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REVIEW

Combined treatment of non-small cell lung cancer using radiotherapy and immunotherapy: challenges and updates

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Abstract

The efficacy of immunotherapy for advanced non-small cell lung cancer (NSCLC) remains unsatisfactory, as the majority of patients either do not experience an objective response or acquire secondary resistance. As a result, several methods to enhance the systemic efficacy of immunotherapy have been investigated, including a large area of active research by combining immunotherapy with radiation therapy (RT). Given the rapidly burgeoning concept of combining immunotherapy and RT for increasing therapeutic benefit, we review the progress in this field thus far and explore further avenues for enhancing this combination. This review commences with a discussion of the only two existing randomized trials (and a pooled analysis) showing that the addition of RT to immunotherapy improves the abscopal response rate, progression-free survival, and overall survival in metastatic NSCLC patients. We then discussed factors and biomarkers that may be associated with a proportionally greater benefit to additional RT, such as low programmed cell death protein ligand 1 (PD-L1) status, tumor mutational burden (TMB), and patient's immune function. Next, the implementation of RT to overcome immunotherapy resistance is discussed, including a mechanistic discussion and methods with which these mechanisms could be exploited. Lastly, the emerging role of low-dose RT is discussed, which

Abbreviations: ACR, abscopal control rate; ARR, abscopal response rate; cGAS, cyclic GMP-AMP synthase; DC, dendritic cell; DCs, dendritic cells; dMMR, mismatch repair-deficient; dsDNA, double-stranded DNA; ICIs, immune checkpoint inhibitors; IFNβ, interferonβ; iNOS$^+$, inducible nitric oxide synthase-positive; iRT, immunoradiotherapy; iRT, immunotherapy combined with radiotherapy; LDI, low-dose irradiation; MDACC, MD Anderson Cancer Center; NKI, Netherlands Cancer Institute; NSCLC, non-small cell lung cancer; OS, overall survival; OXPHOS, novel oxidative phosphorylation; PD-1, programmed cell death protein 1; PD-L1, programmed cell death protein ligand 1; PFS, progression-free survival; RT, radiation therapy; SBRT, stereotactic body RT; STING, stimulator of interferon genes; TAAs, tumor-associated antigens; TMB, tumor mutational burden

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may help to overcome inhibitory signals in the tumor stroma that limit T-cell infiltration. Taken together, given the current state of this rapidly expanding realm, these futuristic strategies may be reflected upon to further enhance the efficacy of immunotherapy for a wider group of patients.

KEYWORDS
immune checkpoint inhibitors, immunotherapy, immunotherapy combined with radiotherapy, low-dose radiotherapy, non-small cell lung cancer, radiotherapy

1 | BACKGROUND

Immunotherapy, most notably immune checkpoint inhibitors (ICIs) such as anti-programmed cell death protein 1/programmed cell death protein ligand 1 (PD-1/PD-L1) compounds, have improved the survival of patients with advanced non-small-cell lung cancer (NSCLC) [1–4]. However, the efficacy of ICIs remains unsatisfactory, as a majority of patients do not experience an objective response [1, 5] and most patients initially develop primary resistance or acquire secondary resistance soon after therapy [6, 7]. As a result, many methods to enhance the systemic efficacy of ICIs have been explored [8–10], and a large area of active research is to combine ICIs with radiation therapy (RT), termed immunoradiotherapy (iRT) [11–13].

The concept of RT enhancing the effects of systemic therapy is known as the "abscopal effect". Although this phenomenon has been known for decades [14], it is also known that RT-induced abscopal responses are rare. The first case detailing an abscopal effect produced by iRT was reported in 2012 [15].

iRT could potentially be utilized for any stage of NSCLC. For metastatic cases, there is randomized evidence to support the addition of RT to immunotherapy alone [16–18]. For locally advanced non-metastatic cases, the randomized PACIFIC trial demonstrated the efficacy of combining definitive RT with subsequent immunotherapy [19]. Lastly, for early-stage NSCLC, there are a number of randomized trials aiming to evaluate stereotactic RT with or without adjuvant immunotherapy (e.g., NCT03110978, NCT03446547, NCT03833154, NCT03924869, NCT04214262). Representative clinical trials for NSCLC are presented in Table 1.

Given this rapidly burgeoning concept of combining immunotherapy and RT for further therapeutic benefit, we herein review the progress in this field thus far and explore further avenues to further enhance this combination. We first provide a discussion of the only two existing randomized trials of immunotherapy with or without RT. Next, we discuss factors and biomarkers associated with a potentially higher benefit to adding RT to immunotherapy. Then, we describe the utility of RT for overcoming immunotherapy resistance. Lastly, we review the emerging role of low-dose RT in efforts to promote immune infiltration of tumor tissue.

