving use of both radiotherapy and immunotherapy in advanced NSCLC.

NCT number	Reference	Radiation	Sequencing	Radiation dose	Immunotherapy	Phases	Enrollment
NCT02239900[a]	66	SBRT	Sequential/ concurrent	50 Gy/4 fx, 60 Gy/ 10 fx, 20 Gy/5 fx	Ipilimumab	1/2	120
NCT02221739	67	RT	Concurrent	30 Gy/5 fx, 28.5 Gy/ 3 fx	Ipilimumab	2	27
NCT02318771	68	RT	Sequential/ concurrent	8 Gy/1 fx, 20 Gy/5 fx	Pembrolizumab	1	40
NCT02492568	69	SBRT	Sequential	24 Gy/3 fx	Pembrolizumab	2	92
NCT02608385	70	SBRT	Sequential	3–5 fx (dose NM)	Pembrolizumab	1	35
NCT03004183	71	SBRT	Sequential	30 Gy/5 fx	Pembrolizumab	2	57
NCT03307759	72	SBRT	Sequential/ induction	NM	Pembrolizumab	1	32
NCT03245177	73	TRT	Induction	60–66 Gy/30–32 fx	Pembrolizumab	1	25
NCT03368222	74	SBRT	Induction	30 Gy/3 fx, 54 Gy/ 3 fx	Pembrolizumab	1	24
NCT03436056	75	SBRT	Induction	30 Gy/3 fx, 54 Gy/ 3 fx	Pembrolizumab	1	24
NCT02444741[a]	76	SBRT/ HFRT	Concurrent/ induction	50 Gy/4 fx, 45 Gy/ 15 fx	Pembrolizumab	1/2	104
NCT02587455	77	RT	Concurrent	NM	Pembrolizumab	1	48
NCT02658097	78	RT	Concurrent	8 Gy/1 fx	Pembrolizumab	2	48
NCT02831933	79	SBRT	Sequential	30 Gy/5 fx	Nivolumab	2	25
NCT03223155	80	TRT	Sequential/ concurrent	3–5 fx(dose NM)	Nivolumab/ipilimumab	1	80
NCT03511391	81	SBRT	Induction	24 Gy/3 fx	Nivolumab/ pembrolizumab	2	97
NCT03044626	82	RT	Concurrent	20 Gy/5 fx	Nivolumab	2	130
NCT03224871	83	HFRT	Concurrent	24 Gy3 fx	Nivolumab/ pembrolizumab	1	30
NCT03176173	84	IGRT	Concurrent	10 fx (dose NM)	Nivolumab/ pembrolizumab/ atezolizumab	2	85
NCT03035890	85	HFRT	Concurrent	24–45 Gy/3 fx, 30–50 Gy/5 fx	Nivolumab/ pembrolizumab/ atezolizumab	NM	33
NCT03313804	86	SBRT/3D-CRT	Concurrent	SBRT: BED > 100 Gy, 3D-CRT: 30 Gy	Nivolumab/ pembrolizumab/ atezolizumab	2	57
NCT03391869	87	RT	Induction	NM	Ipilimumab+nivolumab	3	270
NCT03168464	88	RT	Concurrent	30 Gy/5 fx	Ipilimumab+nivolumab	1/2	45
NCT03431948	89	SBRT	NM	30–50 Gy	Nivolumab+ urelumab/ cabiralizumab	1	60
NCT03509584	90	HFRT	NM	24 Gy/3 fx	Nivolumab (+ipilimumab)	1	24
NCT02400814	91	SBRT	Sequential/ induction/ concurrent	5 fx (dose NM)	Atezolizumab	1	45
NCT02463994	92	HIGRT	Sequential	NM	Atezolizumab	1	12
NCT03050060	93	HIGRT	Induction	NM	Atezolizumab	2	120
NCT03275597	94	SBRT	Sequential	30–50 Gy/5 fx	Durvalumab +tremelimumab	1b	180
NCT02888743	95	RT	Induction	NM	Durvalumab +tremelimumab	2	180
NCT03212469	96	SBRT	Induction	NM	Durvalumab +tremelimumab	1/2	40

