

A review of radiation-induced lymphopenia in patients with esophageal cancer: an immunological perspective for radiotherapy

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Abstract: Radiotherapy is a frequently utilized therapeutic modality in the treatment of esophageal cancer (EC). Even though extensive studies are carried out in radiotherapy for EC, the design of the clinical target volume and the radiation dose is not satisfactorily uniform. Radiotherapy acts as a double-edged sword on the immune system; it has both an immunostimulatory effect and an immunosuppressive effect. Radiation-induced lymphopenia and its potential association with tumor control and survival outcomes remain to be understood. The advent of immunotherapy has renewed the focus on preserving a pool of functioning lymphocytes in the circulation. In this review, we summarize the potential impact mechanisms of radiotherapy on peripheral blood lymphocytes and the prognostic role of radiation-induced lymphopenia in patients with EC. We also propose the concept of organs-at-risk of lymphopenia and discuss potential strategies to mitigate its effects on patients with EC. From an immunological perspective, we put forward the hypothesis that optimizing radiation modalities, radiation target volume schemes, and radiation doses could help to reduce radiation-induced lymphopenia risks and maximize the immunomodulatory role of radiotherapy. An optimized radiotherapy plan may further enhance the feasibility and effectiveness of combining immunotherapy with radiotherapy for EC.

Keywords: anti-tumor immunity, esophageal cancer, lymphopenia, organs-at-risk of lymphopenia, radiotherapy

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Background

Esophageal cancer (EC) is one of the most aggressive tumors and is the sixth leading cause of cancer-related mortality worldwide.¹ EC includes two predominant histological types: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC).

At present, neoadjuvant chemoradiotherapy (CRT) followed by surgery is the preferred treatment for locally advanced EC patients, but definitive CRT is an alternative treatment for non-operative candidates.^{2,3} According to the landmark RTOG 94-05 trial, 50.4 Gy is the standard dose of radiation in Europe and North America for patients undergoing definitive CRT.⁴ Although radiation dose escalations have failed to improve local

control or survival, a dose of 60.0 Gy or more is more prevalent in many Asian countries, including China and Japan.^{5,6}

The nodal clinical target volume (CTV) of external beam radiation therapy (RT) has remained a topic of persistent controversy among radiation oncologists. EC commonly shows lymph node metastases, particularly regional lymph node involvement, even in the early phases of the disease.⁷ The conservative school of thought on this matter promotes the involved-field irradiation (IFI) – that is, irradiation of positive lymph nodes only – because the rationale to include elective nodal irradiation (ENI) is to prevent regional nodal relapse rather than improve survival. The other school of thought favors prophylactically

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Figure 1. The design of IFI and ENI for EC. Red: GTV; Blue: IFI field in the coronal direction; Green: ENI field in the coronal direction.
EC, esophageal cancer; ENI, elective nodal irradiation; GTV, gross tumor volume; IFI, involved-field irradiation.

irradiating the regional lymph node area according to different tumor sites because ENI could be of benefit in eliminating potential subclinical nodal disease.⁸ The design of different radiation fields is shown in Figure 1.

Treatment-related lymphopenia is a common side effect of RT, chemotherapy, and certain drugs, like steroids, that are used in cancer patients. RT may play a primary role in the etiology of treatment-related lymphopenia. Lymphocytes are highly radiosensitive, and exposure to low doses of radiation could lead to a decrease in the number of peripheral blood lymphocytes.⁹ In a study on non-small-cell lung cancer (NSCLC), Campian *et al.* revealed that total lymphocyte counts did not change following neoadjuvant chemotherapy, but steep declines were noted after the initiation of thoracic RT.¹⁰ In another study, researchers found that RT, with or without concomitant chemotherapy, induced substantial and long-lasting immune suppression in patients with cervical cancer.¹¹ The immune system has multiple mechanisms for identifying tumor cells and removing them from the body, most of which involve lymphocytes.¹² Treatment-related lymphopenia is associated with poor prognosis in many types of cancer,^{13–17} but the mechanics remain to be understood.

Lymphocytes play a vital role in antitumor immunity, but radiosensitivity makes them vulnerable

targets during radiation therapy. It seems reasonable to assume that preserving a pool of functioning lymphocytes in circulation might contribute to treatment outcomes. Because there is controversy in the concept of radiation field size and dose, we need an immunological perspective to assist in improving individualized treatment for EC patients. This review, therefore, summarizes current knowledge on RT for EC and attempts to evaluate the potential effect of RT on the immune system, especially the count of peripheral circulating lymphocytes. Topics covered include effects of radiation-induced lymphopenia (RIL) in patients with EC, the organs-at-risk (OARs) of lymphopenia, and the possible risk avoidance of RIL.

