Review Article

Radiotherapy for unresectable locally advanced non-small cell lung cancer: a narrative review of the current landscape and future prospects in the era of immunotherapy

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Abstract: Significant recent advances have occurred in the use of radiation therapy for locally advanced non-small cell lung cancer (LA-NSCLC). In fact, the past few decades have seen both therapeutic gains and setbacks in the evolution of radiotherapy for LA-NSCLC. The PACIFIC trial has heralded a new era of immunotherapy and has raised important questions for future study, such as the future directions of radiation therapy for LA-NSCLC in the era of immunotherapy. Modern radiotherapy techniques such as three-dimensional (3D) conformal radiotherapy and intensity-modulated radiotherapy (IMRT) provide opportunities for improved target conformity and reduced normal-tissue exposure. However, the low-dose radiation volume brought by IMRT and its effects on the immune system deserve particular attention when combing radiotherapy and immunotherapy. Particle radiotherapy offers dosimetric advantages and exhibits great immunoregulatory potential. With the ongoing improvement in particle radiotherapy techniques and knowledge, the combination of immunotherapy and particle radiotherapy has tremendous potential to improve treatment outcomes. Of particular importance are questions on the optimal radiation schedule in the settings of radio-immunotherapy. Strategies for the reduction of the irradiated field such as involved-field irradiation (IFI) and omission of clinical target volume (CTV) hold promise for better preservation of immune function while not compromising locoregional and distant control. In addition, different dose-fractionation regimens can have diverse effects on the immune system. Thus, prospective trials are urgently needed to establish the optimal dose fractionation regimen. Moreover, personalized radiotherapy which allows the tailoring of radiation dose to each individual's genetic background and immune state is of critical importance in maximizing the benefit of radiation to patients with LA-NSCLC.

Keywords: Immunotherapy; lung cancer; radiation therapy

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Introduction

Treatment of locally advanced non-small cell lung cancer (LA-NSCLC) is one of the greatest challenges facing oncologists. The role of chemotherapy and curative-intent radiotherapy is well recognized as the gold standard treatment for LA-NSCLC (1). Nonetheless, conventional treatment options for LA-NSCLC tend to reach a therapeutic plateau with suboptimal clinical outcomes (2,3). Recently, the encouraging results of the PACIFIC trial, a multicenter randomized phase III trial of PD-L1 blockade durvalumab versus placebo in patients with non-progressive LA-NSCLC after concurrent chemoradiotherapy, have heralded a new era of immunotherapy in the treatment of LA-NSCLC (4). The combination of radiotherapy and immune checkpoint inhibitors (ICIs) significantly improved objective response rate, progression-free survival, and overall survival (OS), making it a new treatment paradigm in LA-NSCLC (4,5).

The revolutionary advances in the treatment of LA-NSCLC are the result of the advent of immunotherapy, which offers opportunities to augment antitumor immunity (6). Prominent recent progress in immunotherapies for NSCLC includes the development of ICIs (e.g., anti-CTLA-4 antibodies and anti-PD-1/PD-L1 antibodies), cytokines and cytokine blockers (e.g., GM-CSF, IL-2, and TGF-β blockade), oncolytic viruses (e.g., ADV/HSV-tk), and other targeted immunotherapies (e.g., OX-40 antibodies, Toll-like receptors (TLR) agonists, and IOD1 inhibitors) (6-11). To date, PD-1 inhibitors pembrolizumab and nivolumab, PD-L1 inhibitor atezolizumab, and CTLA-4 blockade ipilimumab combined with nivolumab have demonstrated impressive efficacy in prospective trials and are approved by the FDA for the treatment of metastatic NSCLC (12-15).

Meanwhile, we are also in the midst of one of the most exciting times of radiation oncology, in which technological advances are now enabling more accurate radiation delivery with limited exposure of surrounding normal tissues. The integration of radiotherapy with immunotherapy is expected to revolutionize cancer treatment, given that numerous murine studies have shown the synergistic antitumor effect of this combination strategy (16-18). Mechanistically, radiation can, via various mechanisms, stimulate antitumor immune response (6). For example, radiation-induced immunogenic cell death triggers the release of tumor antigens and damage-associated molecular patterns (DAMPs) and the production of type I interferons (IFNs) (19,20). Moreover, radiation initiates the production of pro-inflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin 1β (IL-1β), which triggers the infiltration of immune cells (e.g., dendritic cells and cytotoxic T cells) and results in an inflammatory tumor microenvironment (21-23). Radiation also enhances systemic immune activation which manifests itself as the abscopal effect, where tumor regression occurs in non-irradiated sites (24). This phenomenon is presumably attributable to the capacity of radiotherapy to convert the tumor into an in situ vaccine (25). Additionally, radiation induces the upregulation of PD-L1 on tumor cells (26), and thus integrating radiotherapy with anti-PD1/PD-L1 antibodies can overcome adaptive immune resistance. Notably, the use of immunotherapy in non-metastatic settings has recently garnered attention as growing preclinical data support the potential utility for ICIs to reduce metastatic relapse from localized disease (27,28). Furthermore, based on the strong evidence from the PACIFIC trial, PD-L1 inhibitor durvalumab has been approved by the FDA for patients with non-progressive LA-NSCLC after concurrent chemoradiotherapy. The success of the PACIFIC trial has increased enthusiasm for the integration of radiotherapy with immunotherapy for the treatment of LA-NSCLC.