Continued

Table 4. Continued

NCT number	Reference	Radiation	Sequencing	Radiation dose	Immunotherapy	Phases	Enrollment
NCT02639026	97	HFRT	Concurrent	24 Gy/3 fx, 17 Gy/ 1 fx	Durvalumab +tremelimumab	1	30

Fx, fractions; Gy, gray; HFRT, hypofractionated radiotherapy; HIGRT, hypofractionated image-guided radiotherapy; IGRT, image-guided Radiotherapy; NM, not mentioned; NSCLC, non-small lung cancer; RT, radiation therapy; SBRT, stereotactic body radiation therapy; TRT, thoracic radiotherapy; WFRT, wide-field radiation therapy; 3D-CRT, 3D conformal radiotherapy.
Note: all trials used consolidation immunotherapy after the combination therapy which is not shown in this table.
[a]Trials with reported results.

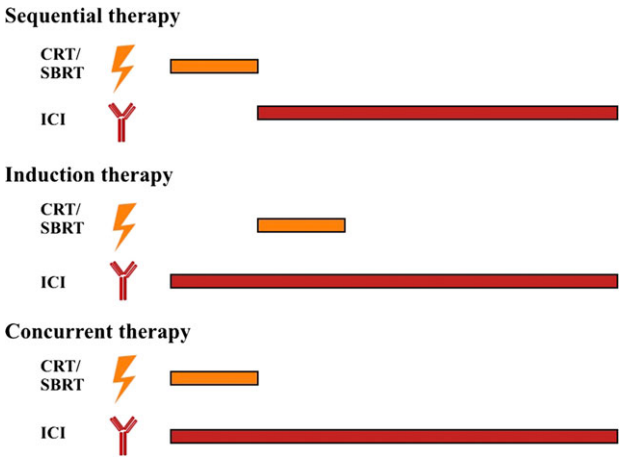

Figure 2. Sequencing modes of combination therapy. Sequential therapy: patients receiving ICI after CRT; Induction therapy: patients receiving CRT after a few cycles of ICI; Concurrent therapy: patients receiving CRT and ICI concurrently.

nivolumab, atezolizumab, and durvalumab. Different combination modes, including induction, sequential, or concurrent therapies have been used (Fig. 2). The clinical trials also adopted various radiation technologies, such as image-guided radiotherapy (IGRT), intensity modulated radiation therapy (IMRT), hypofractionated radiotherapy (HFRT), stereotactic body radiation therapy (SBRT), and wide-field radiation therapy (WFRT)[65–96] (Table 4). The clinical trial NCT02239900 on ipilimumab with concurrent or sequential RT reported that three (10%) of 31 advanced NSCLC patients exhibited partial response (PR) outside the radiation field.[65] For the 19 patients in NCT02444741, six patients (32%) showed PR, seven patients (36%) experienced stable disease, and six patients (32%) progressed after a combination of RT and pembrolizumab.[75]

With the rapidly increasing number of clinical trials regarding the combination therapy, we look forward to further discussions on their results.

