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Lymphocyte-Sparing Radiotherapy

Citation for published version (APA):

Lambin, P., Lieverse, R. I. Y., Eckert, F., Marcus, D., Oberije, C., van der Wiel, A. M. A., Guha, C., Dubois, L. J., & Deasy, J. O. (2020). Lymphocyte-Sparing Radiotherapy: The Rationale for Protecting Lymphocyte-rich Organs When Combining Radiotherapy With Immunotherapy. *Seminars in Radiation Oncology*, *30*(2), 187-193. https://doi.org/10.1016/j.semradonc.2019.12.003

Document status and date:
Published: 01/04/2020

DOI:
10.1016/j.semradonc.2019.12.003

Document Version:
Publisher's PDF, also known as Version of record

Document license:
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Please check the document version of this publication:

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Lymphocyte-Sparing Radiotherapy: The Rationale for Protecting Lymphocyte-rich Organs When Combining Radiotherapy With Immunotherapy

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There is now strong clinical and preclinical evidence that lymphocytes, for example, $CD8^+$ T cells, are key effectors of immunotherapy and that irradiation of large blood vessels, the heart, and lymphoid organs (including nodes, spleen, bones containing bone marrow, and thymus in children) causes transient or persistent lymphopenia. Furthermore, there is extensive clinical evidence, across multiple cancer sites and treatment modalities, that lymphopenia correlates strongly with decreased overall survival. At the moment, we lack quantitative evidence to establish the relationship between dose-volume and dose-rate to critical normal structures and lymphopenia. Therefore, we propose that data should be systematically recorded to characterise a possible quantitative relationship. This might enable us to improve the efficacy of radiotherapy and develop strategies to predict and prevent treatment-related lymphopenia. In anticipation of more quantitative data, we recommend the application of the principle of As Low As Reasonably Achievable to lymphocyte-rich regions for radiotherapy treatment planning to reduce the radiation doses to these structures, thus moving toward "Lymphocyte-Sparing Radiotherapy."
Semin Radiat Oncol 30:187–193 © 2019 Published by Elsevier Inc.

Introduction

The Pacific trial, a randomised phase 3 trial in non-metastatic, advanced non-small cell lung cancer (NSCLC), represented a breakthrough in immuno-oncology (IO) treatment within radiation oncology, convincingly demonstrating that adjuvant IO, after normofractionated chemoradiotherapy, can improve progression-free survival (PFS).[1] Remarkably, the radiotherapy (RT) schedules of the Pacific trial were neither

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FE was partly funded by the Else-Kroener-Fresenius Research Foundation under Grant 2015_Kolleg.14. FE has a research collaboration with Merck KgAa. FE has research and educational grants from Elekta, Philips, Siemens, Sennewald. JD is funded by the NIH and the Breast Cancer Research Foundation. Partially supported by NIH Core Grant P30 CA 008748. PL acknowledges financial support from ERC advanced grant (ERC-ADG-2015, no. 694812 - Hypoximmuno) and the European Program H2020-2017 (ImmunoSABR – no. 733008).

Conflict of interest: Dr. Philippe Lambin reports, within and outside the submitted work, grants/sponsored research agreements from Varian medical, Oncoradiomics, ptTheragnostic, Health Innovation Ventures and DualTpharma. He received an advisor/presenter fee and/or reimbursement of travel costs/external grant writing fee and/or in kind manpower contribution from Oncoradiomics, BHV, Merck and Convert pharmaceuticals. Dr. Lambin has shares in the company Oncoradiomics SA and Convert pharmaceuticals SA and is co-inventor of two issued patents with royalties on radiomics (PCT/NL2014/050248, PCT/NL2014/050728) licensed to Oncoradiomics and one issue patent on mtDNA (PCT/EP2014/059089) licensed to ptTheragnostic/DNAmito, three non-patentable invention (softwares) licensed to ptTheragnostic/DNAmito, Oncoradiomics and Health Innovation Ventures.

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https://doi.org/10.1016/j.semradonc.2019.12.003
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standardised nor optimised, as these were based only on investigator or radiation oncologist choice for each individual patient (total dose 54 Gy-74 Gy). Separately, it has been shown that RT is a double-edged sword regarding immune effects: it has both an immunostimulatory effect but also an immunosuppressive effect.[2] IO might reduce or overrule this RT-related immunosuppression.[1,3] Furthermore, lower doses to the heart, circulating blood pool, and lymphoid organs are associated with reduced immunosuppressive effect.[3,4] It can thus be hypothesised that an optimised RT protocol has the potential to decrease the immunosuppressive effects of RT, for example, by reducing RT-related lymphopenia (LP).