2 | CURRENT STATUS

Currently, there are only two published randomized trials evaluating immunotherapy with or without RT for metastatic NSCLC, the PEMBRO-RT study from the Netherlands Cancer Institute (NKI) [16], and the phase I/II trial from the MD Anderson Cancer Center (MDACC) [18]. The NKI PEMBRO-RT randomized trial showed that anti-PD-1 antibodies combined with stereotactic body RT (SBRT) produced a non-significant trend towards better response rate than anti-PD-1 antibodies alone ($P = 0.07$), particularly in PD-L1-negative patients. In the MDACC trial, although the survival rates of patients treated with SBRT or traditional radiotherapy were not different from the overall population ($n = 80$), SBRT was associated with increased treatment response rate and improved progression-free survival (PFS).

Although a beneficial trend for iRT was found in both studies, it was not statistically significant because of the small sample sizes in both trials ($n = 72$ in NKI trial [16] and $n = 80$ in MDACC trial [18]). As such, a pooled analysis of these two clinical trials was performed to better evaluate response rates and PFS [17]. Overall, 148 patients were included in the final analysis. The iRT cohort was found to experience a higher abscopal response rate (ARR) (41.7%) as compared to the immunotherapy alone group (19.7%) ($P = 0.0039$). There were also significant advantages for abscopal control rate (ACR) (65.3% vs. 43.4%, $P = 0.0071$). The improved control of systemic disease translated to a higher PFS (9.0 months vs. 4.4 months, $P = 0.045$) and overall survival (OS) (19.2 months vs. 8.7 months, $P = 0.0004$) with iRT. Additionally, from that pooled analysis, an exploratory subgroup analysis of different radiotherapy regimens showed that the ARR in

TABLE 1 Representative ongoing or completed clinical trials using PD-1/PD-L1/CTLA-4 inhibitors and RT for NSCLC

ClinicalTrials.gov identifier	Trial Phase	Drug classification	Inventions	Sponsors	Estimated/ Actual study completion date	Status
Early-stage NSCLC						
NCT03801902	1	PD-L1 inhibitors	Arm I: 13 cycles × durvalumab with ACRT (60 Gy in 15 fractions) Arm II: 13 cycles × durvalumab with standard RT (60 Gy in 30 fractions)	National Cancer Institute (NCI)	Dec 31, 2021	Active, not recruiting
NCT03383302	1/2	PD-1 inhibitors	1 year × nivolumab following SBRT (54 Gy in 3 fractions or 55 Gy in 5 fractions)	Royal Marsden NHS Foundation Trust	Jan 01, 2022	Recruiting
NCT03110978	2	PD-1 inhibitors	Arm I: 1-2 weeks × SBRT Arm II: 1-3 cycles × nivolumab with 1-2 weeks × SBRT	M.D. Anderson Cancer Center	Jun 30, 2022	Recruiting
NCT03148327	1/2	PD-L1 inhibitors	Arm I: 5 cycles × durvalumab with SBRT (54Gy, 50Gy or 65Gy in 3, 4 or 10 fractions) Arm II: SBRT (54Gy, 50Gy or 65Gy in 3, 4 or 10 fractions)	Jonsson Comprehensive Cancer Center	Jun 01, 2023	Active, not recruiting
NCT03924869	3	PD-1 inhibitors	Arm 1:17 cycles × pembrolizumab with SBRT (45-54 Gy in 3-5 fractions) Arm 2:17 cycles × placebo with SBRT (45-54 Gy in 3-5 fractions)	Merck Sharp & Dohme Corp	Jul 01, 2026	Recruiting
NCT04271384	2	PD-1 inhibitors	3 cycles × nivolumab with SAR (54 Gy in 3 fractions or 50 Gy in 5 fractions or 60 Gy in 8 fractions)	Hospital Israelita Albert Einstein	Jun 29, 2023	Recruiting
NCT03833154	3	PD-L1 inhibitors	Arm 1:24 months × durvalumab with SBRT (in 3, 4, 5 or 8 fractions) Arm 2:24 months × placebo with SBRT (in 3, 4, 5 or 8 fractions)	AstraZeneca	Oct 31, 2025	Recruiting
Locally-advanced NSCLC						
NCT03801902	1	PD-L1 inhibitors	Arm I: 13 cycles × durvalumab with ACRT (60 Gy in 15 fractions) Arm II: 13 cycles × durvalumab with standard RT (60 Gy in 30 fractions)	National Cancer Institute (NCI)	Dec 31, 2021	Active, not recruiting
NCT04013542	1	PD-1 and CTLA-4 inhibitors	Concurrent therapy:8 cycles × nivolumab and 4 cycles × ipilimumab with 6-7 weeks × RT Maintenance therapy:8 cycles × nivolumab	M.D. Anderson Cancer Center	Feb 01, 2022	Recruiting
NCT03818776	1	PD-L1 inhibitors	Arm I: 13 cycles × durvalumab with Proton beam therapy RT (60 CGyE in 20 fractions) Arm II: 13 cycles × durvalumab with Proton beam therapy RT (69 CGyE in 23 fractions)	Case Comprehensive Cancer Center	Nov 01, 2022	Recruiting