Toxicities of combination therapy

Among studies reporting toxicity data, four trials are about unresectable stage III NSCLC and two trials are conducted with stage IV NSCLC patients. In the updated analyses of the PACIFIC study, grade 3 or 4 AEs were observed in 30.5% of patients who received sequential cCRT and durvalumab, and in 26.1% of patients in the placebo group.[56] As the most frequent AE leading to treatment discontinuation in this trial, pneumonitis occurred in 32.8% (156/475) of patients. Among the 475 patients, 16 patients experienced grade 3–4 pneumonitis and five patients experienced grade 5 pneumonitis.[56] The DETERRED study was designed for sequential and concurrent parts, but only the results of the sequential group have been published. The latest abstract published at the 2018 American Society of Clinical Oncology (ASCO) annual meeting, reported that one patient developed grade 2 radiation-induced pneumonitis which was manageable with steroids.[57] The current combination of cCRT and pembrolizumab in LUN 14-179 reported 11 patients with grade 2 pneumonitis, five with grade 3–4 pneumonitis, and one pneumonitis-associated death from a total of 93 patients. The median interval for pneumonitis occurrence is 8.4 weeks.[58] However, for the first 21 patients recruited in the trial ETOP NICOLAS, the most frequent AEs are fatigue and anemia and no pneumonitis grade ≥3 has been observed. The observation period is only 3 months after RT, so long-term toxicities are not available.[61] The trial NCT02239900 on ipilimumab reported four patients with grade 3 toxicity from 13 patients in the concurrent group, and eight out of 22 patients in the sequential group. No patient experienced grade > 1 pneumonitis in this trial.[65] As for current combination of pembrolizumab, NCT02444741 reported one grade 3 pneumonitis in 19 patients[75] (Table 5). According to the reported data, ICIs remain tolerable when combined with RT in NSCLC patients.

Challenges in combination therapy

The traditional mechanism of RT includes four principles (“four Rs”): repair, reassortment, repopulation, and reoxygenation.[97] Currently, the immunogenic effect of RT is noted, and the combination therapy is expected to deliver better clinical efficacy. However, there are still many questions regarding the optimal combinations to maximize the synergistic effects.

RT dose and fractionation

In the design of the current clinical trials, a wide range of RT dose and fractionation schedules are applied. The

Table 5. Toxicities reported in the combination therapy.

NCT number	Reference	Radiation	Sequencing	Immunotherapy	Stage	Any grade AEs	Grade ≥3 AEs	Any grade pneumonitis
NCT02125461[a]	57	cCRT	Sequential	Durvalumab	III	460/475	145/475	Grade ≤2 (136/475); Grade 3–4 (16/475); Grade 5 (4/475)
NCT02525757[a]	58	cCRT	Sequential	Atezolizumab	III	3/7	2/7	Grade 2 (1/7)
NCT02343952[a]	59	cCRT	Concurrent	Pembrolizumab	III	NM	NM	Grade 2 (11/93),Grade 3–4 (5/93), pneumonitis associated death (1/93)
NCT02434081[a]	62	cCRT	Concurrent	Nivolumab	III	NM	NM	No pneumonitis grade ≥3
NCT02239900[a]	66	SBRT	Concurrent	Ipilimumab	IV	6/13	4/13	No pneumonitis grade > 1
NCT02239900[a]	66	SBRT	Sequential	Ipilimumab	IV	9/22	8/22	No pneumonitis grade > 1
NCT02444741[a]	76	SBRT/HFRT	Concurrent	Pembrolizumab	IV	11/19	3/19	Grade 3 (1/19)

AEs, adverse events; cCRT, concurrent chemoradiotherapy; Fx, fractions; Gy, gray; HFRT, hypofractionated radiotherapy; NM, not mentioned; NSCLC, non-small cell lung cancer; SBRT, stereotactic body radiation therapy.

[a] Trials with reported results.

appropriate selection of RT dose and fractionation to elicit an effective anti-tumor immune response safely is unknown.[98]

Previous preclinical breast and colon cancer models suggest that 24 Gy/3 fx or 30 Gy/5 fx is better than 20 Gy/1f when combined with anti-CTLA4 inhibitor for metastatic lesions.[99] Work on a preclinical model of prostate cancer also suggested that multi-fraction radiation is superior when compared to a single dose of radiation.[100] Furthermore, in preclinical studies of lung cancer and melanoma models, ablative hypofractionated radiotherapy (AHFRT) (23 Gy/2 fx) showed better local control of primary tumors and a stronger systemic anti-tumor effect (abscopal effect) than conventional fractionated radiotherapy (CFRT) of 36 Gy/9 fx with the same biological equivalent dose (BED).[101] At the cellular level, AHFRT suppressed recruitment of MDSCs into tumors and decreased expression of PD-L1, which then activated cytotoxic $CD8^+$ T lymphocytes. AHFRT combines with anti-PD-L1 inhibitor to trigger a more effective anti-tumor effect, showing significant improvement in terms of tumor control and mouse survival.