However, radiotherapy is a double-edged sword to immunotherapy, involving not only the enhanced antitumor activity but also increased normal tissue toxicities and risks of lymphopenia. Radiation-induced normal tissue injury begins with a cascade of molecular events, including reactive oxygen species production and DNA damage (29). The subsequent release of DAMPs induces the release of pro-inflammatory cytokines (e.g., IL-1 and TNF-α) through the activation of the nuclear factor kappa-B (NF-κB) signaling pathway (30), leading to activation of resident immune cells and recruitment of pro-inflammatory cells (31). Given that immunotherapy helps boost the immune system, the combination of radiation with immunotherapy may increase the risk of normal tissue toxicities. In the PACIFIC trial (4), although the combination of radiotherapy and immunotherapy did not increase the incidence of serious side effects (≥ grade 3) such as pneumonitis, it increased the incidence of toxicities at all grades. Among the toxicities caused by the thoracic radiation plus ICI regimen, the most common studied overlapping toxicities are cardiotoxicity and pulmonary toxicity (32-34). In fact, both radiotherapy and immunotherapy carry the potential risk of cardiotoxicity and pulmonary toxicity (35-38). The synergistic interaction between immunotherapy and thoracic irradiation in

increasing the risk of pulmonary and cardiotoxicities has been proven in series preclinical models (32,33). In addition, radiation exposure is also known to have suppressive effects on the immune system (39). Lymphocytes, as part of systematic immune cells, are extremely sensitive to radiation exposure (40), and a single radiation dose of 1–3 Gy has been demonstrated to induce apoptosis in lymphocytes (41). Research has shown that radiation-induced lymphopenia (RIL) occurs in 40% to 70% of patients treated with radiotherapy, potentially attributed to the direct irradiation of lymph nodes and to circulating lymphocytes (CLs) traversing through the radiation field (42). Lymphocytes are heavily involved in the antitumor activity of immune system. The nadir of absolute lymphocyte counts (ALC) during radiotherapy has been shown to be associated with worse survival in NSCLC patients in multiple studies (43-45), and the depletion of lymphocytes is potentially linked with a lower likelihood of response to immunotherapy for NSCLC (46). Therefore, special attention is needed to better preserve lymphocyte function when combining immunotherapy with radiotherapy.

Since the pre-immunotherapy era, radiation therapy has evolved significantly. In fact, the past few decades have witnessed both therapeutic gains and setbacks in the development of radiotherapy for LA-NSCLC. The PACIFIC trial has opened new horizons for the management of LA-NSCLC, but has also raised important questions, including the future directions of radiation therapy for LA-NSCLC in the era of immunotherapy. Current radiotherapy for LA-NSCLC is not yet fully optimized for this combination strategy with many unanswered questions, including the appropriate choice of radiation techniques, appropriate radiation target volumes, and appropriate dose-fractionation regimen to combine with immunotherapy. Here, we review the current data with regard to the radiotherapy techniques (including photon-based radiotherapy and particle beam therapy) and strategies (including reduction of radiation target volumes and dose-fractionation regimen) for the treatment of LA-NSCLC with a particular focus on the future directions and challenges for radiotherapy in the era of immunotherapy. A literature search in MEDLINE and EMBASE was conducted (date of the last search 15 January 2020) to identify English-language publications on radiotherapy for LA-NSCLC and its combination with immunotherapy, supplemented by manual searches of the reference lists of identified articles and relevant reviews.

We present the following article in accordance with the Narrative Review checklist (available at http://dx.doi.org/10.21037/tlcr-20-511).

Radiation techniques

Photon-based radiotherapy

The paradigm shift from two-dimensional radiotherapy (2DRT) to advanced three-dimensional (3D)-based radiation techniques, including 3-dimensional conformal radiotherapy (3DCRT) and intensity-modulated radiotherapy (IMRT), allows for more accurate radiation delivery and limited exposure of adjacent critical structures. The theoretical advantages of 3DCRT mainly lie in its superior conformity in using computed tomography (CT) for treatment planning, considering the larger target volumes caused by the deficiency in precise visualization of target lesions on a 2D radiograph. IMRT can currently be delivered in static mode (fixed-field IMRT), volumetric-modulated arc therapy (VMAT), helical tomotherapy (HT), and other modalities. All forms of IMRT enable the intensity modulation of each beam and further improve target conformity with a substantial decrease in the doses to normal tissues (47-49). Consequently, in contrast to 2DRT, 3D-based radiation techniques can achieve improvement of local control with lower rates of treatment-related toxicity.