(Continues)

TABLE 1 (Continued)

ClinicalTrials.gov identifier	Trial Phase	Drug classification	Inventions	Sponsors	Estimated/ Actual study completion date	Status
NCT04765709	2	PD-1 inhibitors	Part 1: Induction with durvalumab and platinum-based chemotherapy (cisplatin or carboplatin plus vinorelbine or pemetrexed) Part 2: Eligible for durvalumab and RT Part 3: Eligible for durvalumab maintenance	Mario Negri Institute for Pharmacological Research	Jun 01, 2026	Not yet recruiting
NCT03519971	3	PD-L1 inhibitors	Arm I: Durvalumab + platinum-based chemotherapy (cisplatin/etoposide, carboplatin/paclitaxel, pemetrexed/cisplatin, pemetrexed/carboplatin) and RT Arm II: Placebo + platinum-based chemotherapy (cisplatin/etoposide, carboplatin/paclitaxel, pemetrexed/cisplatin, pemetrexed/carboplatin) and RT	AstraZeneca	Nov 13, 2023	Active, not recruiting
NCT03523702	2	PD-1 inhibitors	PembroRT Cohort: 15 cycles × pembrolizumab with 4 weeks × RT ChemoRT Cohort: Chemotherapy (carboplatin and paclitaxel) with 4-7 weeks × RT	Albert Einstein College of Medicine	Sep 01, 2022	Recruiting
NCT04230408	2	PD-L1 inhibitors	Induction chemo-immunotherapy phase: 2 cycles × paclitaxel, carboplatin and durvalumab Concurrent chemo-immuno-radiotherapy phase: RT with paclitaxel, carboplatin and durvalumab Consolidation immunotherapy:12 cycles × durvalumab	Latin American Cooperative Oncology Group	May 01, 2024	Recruiting
NCT03102242	2	PD-L1 inhibitors	Induction immunotherapy:4 cycles × atezolizumab Chemoradiotherapy:6 cycles × carboplatin and paclitaxel with RT (60 Gy in 30 fractions) Adjuvant immunotherapy: 1 year × atezolizumab	Alliance Foundation Trials, LLC	Mar 01, 2020	Active, not recruiting
NCT03237377	2	PD-L1 and CTLA-4 inhibitors	Arm 1: 3 cycles × durvalumab with RT (45Gy in 25 fractions) Arm 2: 3 cycles × durvalumab and tremelimumab with RT (45Gy in 25 fractions)	Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins	Sep 01, 2021	Recruiting

(Continues)

TABLE 1 (Continued)

ClinicalTrials.gov identifier	Trial Phase	Drug classification	Inventions	Sponsors	Estimated/ Actual study completion date	Status
NCT04597671	3	PD-L1 inhibitors	Arm I: Durvalumab with low-dose PCI (15 Gy in 10 fractions) Arm II: Durvalumab with observation	Association NVALT Studies	Nov 01, 2027	Not yet recruiting
NCT03774732	3	PD-1 inhibitors	Arm 1: Pembrolizumab and chemotherapy (carboplatin/paclitaxel, cisplatin/pemetrexed, carboplatin/pemetrexed) Arm 2: Pembrolizumab and chemotherapy (carboplatin/paclitaxel, cisplatin/pemetrexed, carboplatin/pemetrexed) with 3D-CRT (18 Gy in 3 fractions) or SBRT	UNICANCER	May 15, 2023	Recruiting
NCT04577638	2	PD-1 inhibitors	3 cycles × nivolumab with IMRT (66 Gy in 24 fractions)+ 6 months × nivolumab maintenance	Center Eugene Marquis	April 1, 2023	Not yet recruiting
Advanced NSCLC						
NCT03168464	1/2	PD-1 and CTLA-4 inhibitors	Ipilimumab and nivolumab with RT (30 Gy in 5 fractions)	Weill Medical College of Cornell University	Dec 30, 2022	Recruiting
NCT03158883	1	PD-L1 inhibitors	Avelumab with SAR (50 Gy in 5 fractions)	Megan Daly, MD	Jun 01, 2022	Recruiting
NCT03275597	1	PD-L1 and CTLA-4 inhibitors	Durvalumab and tremelimumab with SBRT (30 - 50 Gy in 5 fractions)	University of Wisconsin, Madison	Oct 01, 2022	Recruiting
NCT03035890	Not Applicable	PD-1 or PD-L1 inhibitors	RT (24-45 Gy in 3 fractions or 30-50 Gy in 5fractions) with nivolumab or pembrolizumab or atezolizumab	West Virginia University	Jun 30, 2023	Active, not recruiting
NCT04081688	1	PD-L1 inhibitors	18 cycles × atezolizumab and varlilumab with 2 cycles × SBRT	Rutgers, The State University of New Jersey	Jun 30, 2023	Recruiting
NCT03825510	Not Applicable	PD-1 inhibitors	Nivolumab or pembrolizumab with SBRT	Crozer-Keystone Health System	Aug 28, 2021	Recruiting
NCT03915678	2	PD-L1 inhibitors	Atezolizumab and BDB001 with RT (27-60 Gy in 3-5 fractions)	Institut Bergonié	Mar 01, 2025	Not yet recruiting
NCT03774732	3	PD-1 inhibitors	Arm 1:Pembrolizumab and chemotherapy (carboplatin/paclitaxel, cisplatin/pemetrexed, carboplatin/pemetrexed)	UNICANCER	May 15, 2023	Recruiting