RT target region

T-lymphocytes are radiosensitive. Radiation can not only damage TILs, but also affect T-cells in the peripheral blood that transit through the irradiated field.[102] Larger radiation fields expose more lymphocytes to RT, which may in turn exhaust the T-cells. Methods to reduce RT-induced lymphopenia include reduction in the volume of the radiation field using highly conformal techniques such as SBRT and HFRT. A comment on keynote-001 trial even reported that it might not be necessary to include the entire tumor during RT.[50,103] This is because RT of only a part of cancer might be sufficient to trigger the immune response.[101] There are some clinical trials in which only a part of the large primary tumor (NCT02608385, NCT03217071, NCT03368222, NCT03436056) has been irradiated[52,69,73,74] (Table 6). However, another report revealed that when RT is combined with ICI, it is necessary to avoid irradiation of the lymph node region, as this can affect generation of stronger immune responses.[104] As it is common practice to irradiate the draining lymph nodes in NSCLC, there is a need for further research into this discrepancy in conclusions.

Several previous clinical trials used ICIs and single lesion radiation, but failed to provoke the abscopal effect and clinical benefits.[105,106] In his latest review, Chang presented an opposite opinion.[107] Firstly, that multisite radiation can induce more neoantigen and functional T-cells than single lesion radiation and can modulate the immunosuppressive TME. Secondly, the extent of anti-tumor immunity induced by radiation is not the same when irradiating different sites. Analysis of phase I trial NCT02239900 showed that irradiation of liver metastases in NSCLC patients caused stronger anti-tumor immunity than irradiation of pulmonary metastases.[65] Hence, different organs are not equally immunogenic and irradiation of multisites can increase the probability of successful induction of anti-tumor response. Thirdly, multisite radiation can reduce the tumor burden and enhance efficacy of ICIs. In the subgroup analysis of a phase III trial NCT00861614, the prostate patients with smaller disease burden or who received irradiation to all lesions demonstrated good clinical benefits with combination therapy of ipilimumab and RT.[105] Furthermore, in a preclinical model of melanoma, tumor burden was found to be associated with T-cell reinvigoration. The T-cell invigoration to tumor burden ratio was directly proportional to the PFS after the immunotherapy.[108] Based on these preclinical and clinical studies, the review by Chang suggested enhancing the limited efficacy of combination therapy by delivering RT to as much of the tumor burden as

Table 6. Different design of radiation field in clinical trials.

NCT number	Reference	Radiation	Sequencing	Immunotherapy	Radiation lesion enrollment
NCT02608385	70	SBRT	Sequential	Pembrolizumab	Partially for large tumor or all lesions for oligometastatic patients
NCT03217071	53	SBRT	Induction	Pembrolizumab	50% of primary tumor
NCT03368222	74	SBRT	Induction	Pembrolizumab	Part of a lung lesion
NCT03436056	75	SBRT	Induction	Pembrolizumab	Part of a lung lesion
NCT02444741[a]	76	SBRT/ WFRT	Concurrent/ induction	Pembrolizumab	Primary tumor: 50 Gy/4 fx or 45 Gy/15 fx and metastatic lesion: 5–10 Gy
NCT03223155	80	TRT	Sequential/ concurrent	Nivolumab/ipilimumab	2–4 lesions
NCT03511391	81	SBRT	Induction	Nivolumab/ pembrolizumab	Maximally 3 lesions
NCT03431948	89	SBRT	NM	Nivolumab+ urelumab/ cabiralizumab	Metastatic lesion(s)
NCT03275597	96	SBRT	Sequential	Durvalumab +tremelimumab	All lesion

NM, not mentioned; SBRT, stereotactic body radiation therapy; TRT, thoracic radiotherapy; WFRT, wide-field radiation therapy.