The evolution of radiation techniques from 2DRT to 3DCRT and to IMRT in turn, have revolutionized the treatment of LA-NSCLC. A National Cancer Data Base (NCDB) analysis reported that 3D-based radiation techniques, including 3DCRT and IMRT, were associated with a significantly improved OS compared with conventional 2DRT (3-year OS 22% *vs.* 19%; 5-year OS 14% *vs.* 11%, P<0.0001) (50). In terms of treatment-related toxicity, Yom and colleagues demonstrated a substantial reduction in the rates of grade ≥ 3 radiation pneumonitis at 1 year in patients treated with IMRT compared with 3DCRT (8% *vs.* 32%, P=0.002), despite the larger gross tumor volume (GTV) in the IMRT cohort (51). Liao and colleagues evaluated disease outcomes and rates of toxicity in patients treated with IMRT or 3DCRT combined with concurrent chemotherapy. OS for the IMRT group was superior to that of the 3DCRT group. Moreover, IMRT was associated with reduced rates of grade ≥ 3 radiation pneumonitis (52). Notably, given that the median radiation doses of 63 Gy were the same for both groups in this study (52), the survival benefit of IMRT over 3DCRT may be mainly attributed to reduction in doses to normal

structures and the consequent striking improvements in toxicity profiles. Reducing toxicity is a constantly recurring theme in the field of radiotherapy. Especially in the era of immunotherapy, given the synergistic toxicity of combined radiotherapy and immunotherapy, advanced radiation techniques for safer and more accurate radiation delivery will offer opportunities to reduce rates of toxicity and elevate the success of cancer treatment to a new level when combined with immunotherapy.

However, it is worth noting that improved sparing of organs at risk (OAR) of IMRT might come at the expense of more area being exposed to low-dose radiation. In fact, planning studies have shown that lung volume >5 Gy (V5) increased in the IMRT plans compared with 3DCRT plans (49,53). Another comparative study on the dosimetric features between fixed-field IMRT and HT reported significantly reduced lung V20–30 in the HT plan, together with larger volumes of low dose radiation (lung V5–10) (54). In the pre-immunotherapy era, multiple studies have focused on the potentially increased risk of pulmonary toxicity resulting from larger volumes of normal lung exposed to low-dose radiation (55). Two retrospective studies from the MD Anderson Cancer Center demonstrated that lung V5 was significantly associated with pneumonitis risks in patients receiving concurrent chemoradiotherapy (56,57). Apart from pulmonary toxicity, the risk of lymphopenia caused by the low-dose irradiation effect of IMRT will be of paramount concern in the era of immunotherapy. Tang and colleagues reported that lower dose radiation volume, especially lung V5–10, exhibited a greater association with lymphocyte nadirs than high dose ranges (43). Given the association of lower lymphocytes with poorer clinical outcomes (43,44,58) and the lower likelihood of response to immunotherapy (46), special attention should be given to the low-dose radiation volume offered by IMRT in the era of immunotherapy.

Particle beam therapy

Particle radiotherapy, which mainly includes proton and carbon-ion radiotherapy, has experienced a surge in attention as another promising treatment modality for LA-NSCLC. Compared with photon radiotherapy, the primary strength of particle therapy lies in its dose distribution capabilities. The particles release little energy during travel and deposit most of their energy near the end of their path, which is known as the Bragg peak phenomenon. The unique dose distribution characteristics allow for highly conformal and high-dose delivery to the tumor and the sparing of surrounding normal tissues. These qualities therefore make particle radiotherapy an attractive treatment option for LA-NSCLC in which the target lesion typically lies in close proximity to vital organs such as the heart, spinal cord, and esophagus. Multiple dosimetric studies have demonstrated the superiority of proton therapy over photon therapy in reducing volumes of normal lung tissues receiving low dose ranges and constraining doses for critical structures such as the heart, esophagus, and spinal cord (59,60).

The dosimetric advantages of proton therapy are of particular interest in the burgeoning field of combining cancer immunotherapy and radiotherapy. Currently, uncertainty remains over the potential of proton therapy for better clinical outcomes compared with IMRT in patients with LA-NSCLC. A large retrospective study of the National Cancer Database showed that proton therapy conferred a significant OS benefit in stage II and III NSCLC patients compared with photon therapy. However, the OS difference failed to reach statistical significance in propensity score-matched cohorts (61). In addition, a recent prospective randomized phase II clinical trial comparing passively scattered proton therapy (PSPT) and IMRT failed to demonstrate the advantages of proton therapy over IMRT in terms of reducing toxicity, showing no significant differences in the rates of grade ≥3 radiation pneumonitis or local failure (62). Larger volumes exposed to higher doses (20–80 Gy) and lack of sufficient experience in proton radiation planning may account for the negative clinical outcomes (62). Of note, proton therapy is currently delivered either using PSPT, or pencil-beam scanning (PBS). Compared with PSPT, PBS offers the advantage of better dose distribution and greater sparing of normal structures compared with PSPT (59). However, the uncertainties associated with the range of the proton beam and respiratory motion, along with the tissue density heterogeneity of chest organs, also render implementing PBS proton therapy for LA-NSCLC more challenging and demanding (63).