(Continues)

TABLE 1 (Continued)

ClinicalTrials.gov identifier	Trial Phase	Drug classification	Inventions	Sponsors	Estimated/ Actual study completion date	Status
			Arm 2:Pembrolizumab and chemotherapy (carboplatin/paclitaxel, cisplatin/pemetrexed, carboplatin/pemetrexed) with 3D-CRT (18 Gy in 3 fractions) or SBRT			
NCT03509584	1	PD-1 and CTLA-4 inhibitors	Part 1a: Nivolumab with hypofractionated RT (24 Gy in 3 fractions)(bone metatase) Part 1b: Nivolumab and ipilimumab with hypofractionated RT (24 Gy in 3 fractions)(bone metatase) Part 2a: Nivolumab with hypofractionated RT (24 Gy in 3 fractions)(outside the brain) Part 2b: Nivolumab and ipilimumab with hypofractionated RT (24 Gy in 3 fractions)(outside the brain)	Assistance Publique Hopitaux De Marseille	April 2021	Not yet recruiting
NCT02221739	1/2	CTLA-4 inhibitors	3 cycles × ipilimumab with RT (IMRT or 3-D CRT)(30 Gy in 5 fractions or 28.5 Gy in 3 fractions)	NYU Langone Health	Oct 27, 2015	Completed
NCT03223155	1	PD-1 and CTLA-4 inhibitors	Sequential Arm: SBRT (in 3-5 fractions)and nivolumab and ipilimumab Concurrent Arm: Nivolumab and ipilimumab with SBRT (in 3-5 fractions)	University of Chicago	Dec 01, 2024	Recruiting
NCT02888743	2	PD-L1 and CTLA-4 inhibitors	Arm I:4 cycles × tremelimumab and 13 cycles × durvalumab Arm II: 4 cycles × tremelimumab and 13 cycles × durvalumab with High-dose RT Arm III: 4 cycles × tremelimumab and 13 cycles × durvalumab with Low-dose RT	National Cancer Institute (NCI)	Dec 31, 2021	Active, not recruiting
NCT02463994	1	PD-L1 inhibitors	MPDL3280A + HIGRT	University of Michigan Rogel Cancer Center	Nov 07, 2018	Completed

RT, Radiotherapy; ACRT, Accelerated Hypofractionated Radiotherapy; SBRT, Stereotactic Body Radiotherapy; SAR, Stereotactic Ablative Radiotherapy; PCI, Prophylactic Cranial Irradiation; 3D-CRT, Conformal 3D Radiotherapy; IMRT, Intensity-Modulated Radiotherapy; HIGRT, Hypofractionated Image-guided Radiotherapy.

SBRT patients was higher than that of non-stereotactic dosing. This pooled analysis publication remains the only known study showing an improvement in survival-related endpoints with the addition of RT to immunotherapy for metastatic NSCLC. However, those findings (as well as translational/correlational data [10]) illustrate that, even with iRT, the efficacy was only 41.7%, highlighting the need for further optimization.

3 | PATIENT SELECTION AND BIOMARKERS: UPDATES AND CHALLENGES

It is intuitive that the addition of RT to immunotherapy may not benefit all patients to the same degree. Several clinicopathological factors may be associated with a proportionally greater degree of response to immunotherapy. The standard factors of age, performance status, and disease burden may also play an important role in patient selection, especially given the emerging role of consolidative RT for oligometastatic NSCLC.

Additionally, PD-L1 testing is currently the most accepted biomarker of treatment response for immunotherapy alone [20–22]. It is often hypothesized that RT may benefit patients with low PD-L1 to a greater degree because the response to immunotherapy alone in high expressors of PD-L1 may be considerably higher than that of cases with low PD-L1 expression levels [17, 23]. As a result, because immunotherapy alone seems to provide a higher degree of local effects for the former, RT may be more often required to control disease in the latter.

The tumor mutational burden (TMB) may be an additional important biomarker of immunotherapy response, and a correlation between this marker and response rates to anti-PD-1 or anti-PD-L1 therapy has been demonstrated across several tumor types [24–27]. TMB is defined as the total number of mutations, including both base substitutions and short insertions/deletions, per coding area of the tumor genome. However, TMB is not as widely utilized or validated as compared to PD-L1 status, and it remains currently unclear whether this should be utilized as a robust marker for patient selection.