Radiation-induced lymphopenia

In general, the immune system, especially cellular immunity, is thought to play a central role in cancer suppression.¹⁰ Lymphocytes are the primary carriers of cell-mediated immune mechanisms; they play a critical role in promoting a systemic immune response against tumors.¹⁸ T-cells have efforts of endogenous anti-tumor immunity.¹⁹ CD8+ and CD4+ T-cells can drastically improve the prognosis of patients with EC due to their ability to directly destroy tumor cells or secrete cytokines that activate effector cells.^{20,21} The use of immune checkpoint inhibitor (ICPi) blockers modulates the interaction between T-lymphocytes and tumor cells or macrophages, thereby favoring the re-induction of T cell populations in the tumor environment, which leads to a durable clinical response.²² Recently, with the recognition of ICPi as a potent therapeutic agent in the immunotherapy of cancer, the status of the immune system has been deemed an essential biomarker for responses to novel treatments.²² It is, hence, crucial to maintain an intact adaptive immune system during cancer therapy so as to improve cancer control and enhance the effectiveness of treatment.²³

A lymphocyte is the most radiosensitive cell of the hematopoietic system and is frequently depleted by RT using a 50% lethal dose of 1–2 Gy.^{9,24} Currently, the mechanism of RIL is less clear. A systemic effect of localized postmastectomy radiation upon the long-term lymphocyte counts was described in 1970.²⁵ The study showed that the total lymphocyte counts of irradiated patients decreased, whereas those of unirradiated patients remained unchanged. Lymphopenia was also

demonstrated to exist persistently in irradiated patients for at least 4–8 years after treatment. The hypothesis at that time was that direct radiation damage to the thymus prevented the restoration of the usual level of lymphocytes because targeting the internal mammary lymph nodes resulted in the delivery of reasonably high doses to the anterior thymus and mediastinum. Stjernsward *et al.* also analyzed the change in lymphocyte subpopulations in patients receiving irradiation post-mastectomy.²⁶ As a comparison, the authors used healthy individuals in some cases or patients receiving irradiation that did not include treatment reaching the thymus gland. Per the results obtained, compared with both control groups, the thymus-derived lymphocyte subpopulation decreased significantly, and the bone marrow-derived lymphocyte subpopulation increased relatively in breast cancer patients receiving irradiation. Raben *et al.* later questioned the findings of Stjernsward *et al.*,²⁷ intimating that the volume of irradiated blood concomitant with tumor irradiation should also have been taken into account. In the research conducted by Raben *et al.*, the irradiation of breast cancer resulted not only in the irradiation of the thymus area but also in the irradiation of the large thoracic and cardiac blood vessels. Given the findings they obtained, the authors compared lymphocyte count in peripheral blood in patients receiving pelvic irradiation with patients receiving thoracic irradiation, and found that the irradiation of the pelvic area resulted in lower lymphocyte counts than the irradiation of the mediastinum. Therefore, the authors ruled out irradiation of the thymus as the cause of lymphopenia and suggested that the decrease in lymphocyte count following irradiation results from irradiation impact on the lymphocytes in the blood vessels.

To test the effects of extracorporeal irradiation of the blood, Weeke observed the irradiation of blood *via* a radioactive source placed within a dialysis unit, and showed that the degree of lymphocyte depletion was directly proportional to the radioactive source strength and amount of blood passing through the dialysis unit.²⁸ MacLennan and Kay later revealed that the degree of long-term lymphopenia caused by a given 24–25 Gy of whole-brain irradiation was dependent upon the number of fractions into which the irradiation was divided.²⁹ Because the brain and skull contain little bone marrow or lymphoid tissue, the main bulk of lymphocytes entering the fields during irradiation are those circulating in the blood.²⁹

The number of fractions varied from 5 to 15 in this study, and the mean lymphocyte counts of patients 3 months after receiving this dose in 5 fractions was $1.84 \times 10^9/l$, in 12 fractions it was $1.12 \times 10^9/l$, and in 20 fractions it was $0.64 \times 10^9/l$.²⁹ The observations by the researchers above strongly suggest that the radiation dose for circulating blood and bone marrow may be associated with the severity of RIL.

Effects of RIL on patients with EC

Peripheral circulating lymphocytes have a significant effect on survival outcomes in various types of solid tumors.^{30–32} In the past few years, many immune-inflammation parameters, such as absolute lymphocyte count (ALC), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR), have been confirmed to have prognostic values in several solid tumors treated with RT, but relatively few studies exist on this aspect for EC. Here, we summarize the prognostic value of RIL in six retrospective studies of 2374 patients with EC (Table 1).