With the ongoing improvement of proton therapy technique and knowledge, we believe that proton therapy still has great potential to enhance treatment outcome when combined with immunotherapy. First, proton radiotherapy displays the dosimetric advantage of reduced radiation doses for critical organs, especially in heart exposure, which may confer a long-term survival benefit (62). Second, some research has shown the superiority of proton therapy over photon therapy in reducing the risks of grade 4 lymphopenia (64,65). Reduced volume of low-dose radiation could limit the

radiation exposure of peripheral blood lymphocytes, and thus may serve to increase the efficacy of immunotherapy through lymphocyte sparing. Thus, future randomized studies are needed to show whether the combination of immunotherapy with proton therapy, especially PBS proton therapy, will lead to improved outcomes for patients with LA-NSCLC.

In addition to the above-mentioned dosimetric advantage, particle radiotherapy also exhibits great immunoregulatory potential. Gameiro and colleagues reported that protons significantly down-regulated PD-L1 and induced higher levels of calreticulin expression on the tumor cell surface than photons in different tumor cell lines (66). This result gives support to the promise of proton radiotherapy leading to enhanced T-cell mediated antitumor activity in irradiated tumors, and thus suggests an emerging role for proton therapy in facilitating antitumor response when combined with T-cell mediated immunotherapy. Dendritic cells (DC) serve as specialized antigen-presenting cells and play a pivotal role in initiating antitumor immune response after uptake of tumor antigens (67). Accumulating evidence from preclinical studies indicates that carbon-ion irradiation in combination with DC injection has anti-metastatic effects. Ohkubo and colleagues reported that, compared with carbon-ion irradiation alone, combined carbon-ion irradiation and intratumoral DC injection correlated with a significant decrease in the number of lung metastases in a mouse model (68). In another preclinical study, a significantly enhanced anti-metastatic effect was observed with the combination of low dose carbon-ion irradiation and DC injection, while the combination of photon irradiation required a higher dose to suppress tumor metastasis (69). Moreover, in combination with carbon-ion irradiation, intravenous DC injection was more effective in suppressing lung metastases than intratumoral DC administration (69). In clinical practice, intravenous DC injection is also more suitable with respect to the advantage of carbon-ions in treating deep-seated tumors. The enhanced immunogenicity of tumor cells with up-regulation of calreticulin on cell surfaces and the activation of immature DCs during carbon-ion irradiation may contribute to a synergic interaction between carbon-ion radiotherapy and immunotherapy (69,70).

Reduction of radiation target volumes

Elective node irradiation (ENI)

ENI in NSCLC refers to the radiation of lymph nodes that have not metastasized according to clinical judgment, and can include the bilateral hilar, mediastinum, and even supraclavicular areas. This treatment was originally intended as a means to kill subclinical lesions that may exist in these areas. However, the idea of ENI was later questioned because it was found to increase the volume of the radiotherapy target, thus leading to toxicity and making it hard to improve the therapeutic dose (47). Indeed, in the pre-immunotherapy era, accumulated evidence has suggested that involved-field irradiation (IFI) instead of ENI is a better treatment strategy for LA-NSCLC. A prospective randomized study (71) compared IFI versus ENI for patients with inoperable stage III NSCLC treated with concurrent chemoradiotherapy. 3DCRT was delivered with 1.8–2 Gy/Fx to 68–74 Gy for the IFI arm and with 60–64 Gy for the ENI arm. The results showed that the IFI arm achieved a better OS rate (90% *vs.* 79%, P=0.032) and a better 5-year local control rate (LCR) (51% *vs.* 36%, P=0.032) than the ENI arm. Despite the higher dose, the radiation pneumonitis rate in patients with IFI was actually lower than that in patients with ENI (17% *vs.* 29%, P=0.044). However, given the higher prescribed dose in the IFI arm, whether the better outcome was due to the higher radiation dose or due to IFI, is still disputed. Notably, in another prospective cohort comparing IFI to ENI, a maximum radiation dose, given the condition that OAR could be tolerated in both arms, was given by Chen and colleagues who found a tendency of improved locoregional PFS rate with IFI (34.1% *vs.* 30%, P=0.673), along with a significant increased OS rate (72). Li and colleagues performed a meta-analysis comprising 3 RCTs and 3 cohort studies to compare the incidence of elective nodal failure (ENF) in ENI versus IFI. The results showed no significant difference in the incidence of ENF between IFI and ENI either among RCTs (P=0.46) or cohort studies (P= 0.97), or when RCTs and cohort studies were combined (P=0.64) (73). Additionally, numerous retrospective studies of patients with stage III NSCLC treated with definitive radiotherapy have reported that IFI was associated with an acceptably low ENF rate and a significantly lower risk of higher-grade esophagitis (74-76).