[a]Trials with reported results.

could be safely irradiated.[107] Compared with numerous clinical trials examining radiation of only a single lesion, there are only six clinical trials using multiple site radiation. The NCT03223155, NCT03431948, and NCT03511391 trials were designed to irradiate more than one lesion[79,80,88] (Table 6). Clinical trial NCT02444741 was designed to irradiate metastatic lesions with low doses ranging from 5 to 10 Gy and to irradiate the primary tumor at a dose of 45–50 Gy/5 fx.[73] The NCT02608385 and NCT03275597 trials were designed to conduct SBRT on all lesions for oligometastatic patients[69,93] (Table 6).

RT sequencing

The sequencing of each ingredient is critical for the efficacy of combination therapy. As different ICIs affect different phases of the immune response and different immune cells, the sequencing must be carefully designed to produce the greatest synergistic effect. However, the optimum sequencing is as yet unknown, although concurrent therapy appeared to be superior to sequential therapy in a preclinical colorectal cancer model.[109] The preclinical study reported that administration of an anti-PD-1 inhibitor on day one or five of RT gave better results than administration 1 week after RT.[109] A similar phenomenon occurred when a combination of anti-CTLA-4 inhibitor and HFRT (20 Gy/1f) was administered in preclinical colorectal cancer models.[110] The anti-CTLA-4 inhibitor showed most efficient results when given before the radiation.[110] The difference between preclinical and clinical studies highlights the need to conduct studies to compare the concurrent mode with the sequential mode and the induction mode.

A retrospective analysis of 758 patients who received an ICI (anti-CTLA4 and/or anti-PD1/anti-PDL1) demonstrated that patients who received concurrent therapy had a better OS. Furthermore, the OS was longer when patients received induction ICI for more than 30 days before RT when compared with those receiving ICI within 30 days of RT (20 vs. 11 months).[111] There is also a phase II clinical trial, NICOLAS (NCT02434081), regarding involvement of timepoint of nivolumab concurrently with cCRT in patients with unresectable locally advanced NSCLC.[61] The results published at the 2018 ASCO annual meeting provided evidence that combining nivolumab and RT concurrently in cycle two of chemotherapy was safe and tolerable.

In contrast, the PACIFIC study reported that sequential use of durvalumab from several weeks (up to 6 weeks) after cCRT improved median PFS of 11.6 months when compared with the control group.[56] However, the analysis suggested that beginning durvalumab usage within 14 days of completing cCRT seemed to have greater PFS than starting it after 14 days (up to 42 days).[112] Hence, the synergistic effect is not only correlated with the sequencing, but also with the resting period between RT and ICIs. Various trials with different combination modes are ongoing, and the best combination that triggers a powerful immune response without exhausting the T-cells will be confirmed in the future.

Combination with other immunotherapies

Other non-ICI immunotherapies, such as interleukin-2 (IL-2), granulocyte-macrophage colony stimulating factor (GM-CSF), and cancer vaccines have been used experimentally to gather further information to improve the efficacy of combination therapy.

IL-2 is a cytokine produced by T-cells that promotes T-cell growth. It has been approved by the FDA for treatment of metastatic renal cell carcinoma (RCC) and metastatic melanoma.[113] According to a phase I study that combined high dose IL-2 with SBRT for metastatic RCC and melanoma patients, combination therapy resulted in

better tumor regression than IL-2 alone. The better efficacy is induced by increased activation of effector memory T-cells.[114] Although IL-2 has shown promising efficacy, it has apparent toxicities, including flu-like symptoms, vascular leak syndrome (VLS), pulmonary edema, hypotension, and heart toxicities.[115] Currently, a phase I pilot study NCT03224871 is being designed to add IL-2 to concurrent use of nivolumab or pembrolizumab and RT (24 Gy/3 fx).[78]