Several studies have shown that low blood lymphocyte count at baseline, across a range of cancer types, is a negative predictor of outcome.[5-11] Furthermore, the presence of $CD8^+$ tumour infiltrating lymphocytes on pathology review is a well-established predictor of better overall survival.[12-14] Additionally, preclinical experiments with lymphocyte depletion, i.e. decreased $CD4^+$ and $CD8^+$ counts, have clearly established a causal relationship with reduced efficacy of RT and (radio)-IO.[15]

The effect of RT on LP is well-documented and has been extensively described for several decades.[16,17] Typically, LP is a transient phenomenon with a recovery within 3 months after RT, but in certain cases it can continue to persist even years after treatment[18] which has been correlated to RT dose, RT sites, (hyper)fractionation, adjuvant chemotherapy, and irradiated volume.[4,11-13,19-23] A causal relationship between RT-induced LP and adverse locoregional control or survival has been speculated but not confirmed.[24]

The Radiobiology of Lymphocytes

Lymphocytes are located in the blood (circulating lymphocytes), in reservoir lymphoid organs such as the spleen, and the thymus (in children and teenagers), in lymph nodes, and in the bone marrow, which is continuously producing new lymphocytes. As noted, some tumours are infiltrated by lymphocytes. It is important to appreciate that lymphocytes are a highly heterogeneous cell population comprised of subgroups with different roles in the crosstalk of tumours and the host immune system. The most prominent cell type in anti-tumour immune responses are $CD8^+$ effector T cells,[25] reflected in their prognostic significance[26] and their use in adoptive T cell therapy.[27] T_H1 polarised ($CD4^+$),[28] as well as $CD4^+$ cytolytic T cells, have also been shown to induce strong anti-tumour responses.[29] On the other hand, regulatory T cells[30] and T_H2 polarised $CD4^+$ T cells[31] have mostly been linked to pro-tumour effects.[29] There is contradictory data on the role of T_H17 T cells[29] and cancer in cancer immune responses.[32,33]

It has long been known that lymphocytes are the most radiosensitive cells of the hematopoietic system, as well as the entire body.[34] This radiosensitivity is surprising for a nondividing cell type, but may be related to robust apoptotic response pathways. The lethal dose required to reduce the surviving fraction of circulating lymphocytes by 90% (LD90) is only 3 Gy.[35] 0.5 Gy already leads to significant cell death induction in lymphocytes. Such a dose could easily be reached in standard RT schedules. Yovino et al found that with a standard treatment of 60 Gy in 30 fractions for glioblastoma (GBM) treatment, during every fraction of RT, 5% of circulating cells receive >0.5 Gy,[36] summing up to >95% of circulating cells being exposed to >0.5 Gy over the 6 week treatment. The induced cell death is predominantly apoptosis.[37]

Importantly, different lymphocyte subtypes show distinct radiosensitivity.[38-40] Naive $CD8^+$ effector T cells are more sensitive than memory T cells,[37,40,41] while regulatory T cells are relatively resistant.[40,42,43] Furthermore, the state of T cells, the solid organs and the different location containing $CD8^+$ T cells also influences radiosensitivity.[44,45] T cells that are proliferating are more radioresistant than T cells in other state.[44] With regard to the organs, the parenchymal $CD8^+$ T cells in the solid lymphoid organs (lymph nodes and spleen) are found most radiosensitive, followed by those residing in liver and gut. The $CD8^+$ T cells located intratumourally have a higher radioresistance, an increased motility and IFN-γ secretion compared to circulating $CD8^+$ T cells and T cells in unirradiated tumours.[45] This may be due to changes in the tumour microenvironment wherein TGF-β is a key regulator in making the intratumoural T cells more radioresistant.[45] Similar differential effects have been observed concerning radiation dose rate[46] with high dose rates leading to less lymphocyte death.[47,48] These findings are well in line with clinical observations of decreased naïve T cells and enriched regulatory T cells in patients undergoing RT.[14,49-51]

Analysis of the Clinical Literature

In many trials, the Common Terminology Criteria for Adverse Events (CTCAE) is used to differentiate between LP Grade 1 (<~1000-800/mm^3), Grade 2 (<800-500/mm^3), Grade 3 (<500-200 mm^3), and Grade 4 (<200/mm^3). Clinical factors that are associated with LP and key findings regarding LP for various cancers (GBM, head and neck squamous cell carcinoma, nasopharyngeal cancer, NSCLC, SCLC, breast cancer, esophageal cancer, pancreatic cancer, hepatocellular cancer, cervical cancer)[24] are summarised below.