In the era of immunotherapy, in addition to OS, local-regional control, and toxicity profile, the interaction between radiation and the immune system needs to be further considered when we choose between IFI and ENI. The tumor-immune cycle is divided into the following 7 steps: (I) tumor antigen release, (II) tumor antigen presentation, (III) activation of effector T cells, (IV)

migration of T cells to tumor tissues, (V) tumor tissue T cell infiltration, (VI) recognition of tumor cells by T cells, and (VII) removal of tumor cells (77). Obviously, abnormalities in any one of these steps can lead to an anti-tumor-immune cycle failure and an immune escape, which emphasizes the importance of the integrity of the lymphatic system in the efficacy of radiotherapy plus immunotherapy. Previous studies have demonstrated that direct irradiation of lymph nodes can partially explain lymphopenia, since lymph nodes act as reservoirs of lymphocytes and also as depots for clonal expansion of lymphocytes to specific antigens (78). Additionally, Tang and colleagues reported that larger GTVs exhibited a significant association with lymphocyte nadirs (43). In the case of locally advanced NSCLC for which thoracic radiation is needed, it is especially noteworthy that CLs receive a significant dose of radiation during the passes through the heart, and the entire cardiac output also transits through the pulmonary circulation, thus making it more easily affected by radiation (78). Moreover, there is emerging evidence indicating that the depletion of lymphocytes is linked with poorer outcomes in NSCLC patients (43,44,58) and, notably in the era of immunotherapy, a lower likelihood of response to immunotherapy (46). In preclinical studies, stereotactic radiotherapy with ENI, in combination with immune checkpoint blockade (ICB), restrained immune infiltration, attenuated chemokine expression, and negatively correlated with survival (79).

Overall, IFI can achieve better normal tissue sparing and less toxicity while not compromising locoregional and distant control. More importantly, based on preclinical and clinical evidence, IFI may better synergize with immunotherapy than ENI, thus making it part of powerful radiotherapy strategy in the era of immunotherapy.

Omission of clinical target volume (CTV)

In addition to IFI, omission of CTV is another strategy for reducing target volume. Traditionally, the planning target volume (PTV) was generated by expanding step by step from GTV, CTV, and internal target volume (ITV), and then the radical radiation dose was prescribed to the PTV in the definitive radiotherapy for LA-NSCLC. However, the CTV is defined as the tissue volume that contains the GTV and subclinical microscopic malignant lesions, and several studies have proven that the doses needed to eradicate subclinical diseases are lower than those used to control gross tumors in patients with common epithelial tumors (80-82). From this point of view, the dosage prescribed to the subclinical lesions in the traditional target-contouring might be higher than needed. Previous experience has led to the conclusion that radiation doses of 45–50 Gy could result in high control rates for subclinical disease in patients with epithelial tumors (80,81). With this in mind, we performed a dosimetric study on LA-NSCLC (83) and found that radiotherapy with the IMRT technique could deliver sufficient dose coverage to subclinical regions while reducing the dose to normal tissues when CTV was omitted. CTV omission has also been proven to be acceptable in clinical settings. A retrospective study of 105 stage III NSCLC patients treated with (n=50) or without CTV (n=55) revealed no statistical significance in terms of local recurrence, distant metastasis, progression-free survival (PFS), OS, and grade 3–4 radiation esophagitis, or hematological toxicity between 2 two arms. Notably, the grade 3–4 radiation pneumonia rate was significantly lower in the arm without CTV (P=0.044) (84). Kilburn and colleagues evaluated recurrence sites of 110 stage II-III NSCLC patients treated without CTV and found only 2 CTVretro (PTVs expanded 1 cm) failures, thus indicating that CTV omission appears to be a feasible strategy (85). It is worth noting that larger target volume not only increases the risk of radiation toxicities but can also cause more damage to the lymphatic system. In this regard, omission of CTV has the potential to better preserve lymphocyte function and holds great promise for better synergetic effects when combined with immunotherapy.

Radiation dose and fractionation

Dose escalation and altered fractionation

In the pre-immunotherapy era, the standard radiation dose for unresectable LA-NSCLC was 60–63 Gy in 1.8–2 Gy daily fractions, which was established by the RTOG 7301 trial in 1980 (86). The strategy of dose escalation has shown surprisingly disappointing results in RTOG 0617 as dose escalation from 60 to 74 Gy resulted in worse OS compared to 60 Gy (87). Besides dose escalation, considerable interest has been focused on exploring the potential benefits of unconventional fractionation radiotherapy for the treatment of LA-NSCLC. An individual patient data meta-analysis has shown that hyper-fractionated and accelerated radiotherapy yielded a modest survival benefit in LA-NSCLC patients compared with conventional schedules (88). However, it is worth noting that among all the included trials in this meta-

analysis, the benefit in OS reached statistical significance only in the continuous hyper-fractionated accelerated radiotherapy (CHART) trial, in which patients received radiotherapy without chemotherapy (89). Meanwhile, numerous other phase III trials reported that the hyper-fractionated/accelerated radiotherapy regimen did not achieve significant survival advantage over conventional radiotherapy when combined with induction or concurrent chemotherapy (90-93).