Lastly, another emerging area of further research is whether a given patient's immune function may relate to the benefit from iRT. Adequate immune function (especially lymphocytic function) is required to exert the downstream effects of iRT, and data suggest that patients without adequate immune function (e.g. lymphopenia) are at lower risk of deriving a benefit from iRT [28, 29]. However, the data have not been well validated by larger and more robust datasets.

In summary, enhancing the efficacy of iRT may be done by proper patient selection. The clinicopathological factors mentioned above may assist in performing more careful patient selection for iRT, thereby improving its efficacy and patients' outcomes.

4 | USING RADIOTHERAPY TO OVERCOME IMMUNOTHERAPY RESISTANCE: UPDATES AND CHALLENGES

RT may play an important role in advanced NSCLC based on four major pieces of evidence. First, RT has been associated with improved survival in patients with oligometastatic NSCLC [30, 31]. Second, RT can increase the release and presentation of antigens, thereby enhancing dendritic cell (DC) function, augmenting T cell sensitization, and promoting antitumor immune responses [32–35]. Third, RT can regulate the tumor microenvironment and increase the infiltration of cytotoxic $CD8^+$ T lymphocytes, which play a key role in the antitumor immune response [36]. Finally, RT reduces immunotherapy resistance by reshaping the tumor microenvironment [37]. Traditional theory suggests that ionizing RT mainly damages DNA to kill tumor cells [38, 39]. However, an alternative concept is that radiotherapy can control distant metastatic lesions outside the irradiated field in addition to local control, which was coined the "abscopal effect" and was initially proposed in 1953 [14]. The theoretical basis for this effect is that the tumor cells killed by RT serve as an in situ tumor vaccine by releasing tumor-associated antigens (TAAs). These are then captured by dendritic cells (DCs), which then activate $CD8^+$ T cells that home in to tumors, activate systemic immunogenicity, induce abscopal effects, and control tumor proliferation [40–42] (Figure 1).

Conventionally fractionated radiotherapy has historically been the preferred approach to treat NSCLC, especially in the definitive setting [43]. However, with the improvement of radiotherapeutic technologies, image guidance, and radiation physics, hypofractionated radiotherapy has become widely administered in patients with tumors of appropriate size and location, such as tumors of up to 5-7 cm in size and not overlapping the mediastinal organs such as the trachea or esophagus. Hypofractionation, especially with stereotactic RT, may allow reduced dose exposure to uninvolved areas of the cardiopulmonary system and thus better preserve the absolute lymphocyte count in efforts to induce a stronger abscopal response [29].

Preclinical studies have confirmed that the abscopal effect can occur in immunocompetent settings, but not in immunodeficient conditions [44, 45]. Such studies have revealed that antitumor immunity is the key