Factors That Influences LP

A disbalance in immunosurveillance due to tumour suppressor systems can contribute to LP that is present before treatment.[14] Also immunosuppressive medication or cancer-related treatment can lead to pre- and post-treatment LP, for example, corticosteroids, tyrosine-kinase inhibitors, and immune checkpoint inhibitors.[11,14] In addition, patients with immune-related conditions, such as multiple comorbidities, autoimmune diseases, genetic disorders in innate or adaptive defense, or patients with a poor WHO performance state are known to have worse PFS and overall survival (OS), probably related to a sub-optimally functioning immune system.

Also, treatment factors such as RT and chemotherapy have been shown to influence incidence and severity of LP. Firstly, RT in general results in a lymphocyte reduction.

More specifically, hypofractionation results in less reduction than normo- or hyperfractionation. Yuan et al and Saito et al have found in a breast and a palliative cohort, respectively, that LP was correlated with the number of fractions, independent of overall dose.[52,53] Secondly, irradiating larger Gross Tumour Volumes in NSCLC patients has been associated with lower lymphocyte count but not with lower total leukocyte, neutrophil, or monocyte counts during RT.[24] Thirdly, if lymphopoietic sites or organs containing large blood volumes are within the planning target volume, it will contribute to (longer duration of) LP.[14] Several authors have also found that higher spleen irradiation doses (total dose of 50-60 Gy) were significantly correlated with more patients experiencing LP during RT for hepatocellular cancer or palliative RT.[53-56] Based on these results, Liu et al recommend sparing of the spleen during abdominal irradiation.[54] Furthermore, a lower heart and lung dose resulted in less LP.[57-60] Increasing the heart and long dose, severe loss of cardiopulmonary performance was seen in preclinical studies.[61-66] Lastly, another important factor is the use of concurrent chemotherapy. Concurrent chemotherapy has been shown to have an impact on the severity of LP,[22] whereas adjuvant chemotherapy may prolong the duration of LP.[22] Importantly, different chemotherapy agents differ in LP impact.[14]

Predictive/Prognostic Factors for OS After Radiation Induced LP

Many possible prognostic factors for OS and PFS have been investigated, including the role of LP. Ladbury et al concluded that estimated dose of radiation to immune cells, Karnofsky performance status, not-otherwise-specified histology in NSCLC, lack of completion of chemotherapy,[9,23] and smoking history[23] are negative predictors for OS.

Disadvantageous prognostic factors for PFS and OS are baseline LP,[5-11,14] early LP after chemotherapy treatment (5 or 15 days),[14] LP after RT[14] or LP after IO.[7] Post-treatment LP has been negatively associated with poor tumour specific outcome in multiple cancer types for example, GBM, HNSCC, cervical, esophageal, NSCLC, and pancreatic.[11]

Effect of Combination Treatment (RT + chemo, RT + chemo and/or IO)

As described previously, RT alone can induce or worsen LP. However, combining RT with systemic treatment has an even bigger impact on LP and treatment outcome. Cho et al found that RT + checkpoint inhibitor -treated NSCLC patients with LP pre-IO treatment had a significant poorer PFS (2.2 vs 5.9 months) and OS (5.7 vs 12.1 months)[10] compared to patients who had normal lymphocyte counts before IO treatment. Furthermore they found that RT significantly increased LP before start of IO, however irradiating with SABR, proton beam therapy, hypofractionation or radiosurgery reduced the risk on (increasing) RT-induced LP.[10,14,60] The combination of RT with immunocytokines like IL2, IL7, or IL15 could eliminate LP due to their simulating effect to let the T cells develop, proliferate and survive.[14]

Joseph et al found that after concurrent chemo-radiotherapy the absolute lymphocyte count dropped significantly compared to absolute lymphocyte count pretreatment,[4] but did not alter treatment outcome. In contrast, Grossman et al observed worse tumour control and shorter OS in GBM patients with depleted $CD4^+$ T cell counts pre- and post-chemo-radiotherapy treatment.[67] Furthermore, a prolonged duration of LP was also seen with RT. Similar results were found retrospectively by Wang et al, with almost 50% of SCLC patients experiencing severe LP and 70.4% prolonged LP of 3 months minimum after chemo-radiotherapy.[21] For reasons not currently well understood, LP following RT can last from several months up to several years, whereas LP seen after sepsis or even chemotherapy alone tends to resolve more quickly.[68,69]

It is reasonable to hypothesise that transient LP has a different effect on the outcome than persistent LP. Thus, the negative influence of RT on LP might be abolished by combinatorial approaches with IO, which could result in differences in the timing, the length and probably the grade of LP. This effect also depends on type of IO agent applied. On the other hand, it might indicate that the effect of adding IO to RT schedules lies primarily in a better functioning immune system, which in turn will be crucial to slow down the pace of microscopic disease spread in at least some patients.