GM-CSF is a cytokine that can stimulate tumor cell migration from distant sites, promote maturation of DCs, and enable cross-presentation of tumor cell antigens within TME.[116,117] It is normally produced by macrophages, T-cells, and fibroblasts and is secreted from the tumor cells after radiation.[117] A proof-of-principle pilot study enrolled 41 patients with different cancer types receiving RT (35 Gy/10 fx) to a metastatic lesion and 14 days of GM-CSF. Among these patients, 11 (26.8%) exhibited a clinical response distant to the radiation site.[118] The addition of GM-CSF or its gene vaccine (GVAX) to the combination therapy is being investigated in pancreatic cancer and other cancers (NCT02663440, NCT02677155, NCT02648282).[119–121]

As a critical approach to strengthen the anti-tumor immunity, cancer vaccines present tumor antigens on recombinant viral vectors. A phase II trial in 33 prostate cancer patients with prostate-specific antigen (PSA) ≥10 or a Gleason score of ≥7 or stage T2b to T3 receiving PSA-vaccine (adenoviral vector-mediated herpes simplex virus thymidine kinase, AdHSV-tk) and IMRT has been conducted.[122] After treatment, a sustained long-term PSA-specific T-cell response occurred and persisted for up to 8–12 months. Other trials reported that combination of cancer vaccines with CRT showed no dose-limiting toxicities in pancreatic cancer (NCT00638612) and malignant gliomas (NCT00751270).[123,124] There are various cancer vaccines applied in NSCLC, including BLP-25 anti-mucin 1 (MUC1), belagenpumatucel-L, TG4010 (modified virus of Ankara–MUC1–IL-2), CIMAvax epidermal growth factor (EGF), melanoma antigen-encoding gene A3 (MAGEA3), GM.CD40L, and NYESO.[125] Currently, there are two ongoing clinical trials in NSCLC patients, NCT03004183 and NCT02831933, using an oncolytic virus (adenovirus-mediated expression of herpes simplex virus thymidine kinase, ADV/HSV-tk) together with combination therapy of SBRT (30 Gy/5 fx) and ICI.[70,78]

Patient selection biomarkers

Selection of suitable patients for combination therapy is crucial. Previous studies have indicated that PD-L1 expression, tumor-infiltrating lymphocytes (TILs), TMB, neoantigens, and DNA MMR deficiency are candidate biomarkers for ICIs. However, currently, there are no validated biomarkers for responses to combination therapy. In the PACIFIC trial, the unstratified HR for disease progression or death is significantly different between the durvalumab group and control group in people with >1% PD-L1 expression (HR: 0.46, 95% CI: 0.33–0.61) and the HR showed no significant differences in people with <1% PD-L1 expression.[56] On the other hand, patients with higher TMB, particularly those with MMR deficiency could be particularly sensitive to radiotherapy, causing a stronger anti-tumor immune response.[126] Furthermore, patients with lower tumor burden had better response with combination therapy.[105] When selecting the patients, the tumor doubling time should be evaluated and patients with a short tumor doubling time may not be suitable for combination therapy. Other possible factors for patient selection are unknown and may involve multiple levels, including genome, transcriptome, proteome, immunome, or microbiome.

Conclusions

Emerging preclinical and clinical data support combination of ICIs with RT. The results of ongoing trials have reported the safety and efficacy data of combination therapy; however, the optimal arrangement of the two therapies has not been identified, especially the design of RT. Firstly, the studies show that multi-fraction is better than single-fraction, and AHFRT is better than CFRT. Secondly, irradiation of more immunogenic metastatic lesions rather than the primary tumor may bring better clinical benefits because of immunogenic differences between different organs. Otherwise, the importance of accurate gross tumor volume (GTV) has been weakened, as the irradiating part of the tumor still could trigger anti-tumor immunity. Thirdly, studies on sequencing of combination therapy have controversial results. Fourthly, development of biomarkers to predict the response to combination therapy would help to identify the target patients who are most likely to benefit from treatment, which would improve the cost-effectiveness of ICIs. More studies are needed to understand the synergistic effect of the combination therapy. Finding the best way to maximize the immunity of hosts is the next step in the area of immuno-oncology.

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Conflict of interest statement

None declared.

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