Short-term therapeutic evaluation

Two articles have reported correlations between immune-inflammation parameters and pathologic complete response (pCR), as shown in Table 1. Barbetta *et al.* assessed the before and after CRT variations of NLR (Δ NLR) as a predictor of treatment response in patients with ESCC treated with CRT with or without surgery.³³ Among surgical patients, 43% with pCR showed significantly lower median Δ NLR than patients with residual disease. High Δ NLR was a negative predictor of treatment response. On their part, Fang *et al.* examined patients with stages I–IVa EC ($n = 313$) treated with neoadjuvant CRT and then surgery.¹⁸ For patients with pCR, the median ALC nadir during CRT was significantly higher than that of patients without pCR. These two investigations both reiterate the fact that high levels of peripheral blood lymphocytes have a positive effect on short-term tumor treatment efficacy.

Recurrence and metastasis

Many researchers have reported determining findings on recurrence and metastasis in EC. Barbetta *et al.* reported Δ NLR as a prognostic factor for recurrence in their study.³³ The median follow-up time after CRT was 5.2 years, there

Table 1. Summary of studies of RIL in patients with EC.

Author	Design/ period	n	TNM stage	NLR cutoff (low/high)	PLR cutoff (low/high)	ALC (definition)	Blood exam timing	Treatment	Prognostic value analyses	Endpoint
Davuluri <i>et al.</i> ¹²	Retrospective (2007–2013)	504	I–III	NR	NR	Grade 4 ALC nadir: ALC < 200 cells/ml	Before, during (weekly), and 1 month after CRT	Neoadjuvant or definitive CRT ± surgery	Grade 4 ALC nadir	OS, PFS, DSS, DMFS
Barbetta <i>et al.</i> ³³	Retrospective (2007–2013)	217	I–III	ΔNLR	NR	NR	Before and after CRT	61.3%: CRT 38.7%: Neoadjuvant CRT + surgery	ΔNLR	pCR, recurrence, DFS
Fang <i>et al.</i> ¹⁸	Retrospective (2007–2013)	313	I–IVa	NR	NR	High ALC > 0.35 × 10 ³ /μl	Before, during (weekly), and one month after CRT	Neoadjuvant CRT + surgery	ALC	pCR
Deng <i>et al.</i> ³⁴	Retrospective (2004–2015)	755	I–III	NR	NR	Grade 4 ALC nadir: ALC < 200 cells/ml	Before, during (weekly), and at the first follow-up visit after CRT	Definitive or neoadjuvant CRT	Grade 4 ALC nadir	OS, LRFS, DMFS, DSS
Shiraishi <i>et al.</i> ²³	Retrospective (2005–2016)	480	I–IVa	NR	NR	Grade 4 ALC nadir: ALC < 200 cells/ml	During CRT	Neoadjuvant CRT + surgery	Grade 4 ALC nadir	OS, PFS, DMFS
Wu <i>et al.</i> ³⁵	Retrospective (2013–2017)	105	Ia–IIIc	4.35 (52/53)	236 (52/53)	ALC% cutoff set at 16%	1 week before the first and after the last radiotherapy or chemotherapy cycle	Concurrent CRT	PLR, NLR, ALC%	OS, TPD, TM

ALC, absolute lymphocyte count; CRT, chemoradiation therapy; DFS, disease-free survival; DMFS, distant metastasis-free survival; DSS, disease-specific survival; EAC, esophageal adenocarcinoma; EC, esophageal cancer; ESCC, esophageal squamous cell carcinoma; LRFS, local-regional recurrence-free survival; N, number of patients; NLR, neutrophil-to-lymphocyte ratio; ΔNLR, posttreatment minus pretreatment NLR; NR, no report; OS, overall survival; pCR, pathologic complete response; PFS, progression-free survival; PLR, platelet-to-lymphocyte ratio; RIL, radiation-induced lymphopenia; TM, time to metastasis; TPD, time to progressive disease.