Modelling Approaches to Predict the Incidence and Severity of LP

Taking into account the negative effect of LP on clinical outcomes, it is important to identify high-risk patients timely and possibly adapt the treatment. Models predicting grade 4 RT-induced LP during chemo (radio) therapy for esophageal cancer, or acute and late LP for prostate cancer have already been published,[70,71] although the prostate model is yet to be validated.[19,72] Also for NSCLC, a predictive risk model has been developed where clinical and genetic factors, for example, lung V5 > 48%, age >65 years, >40 pack-years, and XRCC1 rs25487 AA genotype, are associated with severe RT-induced LP.[73]

Several recent analyses have indicated that irradiation of cardiovascular structures may lead not just to heart-related morbidities but to unexplained reductions in OS following RT for NSCLC. A key question is whether this is mediated primarily through immune suppression. Contreras et al showed that adjuvant chemotherapy and heart V50 > 25% are associated with LP at 4 months post-RT.[3] Thor et al observed that out-of-treatment-field regional recurrence was statistically linked to LP at 2 months post-RT.[74] However, details of the relationship between patient/disease/treatment factors and LP, as well as the impact on disease progression remain elusive and need further study.

Recommendations for Clinical Trials

There is a large body of literature evidence showing that incidence and severity of LP are associated with patient and

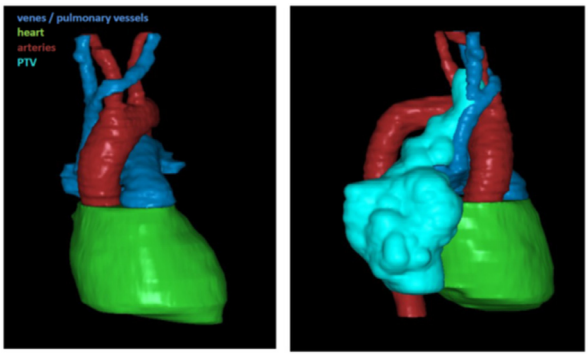

Figure 1 Example of segmentation for lung cancer treatment: left: delineation of the Lymphocyte-related Organs At Risk (LOAR), right delineation of the LOAR and the planning target volume.

treatment characteristics, but also showing the importance for clinical outcomes. Moreover, we have identified 437 trials listed in clinicaltrial.gov combining IO with RT, September 2019, indicating that combining RT with IO is being increasingly adopted as treatment strategy. To improve clinical outcomes, but also to gain the most of RT-IO combination treatment, it is of utmost importance to establish recommendations for RT planning with regard to lymphocyte dose. However, as indicating absolute dose constraints is not (yet) possible, we propose to apply the As Low As Reasonably Achievable principle to Lymphocyte-related Organs At Risk (LOARs) without compromising irradiation of the planning target volume (see Figs. 1 and 2) and keeping the constraints for "conventional" organs at risk such as lung, heart and spinal cord, as recommended in clinical protocols (Fig. 3). Furthermore, systematic recording of dose-volume and dose-rate statistics for those LOARs, as well as longitudinal lymphocyte counts is recommended. These data, routinely available at most treatment centres, would allow the design of strategies to predict and to some extent prevent RT-induced LP. It would also help to answer the main remaining hypothesis whether maintaining and/or restoring optimal lymphocyte counts may improve treatment RT outcomes, or increase the efficacy of IO.

These data can only be obtained if relevant organs are systematically delineated. These include the large vessels, heart,

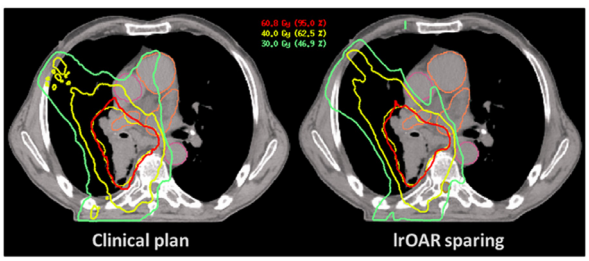

Figure 2 A standard dose distribution of a clinically applied radiation treatment plan (left), and an example of an optimised radiation plan applying the As Low As Reasonably Achievable (ALARA) principle (right), demonstrating that sparing of LOAR is feasible without compromising dose coverage of the target volume or increasing dose to OARs important in clinical radiotherapy planning.