In the era of immunotherapy, the optimal dose-fractionation regimen remains to be clarified. The current dose-fractionation regimen is based on the linear-quadratic (LQ) model, which calculates the optimal dose-fraction to eliminate a certain type of tumor while sparing its surrounding normal tissues. However, the LQ model only focuses on the radiation killing of tumor cells, while neglecting the role of the immune system in antitumor activity. The immune system is of critical importance to tumor control after radiotherapy, while radiation exposure can have destructive effects on lymphocytes (40,94,95). Increased total dose, lung V5, twice-daily fractionation, and extended radiotherapy duration were found as risk factors for lymphopenia. On the other hand, enhanced antigen presentation is another known effect of radiotherapy (96-99). Varying doses of radiation (1–100 Gy) in a single fraction or in a short-course fraction regimen can induce diverse immunogenic effects. A recent preclinical study demonstrated that hypo-fractionated radiation was more immunogenic compared to the conventional fractionated regimen (100). Vanpouille-Box and colleagues found that radiation doses above a certain threshold (12–18 Gy) attenuated tumor immunogenicity by inducing DNA exonuclease Trex1 and degrading cytosolic DNA (101), thus suggesting that fractionated doses should not surpass the threshold for Trex1 induction.

In addition, one of the hurdles to the success of immunotherapy is immunosuppressive tumor microenvironment (TME). Both conventional fractionation radiotherapy and stereotactic body radiotherapy (SBRT) can "de-bulk" the tumor (23,102), thus leading to direct destruction of the TME. Radiation also induces phenotypic changes in immunosuppressive cell populations in the TME including myeloid-derived suppressor cells (MDSCs), M2 tumor-associated macrophages (TAMs), and regulatory T (Treg) cells. For example, radiation has been reported to induce the infiltration of M2-like TAMs (103-105), which display pro-survival and pro-angiogenic activities, whereas other studies have shown that low-dose radiation (2 Gy 1–2 fx daily) promotes the polarization of TAMs from M2 to NOS+ M1 and improves anti-tumor immune response (106,107). Filatenkov and colleagues reported that immunosuppressive TME was transformed by a single 30 Gy dose of radiation that decreased the infiltration of MDSCs (108). In contrast, the addition of conventional daily fractionated (10×3 Gy) to the single dose of 30 Gy resulted in significantly increased infiltration of MDSCs (108). Moreover, Lan and colleagues found that compared with conventional daily low-dose fractionated radiotherapy, hypo-fractionated radiotherapy inhibited hypoxia within primary tumors, decreased vascular endothelial growth factor (VEGF) expression, and reduced the recruitment of MDSCs into tumors (109).

Taken together, these findings suggest that different dose-fractionation regimens can have diverse effects on the immune system. Thus, in the era of immunotherapy, the dose-fractionation schedules should be re-evaluated to determine the optimal radiation regimen. Notably, the combination of ICB and non-conventional dose fractionation radiotherapy is currently being explored by several ongoing clinical investigations (NCT04081688; NCT03801902; NCT03589547; NCT03237377).

Individualized radiation dose

The development of precision medicine has revolutionized systemic therapy for lung malignancies. To date, although accumulating evidence has demonstrated considerable heterogeneity of radiosensitivity and radiotoxicity between patients with the same tumor histology and disease stage but different molecular background (110-113), radiation dose protocols are still uniform for all LA-NSCLC patients (one-size-fit-all).

Tailoring of the radiation dose to individualized patient-tumor radiosensitivity holds great promise as an effective radiotherapy strategy. Radiosensitivity of tumor cells can be strongly impacted by molecular variations on the genomic, transcriptional, and translational levels. Genetic mutations or single nucleotide polymorphisms (SNPs) of DNA repair response-associated genes (such as p53, ATM, BRCA1, BRCA2, ERCC1, XRCC3, and Rad51) have been repeatedly found to be associated with radiosensitivity in lung cancer (114-118). In addition, mutations or SNPs of crucial oncogenes (such as EGFR and ALK) (119-122) or critical radiation-modulating genes (such as TGF-β) (123-125) have recently been shown to influence tumor radiosensitivity. Moreover, comprehensive analyses of

radiosensitivity-associated genes and proteins in lung cancer (126) and other solid tumors (127,128) have been carried out to identify potential biological predictors of radiosensitivity. Accompanying the gradually deepening understanding of the mechanisms and biomarkers of radiosensitivity, gene-expression classifiers that incorporate a handful of vital genes to predict radiosensitivity in specific tumor types or across various human cancers have become available (129,130).