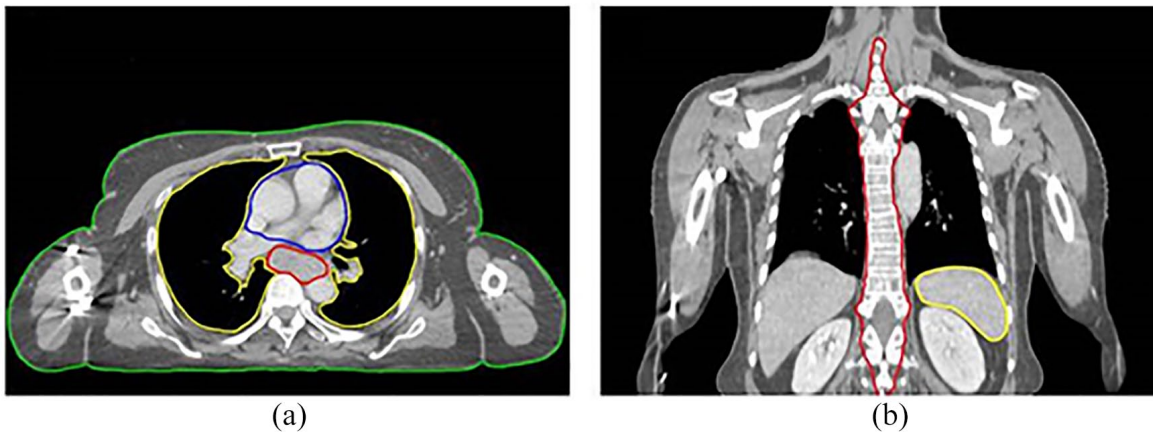


Figure 2. OARs of lymphopenia. (A) Red: GTV; blue: heart and blood vessel; yellow: lung; green: body. (B) Red: spinal cord; yellow: spleen. As the thymus gland gradually shrinks with age, it is not shown here. GTV, gross tumor volume; OARs, organs-at-risk.

were recurrences in 106 patients, and 43 patients died without recurrence. High Δ NLR was significantly associated with an increased risk of recurrence. In a study of 755 patients with stages I–III EC who received concurrent CRT with or without surgery,³⁴ Deng *et al.* obtained ALC before, during, and at first follow-up after CRT. Distant metastasis-free survival (DMFS) and local-regional recurrence-free survival (LRFS) were analyzed as the two endpoints. The researchers found that a Grade 4 ALC nadir during CRT was associated significantly with LRFS and DMFS. Per Davuluri *et al.*'s analysis of weekly ALC changes during the treatment of 504 patients with stages I–III EC subjected to neoadjuvant or definitive CRT,¹⁵ a Grade 4 ALC nadir during CRT predicted worse DMFS ($p < 0.001$), suggesting that there could be a role for host immunity in disease control. Shiraishi *et al.* reported lymphopenia in 480 EC patients undergoing neoadjuvant CRT,²³ finding that a Grade 4 ALC nadir was significantly associated with reduced DMFS. Wu *et al.* evaluated PLR, NLR, and lymphocyte percentage before and after the concurrent CRT of stages IA–IIIC EC patients.³⁵ The authors chose the time to metastasis (TM) as one of the endpoints in their reports, and their results demonstrated that lower lymphocyte percentage, higher PLR, and higher NLR were significantly associated with reduced TM. Through the studies herein discussed, there is an indication of a potential relationship between lymphocyte counts and a patient's recurrence and metastasis.

Survival outcomes

Among the six studies in Table 1, only one research did not establish the relationship between immune-inflammation parameters and survival outcomes, with four studies utilizing overall survival (OS) as their observation endpoint. Suggestively, high NLR, high PLR, and low ALC tend to be associated with poor survival outcomes in general. Many published reports have indicated that a cancer-associated inflammatory response is linked to worse long-term results in a variety of solid tumors, including EC. Lymphocytes are at the center of these different anti-tumor immune-inflammation parameters. Therefore, per the reports we have listed, the protection and maintenance of a high level of lymphocyte counts during a treatment process may be beneficial to the survival outcomes of EC patients.

Organs-at-risk of lymphopenia

Hematological toxicities caused by RT include acute side effects stemming from the depletion of peripheral blood progenitor cells and chronic injury caused by alterations in vasculature and fibrosis in lymphoid organs, like bone marrow, thymus, and spleen.^{36,37} As a result, we can separate the OARs of lymphopenia for the RT of patients with EC into two categories. One class of OARs includes the heart, lungs, prominent blood vessels, and body with abundant blood circulation (Figure 2A). The other category of OARs consists of lymphoid and hematopoietic tissues, such as the spleen and bone marrow that may

affect the development of lymphocyte hematopoiesis and maturation (Figure 2B).

For the first category of OARs, lymphocytes receive a significant dose of radiation through large blood vessels that are contained in the radiation field during transit. This process includes systemic and pulmonary circulations. In line with this category of OARs, Tang *et al.* analyzed the relationships between lung dose-volume histogram (DVH) parameters with lymphocyte nadirs in 771 NSCLC patients and observed that lymphocyte nadirs were associated with the percentage of lung dose receiving 5 Gy (lung V5).³⁸ In addition, the authors pointed out that the entire cardiac output was channeled through the lungs, causing increased circulating lymphocyte exposure. Saito *et al.* retrospectively assessed patients with various cancers who had undergone palliative RT using OARs based on body surface contour to evaluate their predictive value,³⁹ and found that higher body dose-volume parameters and a more significant number of RT fractions were predictive of Grade 3 lymphopenia (absolute lymphocyte count $<500 \times 10^6/l$).