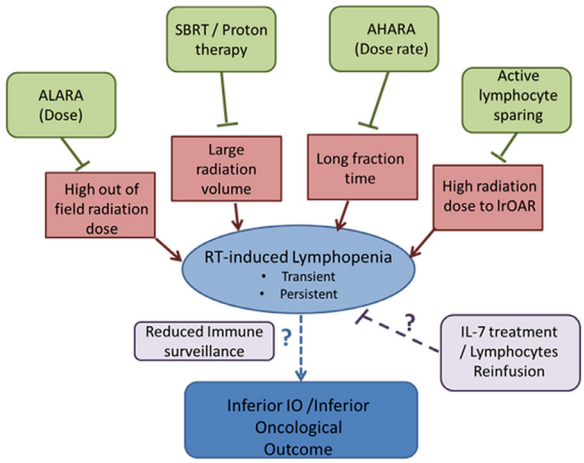

Figure 3 Hypothetical model linking radiation to lymphopenia and to inferior oncological outcomes.

and any irradiated lymphoid organs such as bone marrow (eg, pelvic bones, vertebrae, large long bones), nodal regions not included in the clinical target volume, spleen, and thymus in children. To facilitate the segmentation of large vessels, we propose to explore the use of contrast-enhanced computed tomography, acquiring data during the early blood dominated phases. Automatic segmentation methods based on deep learning will certainly facilitate this process.[75] Dose, fractionation, dose rate, and mean doses to LOARs should be reported as a minimum. Blood can be seen as a "moving OAR", therefore long irradiation times should be avoided. Instead, high-dose rate irradiation, following the principle of "As High As Reasonably Achievable" should be favoured, for example, using flattening filter-free irradiation.[76,77]

Prospects

As it is clear that the role of the immune system is very important for clinical outcomes, much research currently focuses on unraveling the complex interplay between treatment characteristics and the immune system and how to influence this relationship. In an attempt to preserve the immune system from the effects of radiation and chemotherapy, lymphocytes were isolated before treatment, stored, and administered again to the patient upon treatment completion (NCT01653834).[67] Interestingly, the promising therapeutic effect of immunoadjuvant therapy with IL7 (essential for lymphocyte proliferation and survival) has been explored in for example, immunocompromised patient and in some cancer trials, however the data regarding IL7 and LP during, pre, and post cancer treatment are scarce.[14,78-82]

New imaging methods may also become important. New magnetic resonance (MR) sequences may enable the investigator to quantify blood volume in vessels and organs using non-contrast MR imaging such as a venography technique or velocity-selective pulse trains.[83,84] These new approaches will allow us not only to quantify blood volume without contrast in the vascular system but also in organs such as liver, brain and

spleen. New positron emission tomography tracers that can precisely track $CD8^+$ T cells are also under development.[85] Furthermore, the combination of new strategies and precise technological developments,[20] such as a MR linear accelerator (MR-linac),[86] will make it possible to not only more precisely identify and track LOARs, but also avoid or restrict radiation dose to these LOARs. To facilitate comparable analyses, new autosegmentation and artificial intelligence methods could be distributed using portable container technology to extract dosimetric characteristics of the LOARs.[87]

Conclusion

The breakthrough improvement in outcomes by IO alone, or in combination with RT, has renewed the interest of the scientific community in strategies to predict and avoid RT-associated LP that may be immunosuppressive. There is a convergence of preclinical and clinical evidence correlating unintentional irradiation of LOARs with LP and poor outcomes. Preclinical studies definitively show an established causal relationship between lymphocyte depletion and the effectiveness of IO. However, accurate, individualised normal tissue complication probability models for LP are currently lacking. Therefore, we propose that the As Low As Reasonably Achievable principle should be applied to LOARs, and dose rates should be kept as high as practical possible to spare peripheral blood lymphocytes, in particular in the context of clinical trials combining RT with IO. Furthermore, we urge investigators of clinical RT trials with an immune component to systematically record the potentially-relevant dosimetric and hematopoietic parameters. Such unique data will hopefully lead to predictive models that will allow us to predict and prevent RT-induced LP in an individualised approach for each patient in order to answer the key unresolved question: whether maintaining and/or restoring optimal lymphocyte counts independently improves RT or IO outcomes.

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