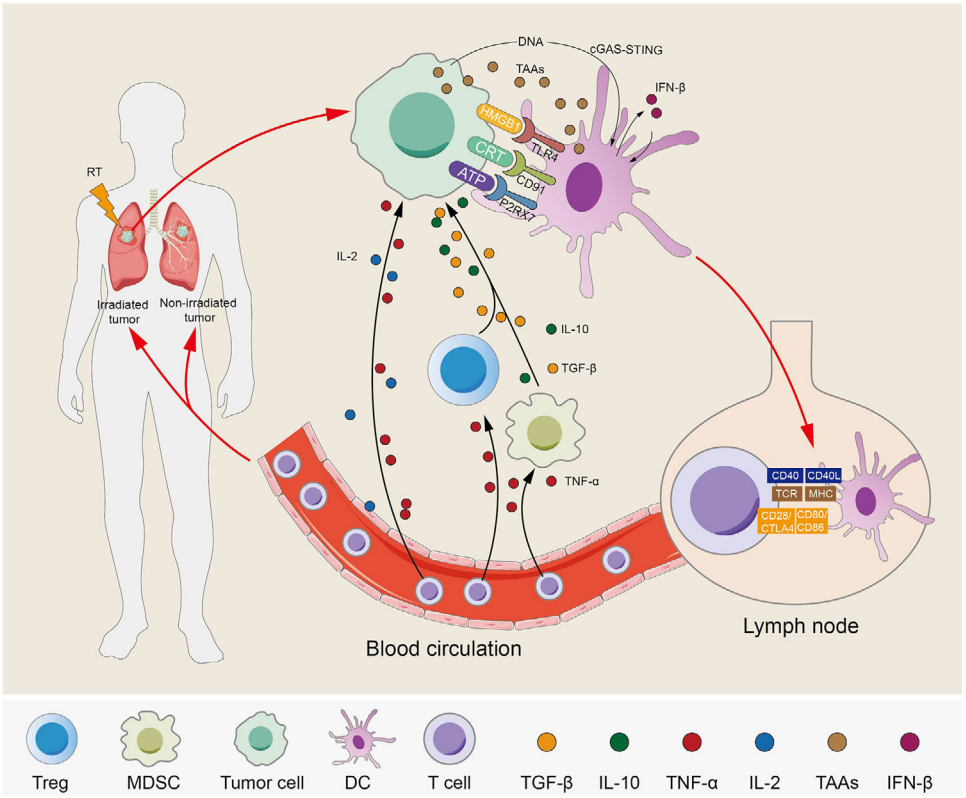

FIGURE 1 Radiotherapy-induced effects on tumor cells. Radiotherapy (RT) induces immunogenic death of tumor cells which increases the release of tumor-associated antigens (TAAs) and damage-associated molecular patterns such as high-mobility group box 1 (HMGB1) and adenosine triphosphate (ATP), and enhances the surface expression of calreticulin (CRT). Secretion promotes the activation and maturation of dendritic cells (DCs) through their corresponding receptors. DCs that sense cancer cell-derived DNA induce interferon-β (IFN-β) production through the cyclic GMP-AMP synthase (cGAS)- stimulator of interferon genes (STING) pathway. In turn, IFN-β promotes the activation and maturation of DCs. DCs take up tumor-associated antigens (TAAs) and migrate to draining lymph nodes and then present the TAAs on major histocompatibility complex class I (MHCI) to T cells through the T-cell receptor (TCR), which requires the costimulatory molecules CD80/86-CD28/cytotoxic T lymphocyte-associated protein 4 (CTLA4) and CD40L-CD40. Otherwise, these are not sufficient to cause T cell activation and proliferation in the absence of costimulatory signals. Activated T cells are transported to irradiated lesions and distant nonirradiated lesions through the blood circulation. At the same time, tumor cell immunogenic death leads to the release of cytokines, the immune-promoting factors tumor necrosis factor-α (TNF-α) and interleukin-2 (IL-2) recruit activated T cells to kill tumor cells through upregulated MHCI, and the immunosuppressive factors such as TGF-β and IL-10 recruit immunosuppressive cells such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) to inhibit immune effects. However, activated T cells cause the apoptosis of Tregs and MDSCs through cytokines such as TNF-α. Abbreviations: Radiotherapy, RT; tumor-associated antigens, TAAs; high-mobility group box 1, HMGB1; adenosine triphosphate, ATP; calreticulin, CRT; dendritic cells, DCs; interferon-β, IFN-β; cyclic GMP-AMP synthase, cGAS; stimulator of interferon genes, STING; tumor-associated antigens, TAAs; major histocompatibility complex class I, MHC1; T-cell receptor, TCR; Cytotoxic T lymphocyte-associated protein 4, CTLA4; tumor necrosis factor-α, TNF-α; interleukin-2, IL-2; Myeloid-derived suppressor cells, MDSCs; P2X7 receptor, P2RX7; transforming growth factor-β, TGF-β; regulatory T cells, Tregs

factor affecting the efficacy of radiotherapy. For example, in a syngeneic mouse model of fibrosarcoma, the radiotherapy dose needed to control tumors in immunocompetent mice was lower than that needed for immunodeficient mice [44]. Similarly, in another investigation, mouse melanoma B16 tumors implanted into immunocompetent hosts responded to high doses of radiation but tumors grown in immunocompromised hosts did not respond to radiotherapy and were more susceptible to metastasis [46].

Distinct immune therapies might differentially affect primary and abscopal tumor responses. For example, vaccination is an emerging field of research that has brought promising results for the future of immunotherapy. It is feasible to boost RT-induced immune responses and to achieve immunosuppression by inhibiting immunosuppressive molecules, such as PD-1, with activated whole tumor cell vaccines [47]. Additionally, recent studies have reported that elevated levels of novel oxidative phosphorylation (OXPHOS) in tumors are an important factor associated with immunotherapy efficacy [48, 49]. Furthermore, anti-PD-1 antibodies or radiotherapy can increase OXPHOS levels [49–51]. Therefore, combination treatment

with radiotherapy and OXPHOS inhibitors could also be an effective strategy against PD-1 resistance in NSCLC [52].

Preclinical studies have demonstrated that high-dose radiation may cause the accumulation of endogenous cytosolic DNA, resulting in the activation of the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway [53, 54]. DCs in the tumor microenvironment can take up double-stranded DNA (dsDNA) from dying tumor cells after radiotherapy, although the detailed mechanisms remain debatable, subsequently activating the cGAS-STING interferonβ (IFNβ) pathway in DCs and evoking an immune response [53, 55, 56]. However, following radiotherapy, cancer cells also produce adequate cytosolic dsDNA which comes from damaged mitochondria, binds endogenous cGAS, activates the downstream STING-IFNβ pathway, and promotes tumor immunity [57, 58]. Additionally, an intact cGAS-STING pathway in irradiated tumor cells is indispensable for irradiation-provoked abscopal effects. Although anti-PD-1/PD-L1 immunotherapy depends on adequate T cell function in the tumor microenvironment, Fu et al. [59] demonstrated that, in the context of a mismatch repair-deficient (dMMR) background, a competent cGAS-STING-IFNβ pathway within tumor cells is required for tumor suppression.