The second category of OARs primarily includes organs rich in lymphoid and hemopoietic tissues. Saito *et al.* assessed the dosimetric predictors of treatment-related lymphopenia during CRT for EC and showed that higher spleen dose-volume parameters were associated with severe lymphopenia during CRT.⁴⁰ An increase of 1 Gy in mean splenic dose predicted a 2.9% decrease in nadir ALC. Also, a study published in *Radiotherapy and Oncology* prospectively identified clinical and dosimetric predictors of both acute and late hematologic toxicity in chemo-naïve patients treated with whole-pelvis RT for prostate cancer.⁴¹ The results revealed that higher bone marrow V40 was associated with a higher risk of acute Grade 3 (ALC at nadir $<500 \times 10^6/l$) or late Grade 2 lymphopenia (ALC at 1 year after RT conclusion $<800 \times 10^6/l$ and $\geq 500 \times 10^6/l$). In addition, Newman *et al.* reported the dosimetric relationship between vertebral irradiation and the lymphopenia of EC patients treated with CRT.⁴² Multivariable linear regression showed that lymphopenia was associated with a greater volume of vertebral bodies receiving radiation during CRT.⁴² We can, therefore, surmise that the irradiation of lymphoid organs, such as the bone marrow, spleen, and regional lymph nodes, is a potential promoter of lymphopenia. According to the discussed studies, it might have a certain

protective effect on lymphocytes if the irradiation of these OARs during RT could be avoided.

Risk avoidance of RIL in EC

The identification of the irradiation of lymphocyte OARs as a tool in causing lymphopenia facilitates the advancement of strategies to evaluate and adopt radiation treatment plans. The protection of lymphocyte OARs using approaches, such as the proton beam therapy (PBT) and the reduction of radiation doses, fractions, and field sizes, may help minimize RIL.

Radiation technology—proton beam therapy

The mechanisms of cell-kill for all forms of radiation are similar, but proton particles predictably lose energy as they pass through the body compared with photon beams; this property could be exploited to optimize clinical outcomes.⁴³ Consequently, the depth of the termination of the proton beam is controllable, and the radiation dose beyond the target region can be restricted. Due to the esophagus's central location near several OARs of lymphopenia, including the heart, lungs, and spine, the ability of PBT to conform high radiation dose to the tumor volume while reducing the unintentional radiation dose to adjacent healthy tissues has the potential to decrease RIL.⁴⁴

An example of the potency of PBT is illustrated in a study performed at the MD Anderson Cancer Center that retrospectively evaluated 480 patients with EC treated with preoperative concurrent CRT using PBT or intensity modulated radiation therapy (IMRT) with or without induction chemotherapy followed by surgery.²³ The purpose of the analysis was to compare the relative risk of RT-induced lymphopenia between PBT and IMRT. Findings from the study revealed that a higher proportion of IMRT patients (40.4%) developed Grade 4 lymphopenia during neoadjuvant CRT, compared with PBT patients (17.6%, $p < 0.0001$). Per the multivariable analysis of the research, PBT was associated significantly with a reduced risk of Grade 4 lymphopenia. In another study, the assessment of patients treated with definitive CRT and either PBT or IMRT for EC showed that PBT reduced the risk of severe, treatment-related lymphopenia, particularly in tumors of the lower esophagus.⁴⁵ In Routman *et al.*'s comparison of Grade 4 lymphopenia in patients who received concurrent CRT using PBT or photon RT with EC,⁴⁶ the authors found that photon

RT was associated with a significantly higher risk of Grade 4 lymphopenia, compared with PRT. These reports demonstrate that PRT might be one such way to decrease RIL in EC patients.

In another study, Hirano *et al.* retrospectively reviewed 37 patients with Stage III thoracic ESCC who had received PBT with or without concurrent chemotherapy,⁴⁷ comparing the dose distributions of PBT with those of dummy 3D-CRT and IMRT, primarily focusing on the doses to OARs, such as healthy lungs and heart tissue. The authors' results indicated that PBT significantly reduced the doses to the lungs and heart, compared with 3D-CRT or IMRT. As the significantly reduced doses exit through the lungs and heart, the physics of PBT is ideally more suited for EC than that of photon RT.⁴⁶ PBT would, therefore, seem to hold significant clinical advantages over traditional photon RT.