Genomic-adjusted radiation dose (GARD), one of the most prominent radio-sensitivity prediction algorithms, was derived from the gene-expression-based radiation-sensitivity index and the linear quadratic model. A total of 10 genes were identified and validated by training a linear regression model to predict the experimental survival fraction at 2 Gy for 48 cancer cell lines from 9 different disease sites (129,131,132). Afterward, the radiosensitivity index (RSI) was calculated using a mathematic algorithm incorporating all of 10 genes with distinct weights. Finally, the GARD score was calculated using the linear quadratic model, the individual RSI, and the standard radiation dose/fractionation. GARD scores of patients from 5 different cohorts consisting of primary tumors from 20 disease sites were measured and demonstrated to be independently associated with clinical outcome in breast cancer, lung cancer, glioblastoma, and pancreatic cancer (133). Based on these data, an individualized radiation dose could be suggested both for the primary tumors (134) and lymph nodes (135). However, the GARD score has not been widely used in clinical practice due to the lack of sufficient validation in randomized clinical trials.

In addition, radiation-induced toxicity, which is mainly determined by the biologic and genetic background of surrounding normal tissues, should also be taken into consideration when personalizing radiation dose. A list of germline SNPs has been found to be associated with acute and long-term radiotoxicity (112,136,137). Chemotherapy (especially irinotecan) dose modification according to highly recognized SNPs has been validated in several clinical trials among metastatic patients receiving chemotherapy alone or locally advanced patients receiving chemoradiotherapy (138-140). Similarly, personalized radiotherapy strategies such as the tailoring of the radiation dose to each individual's genetic background aimed to reduce radiation-induced toxicity are particularly attractive.

In the era of immunotherapy, particular attention should be paid to the tumor immune microenvironment and systematic immune state when personalizing the radiation dose, since the potency and durability of the anti-tumor immune response induced by radiotherapy are significantly influenced by these critical factors (101,141). In preclinical models, the radiation dose greater than 12 Gy could attenuate the anti-tumor immune response by degrading DNA that accumulated in the cytosol upon radiation. On the other hand, an enhanced anti-tumor immune response was observed when radiation dose was elevated in the range of 0–8 Gy (101). Therefore, hypo-fractionated radiation at a dose of 5–10 Gy per fraction was speculated to be better than conventionally fractionated schemes of 1.8–2.2 Gy fractions (141). In fact, prospective clinical trials evaluating the combination of anti-PD-1 blockade with SBRT at a dose in that range for the treatment of metastatic lung cancer have yielded promising results (142,143). However, low-dose per-fraction radiation was found to recruit more cytotoxic T cells into the TME than high doses in another preclinical study (106). One recent retrospective study showed that higher doses (estimated dose of radiation to immune cells larger than 6.1 Gy) of radiation to the immune system were associated with tumor progression and death after the definitive treatment of stage III NSCLC (143). Additionally, one systematic analysis of patients treated with radiation and ipilimumab demonstrated that low fractional doses of radiation were associated with a more favorable systemic response (144). Taken together, the optimal radiation dose in the settings of radio-immunotherapy remains controversial (142), and it is likely to be dependent on biological features of tumor cells and the immune state of each individual patient. A number of questions need to be answered before we can prescribe an individualized radiation dose in the hope of provoking the most potent and durable anti-tumor immune response.

Conclusions and future directions

The era of immunotherapy is destined to be a time of great challenge but also wonderful opportunity for the use of radiation therapy for LA-NSCLC. Immune-sparing strategies and the minimization of radiation exposure to adjacent critical structures will be key to a successful combination of thoracic radiotherapy and immunotherapy (*Figure 1*). Advanced radiotherapy techniques such as 3DCRT and IMRT offer opportunities for improved target conformity and reduced normal-tissue exposure. However, the low-dose radiation volume brought by IMRT and its effects on the immune system deserve particular attention. The dosimetric advantages and immunoregulatory potential