In brief, better knowledge of the immune mechanisms of radiotherapy may result in more efficient and effective use of RT, and additionally provide information for the design of iRT studies, opening up new avenues for cancer therapy.

5 | LOW-DOSE RT TO BOOST THE EFFICACY OF iRT: UPDATES AND CHALLENGES

A major deterrent to the efficacy of immunotherapy is that the tumor microenvironment is suboptimally conducive to T cell engraftment. Vascular barriers, lack of appropriate cytokines, and stromal immunosuppressive factors may play important roles in inhibiting T cell infiltration and exerting an antitumor effect [60, 61]. High-dose RT (conventionally fractionated or hypofractionated (including SBRT)) helps to induce the production and release of cytokines and chemokines by killing tumor cells, thereby creating an inflammatory microenvironment in the context of immunogenic cell death to promote T cell infiltration [62–64]. However, high-dose RT cannot largely address potent immunosuppressive factors such as the inhibitory tumor stroma. As a result, there has been a recently posited theory that low-dose irradiation (LDI) may address these limitations. There is no standard definition of LDI but it most commonly involves intentional delivery of 0.5-2 Gy per fraction up to 1-10 Gy total dose [65–68], which is canonically thought to be non-tumoricidal.

Radiobiological data of LDI also support additional synergistic effects of LDI. Studies using dynamic microscopic imaging, a technique that allows experiments to be visualized in real-time, have confirmed that X-rays ranging from 0.1 Gy can also kill some tumor cells owing to a phenomenon called radiotherapy hypersensitivity [69–71]. In some clinical studies, LDI used in combination with chemotherapy and administered at a dose to induce radiation hypersensitivity has achieved surprising rates of tumor control [72–74]. Therefore, the combination of LDI with other treatments, such as chemotherapy or immunotherapy, may be a new therapeutic approach for patients.

Recent studies in mouse models have shown that a single fraction of LDI can reshape the tumor microenvironment, including the polarization of M1 macrophages [75, 76]. Inducible nitric oxide synthase-positive (iNOS$^+$) M1 macrophages can produce chemokines to recruit effector T cells and cause normalization of tumor vessels and the inflammatory response, inducing T cell infiltration [77–79], as shown in Figure 2. The clinical benefit conferred by LDI-mediated remodeling of macrophages was verified in a retrospective study of pancreatic cancer patients who had previously received LDI as neoadjuvant therapy. In these patients, LDI could significantly increase the ratio of iNOS$^+$ macrophages to CD8$^+$ T cells, and reduce the average diameter of tumor blood vessels [77]. By contrast, LDI can actually attenuate inflammatory lesions, as observed in patients with benign inflammation or degenerative diseases caused by autoimmune T cells [80]. Therefore, further clinical studies comparing the effects of LDI on the tumor microenvironment are required to confirm the findings in mouse models and establish an optimal range of LDI doses that can be used to reshape macrophages and improve T cell infiltration.

Although it has been proven that in situ vaccine effects and abscopal effects are triggered by relatively high doses of hypofractionated radiotherapy, no direct evidence has indicated that LDI can trigger the same effects. However, LDI can remodel the tumor microenvironment and facilitate T cell homing in patients lacking tumor-infiltrating CD8$^+$ T cells [77, 81]. LDI may be important in the preparation phase of inducing T cell homing in combination with ICI therapy, as described above. Comparing the efficacy of different LDI schemes in combination with ICIs must be investigated in future clinical trials. Such strategies could be used as a palliative option for patients who are refractory to other treatments, including ICIs, which can reshape the tumor microenvironment to induce new antitumor responses. Eventually, a combination of high-dose SBRT used to trigger an in situ vaccine effect in a few