Radiation dosage and fractions

Yovino *et al.* devised a typical glioblastoma plan (8-cm tumor, 60 Gy/30 fractions) to estimate the radiation dose that lymphocytes receive from the radiation field.⁴⁸ Per this model, a single radiation fraction was found to deliver 0.5 Gy to 5% of circulating cells, and 99% of circulating blood received at least 0.5 Gy (mean dose of 2.2 Gy) after 30 fractions. Concurrently, a decrease in the fractionation of RT has been confirmed in some studies to reduce the incidence of lymphopenia risk. Crocenzi *et al.* analyzed the impact of neoadjuvant standard fractionated and hypofractionated CRT on lymphocytes in patients with locally advanced and borderline resectable pancreatic adenocarcinoma,⁴⁹ and found that standard fractionated CRT (a total dose of 50.4 Gy in 28 fractions over 5.5 weeks) resulted in a significant loss of lymphocytes, and hypofractionated CRT (a total dose of 30 Gy in 3 fractions over 1 week) was associated with reduced systemic loss of T cells. These studies suggest that the reduction of the duration of exposure or short-course radiation with a hypofractionated schedule could mitigate normal tissue exposure and reduce the risk of RIL. For thoracic RT, the RTOG-0617 trial showed that higher doses were associated with worse OS and might be potentially harmful.⁵⁰ According to Ladbury *et al.*,⁵¹ higher doses of radiation to the immune system (calculated as a function of the number of radiation fractions and mean doses to the lung, heart, and the rest of the body based on a model developed by Jin *et al.*)

were associated with tumor progression and worse OS after the definitive treatment of stage III NSCLC.⁵² It is, therefore, reasonable to infer that the standard radiation dose of 50.4 Gy for EC has fewer effects on lymphocytes than a dose of over 60 Gy. Using a higher radiation dose for EC does not always guarantee a better outcome since the response rate and survival outcomes do not necessarily improve.

Radiation target volume

Many studies have performed detailed analyses of radiation field size parameters as predictors of lymphopenia risk in recent years. Tang *et al.* analyzed the relationships between GTVs and lymphocyte nadirs in 771 NSCLC patients who had received definitive RT and found that larger GTVs correlated with fewer lymphocyte nadirs.³⁸ In 2018, further proof of the effects of field size on lymphopenia risk was provided by a study on 210 glioblastoma patients who were treated with standard-field RT (T1 enhancement + surgical cavity + T2 abnormality + 1.3–2.5 cm margin) *versus* limited-field RT (T1 enhancement + surgical cavity + 1.8–2 cm margin).¹³ The results revealed that V25 Gy of the brain was an independent predictor of acute severe lymphopenia in glioblastoma patients receiving 60 Gy of RT with concurrent temozolomide and that a reduction in the volume of the brain irradiated may lead to less treatment-induced lymphopenia. Ellsworth *et al.* also discussed field size effects on RIL,⁵³ finding that RIL risk was associated with field size, dose per fraction, and fraction number.

As mentioned earlier, one of the most controversial points in the radiation target volume of EC is whether to opt for ENI or IFI. ENI involves delivering RT to the primary tumor, as well as the irradiation of clinically uninvolved regional lymph nodes at risk of micrometastases of the treated disease.⁵⁴ The evidence of ENI was derived from prophylactic lymph node dissection in Japan. At operation, metastasis was found frequently in the supraclavicular nodes.⁵⁵ Therefore, the researchers used a three-field lymph node dissection approach to eliminate subclinical metastases, which resulted in better survival following a reduction in the regional lymph node recurrence rates and elimination of micrometastases.^{56,57} In the RTOG 85-01 randomized trial,² the irradiation of the entire esophagus, from the supraclavicular fossae to the esophagogastric junction for EC with the initial tumor length plus a 5 cm

margin, was recommended. In the RTOG 94-05 trial,⁴ an area with a margin of 2–5 cm surrounding the gross tumor volume (GTV) was defined as the CTV; the primary tumor and regional lymph nodes were included. For tumors of the cervical esophagus, supraclavicular lymph nodes were covered in the RT field. However, the results of these two trials did not improve regional control or survival significantly. A three-dimensional conformal radiation therapy (3D-CRT) technology was subsequently applied in the RTOG 01-13 trial⁵⁸; CTV included GTV, a 3 cm cephalad and caudad margin beyond GTV, and locoregional lymph nodes. Planning target volume (PTV) in this trial was defined as having 2 cm margins around CTV and 2 cm superior and inferior margins beyond CTV.