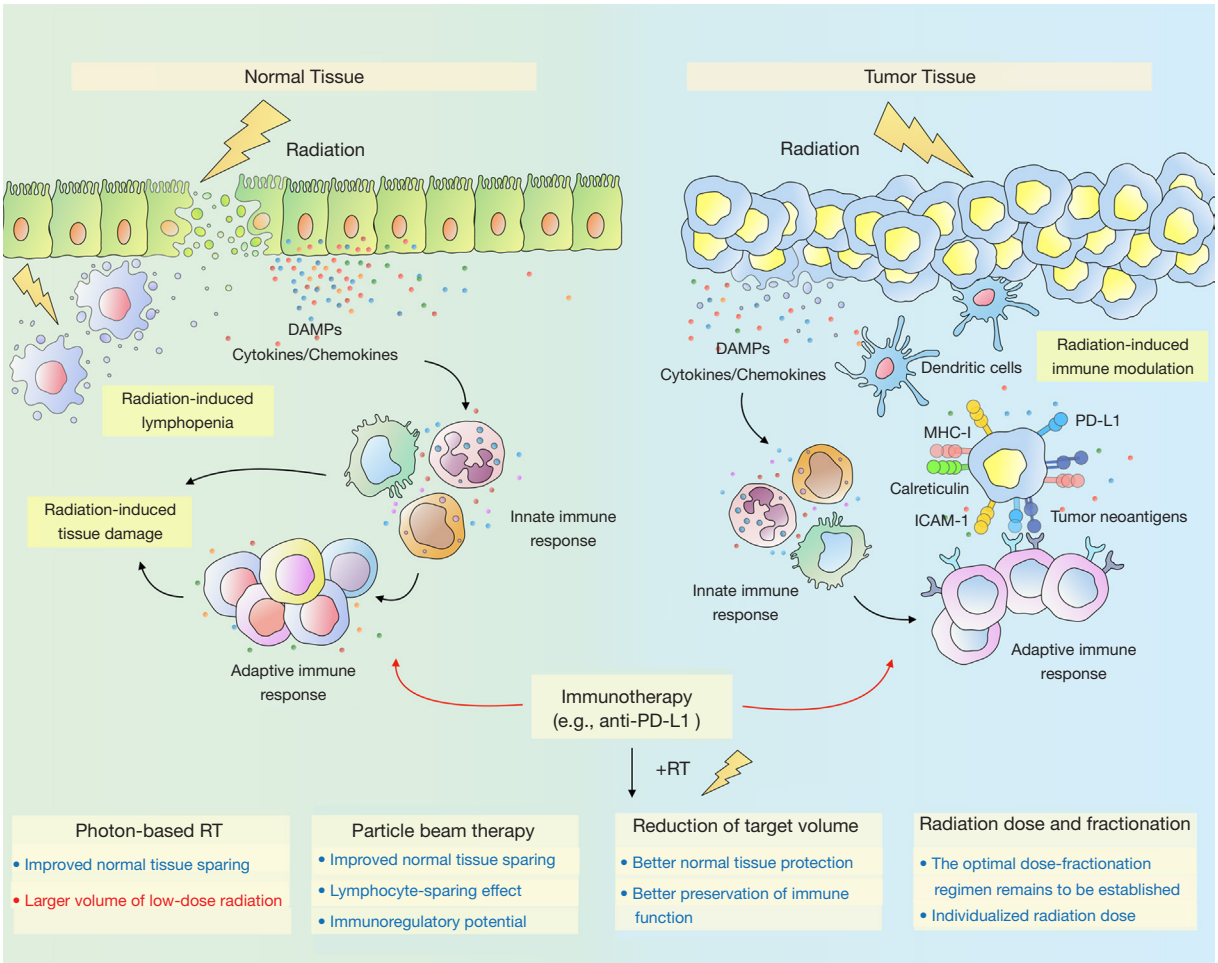

Figure 1 Schematic representation showing radiation-induced immune modulation in the tumor microenvironment and radiation-induced normal tissue toxicity. Radiation can augment antitumor immune response through diverse mechanisms. For example, radiation stimulates the release of tumor antigens and DAMPs, initiates the production of pro-inflammatory cytokines, and triggers the upregulation of immunomodulatory surface molecules (e.g., ICAM-1 and MHC-I), leading to the infiltration of immune cells such as dendritic cells and cytotoxic T cells. Additionally, radiation induces the upregulation of PD-L1 on tumor cells, and thus integrating radiotherapy with anti-PD1/PD-L1 antibodies can overcome this resistance mechanism. Different dose-fractionation regimens can have diverse effects on the immune system. Further investigation is needed to establish the optimal radiation regimen. On the other hand, radiation-induced damage to tissue-resident cells triggers the release of DAMPs and pro-inflammatory mediators, allowing activation of resident immune cells and recruitment of inflammatory cells, which, in turn, amplifies the ongoing inflammatory response. Moreover, radiation can have destructive effects on lymphocytes and result in radiation-induced lymphopenia. Thus, immune-sparing strategies and the minimization of radiation exposure to normal tissues will be key to a successful combination of radiotherapy and immunotherapy. DAMPs, damage-associated molecular patterns; ICAM-1, intercellular cell adhesion molecule-1; MHC-I, major histocompatibility complex class I; PD-1, programmed cell death protein-1; PD-L1, programmed death ligand 1; RT, radiotherapy.

of particle radiotherapy make it an attractive option in the era of immunotherapy, which merits further investigation. Strategies for the reduction of radiation target volumes such as IFI and CTV omission can achieve better normal tissue-sparing while not compromising locoregional and distant control. More importantly, both IFI and CTV omission could better preserve immune function. Thus, there is immense potential for IFI and omission of CTV to achieve better synergetic effects in the era of immunotherapy. The optimal dose fractionation regimen in the settings of

radio-immunotherapy remains to be established in future prospective trials. Personalized radiotherapy might be of particular interest in allowing the administration of individualized radiation dose according to each patient's genetic background and immune state.

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Footnote

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