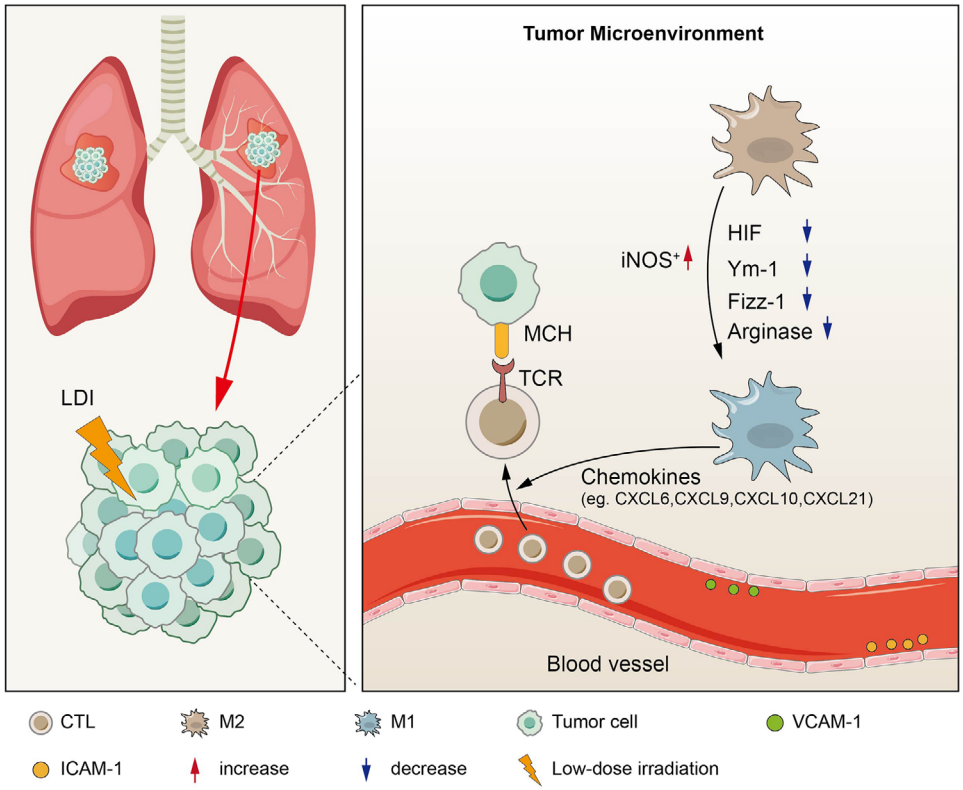

FIGURE 2 Low-dose irradiation remodels the tumor microenvironment. Two main mechanisms exist by which radiation enhances tumor-infiltrating lymphocytes (TILs). One is increased expression of chemokines that enhance immune cell migration and invasion, and the other relates to changes in the vascular endothelium that enhance immune cell extravasation. Low-dose irradiation (LDI) induces M1 macrophage polarization by regulating the corresponding molecules, such as inducible nitric oxide synthase-positive (iNOS$^+$), Hypoxia-inducible factor-1 (HIF-1), chitinase-like-3 (Ym-1), Found in the inflammatory zone-1 (Fizz-1), Arginase, and iNOS$^+$ M1 macrophages, which produce chemokines to recruit effector T cells and cause T cell infiltration. LDI increases vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) expression in human vascular endothelial cells, causing normalization of tumor vessels. Abbreviations: low-dose irradiation, LDI; tumor-infiltrating lymphocytes, TILs; inducible nitric oxide synthase-positive, iNOS$^+$; Hypoxia-inducible factor-1, HIF-1; chitinase-like-3, Ym-1; found in inflammatory zone-1, Fizz-1; M1 macrophages, M1; M2 macrophages, M2; vascular cell adhesion molecule 1, VCAM-1; intercellular adhesion molecule-1, ICAM-1; major histocompatibility complex, MHC; T-cell receptor, TCR; cytotoxic T-lymphocyte, CTL; C-X-C motif chemokine, CXCL

metastatic lesions, together with LDI used to target other metastatic lesions to promote T cell attack, could maximize the abscopal effect.

Although LDI cannot kill tumor cells, it can activate immune cells and regulate the tumor microenvironment to improve the efficacy of immunotherapy. A recently completed clinical trial in which SBRT in combination with ipilimumab was used to treat advanced metastatic lesions found that tumors exposed to LDI (due to proximity to the target tumor) were more likely to respond than lesions distant from the targeted tumor [82]. Based on this finding, our team has developed a new treatment paradigm combining high-dose radiotherapy and LDI to promote the efficacy of systemic immunotherapy [66]. In this model, high-dose radiation aims to increase antigen release and presentation and promote immune cell activation, while LDI aims to promote immune cell infiltration into the stroma and tumor bed of distant tumors. The results of a nonrandomized phase II trial using both LDI and high-dose RT in conjunction with immunotherapy showed that the areas of disease exposed to LDI more often responded locally than those not subjected to LDI [65].

Taken together, LDI offers an emerging approach to address the known mechanistic limitations of higher-dose RT as part of an iRT paradigm. Much more extensive investigation is required, but nevertheless, this approach remains an important method to base future study.

6 | FURTHER CHALLENGES

Although iRT shows promising efficacy for clinical application, the treatment efficacy still needs to be further optimized. Additionally, exploring the optimized dose and

fractionation of radiotherapy, sequencing of therapies, and further exploring the mechanistic interaction between radiotherapy and immunotherapy may provide more effective combined therapeutic options in the future.

7 | CONCLUSIONS

This review assessed and discussed several strategies to enhance the efficacy of combining RT and immunotherapy for advanced NSCLC. These include better elucidation on clinical and pathologic biomarkers that may improve patient selection, along with an increased mechanistic understanding of using RT to overcome immunotherapy resistance, as well as low-dose RT to enhance immune infiltration into tumors. Taken together, given the current state of this rapidly expanding realm, these futuristic strategies may be reflected upon to further enhance the efficacy of immunotherapy for a wider group of patients than currently exists.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

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