Similarly, many studies have investigated the recurrence pattern and survival outcome of ENI and have found that ENI serves to prevent localized regional failure rather than improve the long-term survival of patients with EC.^{59–62} In summary, there is no universal recommendation for the radiation field size selection for EC patients. Because the target volume of EC covers many large blood vessels and lymphoid tissues in the cervicothoracic region, it is reasonable to infer that if we reduce the target volume, the exposure of peripheral circulating lymphocytes to radiation will decrease. Large target volumes of ENI can increase treatment-related toxicities, such as hematological adverse events, acute radiation esophagitis, and late cardiopulmonary toxicities.⁶³ In addition, greater PTV is associated with an increased risk of grade 4 lymphopenia in EC.⁴⁵ Currently, there is not much evidence on the effects of field size on lymphocytes subjected to RT in patients with EC. Nevertheless, a small target volume could miss some subclinical disease, but with the help of modern imaging technology, IFI does not reduce OS compared with ENI. Many studies have demonstrated that the predominant failure pattern after IFI is still the in-field and distant disease rather than the out-field regional failure.^{64–67}

Prospects

Combining radiotherapy with immunotherapy in EC

Since the approval of anti-CTLA4 therapy (ipilimumab) for unresectable or metastatic melanoma on 25 March 2011,⁶⁸ the development of

anti-cancer immunotherapy has accelerated. The success of immunotherapy has improved the prognosis of some types of malignancies considerably,^{69,70} which has greatly encouraged researchers to combine the treatment with other conventional treatment strategies to further improve their efficacy. Among the treatment blends, the combination of RT and immunotherapy is considered promising.⁷¹

Immune checkpoints are inhibitory pathways hardwired into the immune system that are crucial for the maintenance of self-tolerance and also protect healthy tissues from damage when the immune system is responding to pathogenic infection.¹⁹ A growing number of clinical trials have confirmed that blocking inhibitory immune regulatory proteins ultimately results in immune activation against tumor cells.⁷² RT can effectively alter and eliminate both tumor cells and the surrounding stromal cells.⁷³ RT has a unique advantage against tumor immune escape mechanisms by increasing antigen visibility.⁷¹ This advantage includes the enhancement of the clearance of damaged tumor cells by antigen-presenting cells, thereby promoting the activation of T-cells;⁷¹ the upregulation of the expression of MHC-I on tumor surfaces to enhance the visibility of tumors to cytotoxic T-cells,⁷⁴ thus inducing the T-cell mediated abscopal effect and the possibility of RT-induced DNA damages generating neoantigen and enhancing the immune surveillance.^{75,76} An RT-induced modulation of tumor microenvironments can promote the recruitment and infiltration of immune cells and stimulate the recognition and killing of tumor cells by the immune system.⁷⁷ Preclinical studies of mouse models by Deng *et al.* demonstrated that PD-L1 expression is upregulated in tumor microenvironments after RT, and combining of RT with PD-L1 checkpoint blockade could synergistically reduce the local accumulation of tumor-infiltrating myeloid-derived suppressor cells.⁷⁸ According to Chen *et al.*, RT increased PD-L1 expression in human EC cells through *in vitro* experiments.⁷⁹ In addition, many studies have shown that RT can not only increase tumor antigen presentation but also enhance checkpoint inhibitor-induced antitumor immune responses.^{74,80,81} The above mechanisms are the rationale for combining RT with ICPI.

In this review, we have mainly summarized the immunosuppressive effect of RIL in patients with EC. It is crucial to maintain an intact adaptive

Table 2. Ongoing clinical trials of radiotherapy combined with immunotherapy for EC.

Clinical Trial. Gov number.	Phase/line	Condition	Target	Arms
NCT 03044613	I/Neoadjvant	Operable Stage II/III Esophageal Gastroesophageal Junction Cancer	PD-1	Arm A: Nivolumab + carboplatin/ paclitaxel + radiation Arm B: Nivolumab + relatlimab + carboplatin/ paclitaxel + radiation
NCT 02830594	II/Salvage	Adenocarcinoma of the gastroesophageal junction, gastric cancer, EC, metastatic malignant neoplasm in the stomach	PD-1	Single-arm: pembrolizumab + palliative radiation
NCT 02642809	I/1st, 2nd	Metastatic EC	PD-1	Single-arm: pembrolizumab + radiation (brancytherapy 2 fractions \times 8 Gy)
NCT 03087864	II/1st	Stage II/III EC	PD-L1	Single-arm: atezolizumab + carboplatin + paclitaxel + radiation (23 \times 1.8 Gy)
NCT 02844075	II/Neoadjvant	ESCC	PD-1	Single-arm: pembrolizumab + taxol + carboplatin + radiation (21 \times 2.1 Gy) + surgery
NCT 03064490	II/Neoadjvant	Locally advanced EC and gastric cancer	PD-1	Single-arm: weekly neoadjuvant pembrolizumab + concurrent CRT [carboplatin/ paclitaxel + radiation (25 \times 1.8 Gy)] + surgery
NCT 03278626	I, II/1st	Locally advanced ESCC	PD-1	Single-arm: Nivolumab + Carboplatin/ paclitaxel + Radiation (28 \times 1.8 Gy)
NCT 03544736	I, II/-	EC	PD-1	Cohort A: Nivolumab + palliative radiation (2 Gy/ day, to a total of 30–50 Gy) Cohort B: Nivolumab + definitive chemoradiotherapy [carboplatin/ paclitaxel + radiation(23 \times 1.8 Gy)] Cohort C: Nivolumab + neoadjuvant chemoradiotherapy [carboplatin/ paclitaxel + radiation (23 \times 1.8 Gy)] + surgery
NCT 03437200	II/-	Inoperable EC	PD-1	Arm A: Nivolumab + FOLFOX + radiation (25 \times 2 Gy) Arm B: Nivolumab + Ipilimumab + FOLFOX + radiation (25 \times 2 Gy)
NCT 03490292	I, II/neoadjvant	Resectable EC Gastroesophageal cancer	PD-L1	Single-arm: Avelumab + Carboplatin/ paclitaxel + radiation (25f)
NCT 02520453	II/adjvant	EC	PD-L1	Neoadjuvant concurrent CRT + surgery + durvalumab <i>versus</i> neoadjuvant concurrent CRT + surgery + placebo
NCT 03377400	II/1st, 2nd	ESCC	PD-L1	Single-arm: durvalumab/ tremelimumab + concurrent radiotherapy (5FU/ CDDP)

CDDP, cisplatin; CRT, chemoradiation therapy; EC, esophageal cancer; ESCC, esophageal squamous cell carcinoma; 5FU, 5-fluorouracil; NCT, national clinical trial.

immune system during cancer therapy to enhance the effectiveness of immunotherapy and improve cancer control.²³ For a better combination of RT with ICPI, it is necessary to reduce the immunosuppressive effect of RIL. In Marciscano *et al.*'s preclinical model to evaluate the immunological differences of RT strategies with or without ENI and highlight the effect of field size on lymphocyte subpopulation from an immunological perspective⁵⁴; the authors found that RT correlated with the up-regulation of an intratumoral T-cell chemoattractant chemokine signature (CXCR3, CCR5-related), which resulted in a robust infiltration of multiple subpopulations of lymphocytes. The addition of ENI reduced the expression of chemokines, inhibited immune infiltration, and adversely impacted survival when combined with ICPI. In yet another study, Pike *et al.* reported that prolonged courses (>five fractions) of RT increased the risk of severe lymphopenia, which was associated with more reduced survival in patients treated with ICPI.⁸² A study by Won Jin Ho demonstrated that pretreatment ALC is significantly associated with the response to PD1 inhibitors in patients with recurrent and/or metastatic head and neck squamous cell carcinoma.⁸³ Patients with pretreatment ALC < 600 cells/ μ l were found to have significantly shorter PFS than patients with pretreatment ALC \geq 600 cells/ μ l.

More and more emerging evidence is showing that the choice of radiation dose, fractionation, and target volume plays a crucial role in the combination of RT with other treatment measures. It would, therefore, be beneficial to know whether some radiation regimens (optimum dose, fractionation, target site, timing, and target volume) have superior effects over others in terms of immune stimulation. We can infer from the studies discussed in this review that small radiation field sizes and shorter radiation courses appear to promote combinatorial efficacy in RT and immunotherapy.

Currently, immunotherapy is not the standard treatment for EC. It is, to that effect, unwise to change the radiation regimen blindly. A few clinical trials using RT combined with immunotherapy for EC are underway (Table 2).^{77,84,85} We expect to explore a better RT regimen through these clinical trials to maximize the anti-tumor response from the combination of RT with immunotherapy. The ultimate challenge is to integrate cancer immunotherapy into RT optimally. RT could quite possibly be an immunologic adjuvant if the RT plan is right.⁸⁶

Conclusion

RT can cause lymphopenia, and RIL is associated significantly with a poor outcome in EC patients. Tailoring RT regimens to spare the immune system may be an important future direction to improve prognosis in the EC population. Hence, it is critical to find effective treatment strategies to prevent or mitigate RIL in patients with EC. The RT target volume of EC is extensive, including the cervical, mediastinal, and upper abdominal nodes, which could be contained in multiple OARs of lymphopenia. To date, combining RT with immunotherapy has not only proven effective in preclinical studies but has also shown promise in clinical trials. To optimize RT regimens in the context of immunotherapy, several factors need to be considered: target volume, optimal dose and fractionation, and timing. We hope that the optimization of RT plans will further enhance the effectiveness of this combination. From the current evidence, the use of IFI, a radiation dose of 50.4 Gy, and the PBT technology could embody reasonable treatment strategies to reduce the incidence of RIL in EC. Further research is required to test this hypothesis.

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Author contributions

MHL and JMY designed the study. XW drafted the manuscript. PLW, ZXZ, QFM coordinated, edited, and finalized the drafting of the manuscript. All authors read and approved the final manuscript.

Availability of data and material

The dataset supporting the conclusions of this article is included within the article.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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