Review Article

Proton beam therapy and immunotherapy: an emerging partnership for immune activation in non-small cell lung cancer

Howard J. Lee Jr, Jing Zeng, Ramesh Rengan

University of Washington Medical Center, Seattle, WA, USA

Contributions: (I) Conception and design: R Rengan, J Zeng; (II) Administrative support: R Rengan; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Jing Zeng. University of Washington Medical Center, Seattle, WA, USA. Email: jzeng13@uw.edu.

Abstract: Proton beam therapy (PBT) is becoming an increasingly common option for patients undergoing radiation therapy (RT). With the concurrent emergence of immunotherapy as an effective systemic treatment for historically treatment-resistant disease such as advanced non-small cell lung cancer (NSCLC), the combination of RT's immunoadjuvant effects with immunotherapy is gaining widespread attention. However, pre-clinical and clinical studies have shown potential immunosuppressive mechanisms associated with conventional RT that may restrict its immunogenic potential. Protons, as charged particles, exhibit both dosimetric and biological differences in normal and cancer cells that may be able to not only enhance the immunoadjuvant effects of RT, but also reduce immunosuppressive mechanisms. Here, we review the rationale, preclinical and clinical evidence, and ongoing efforts in combining PBT with immunotherapy in cancer treatment with a focus on NSCLC.

Keywords: Proton radiation; lung cancer; immunotherapy

Submitted Dec 24, 2017. Accepted for publication Mar 19, 2018.
doi: 10.21037/tlcr.2018.03.28
View this article at: http://dx.doi.org/10.21037/tlcr.2018.03.28

Introduction

In the landscape of cancer treatment, cytotoxic chemotherapy has been the mainstay of systemic treatment options for decades. Surgery and radiation therapy (RT) comprise the other two pillars of cancer treatment whose primary goal is to provide local control. However, immunotherapy, especially checkpoint inhibitors, has recently emerged as a major addition to the systemic treatment armaments physicians have at their disposal. This comes at a time when proton centers are becoming increasingly prevalent around the world to provide another method of delivering precision RT. Emerging preclinical and clinical evidence show that the combination of RT and immunotherapy can yield exceptional local and systemic outcomes for a subset of patients. A growing body of work suggests that the distinct radiobiological and dosimetric properties of proton beam therapy (PBT) could combine with immunotherapy to improve the outcomes of patients with difficult to treat tumors such as advanced non-small cell lung cancer (NSCLC).

The evolving role of immunotherapy

In 2010, a landmark study demonstrated a survival benefit in metastatic melanoma, a historically rapidly deadly diagnosis, using immune checkpoint blockade targeting the immunoregulatory molecule CTLA-4 (1). Global interest in cancer immunotherapy surged as a result and a second class of checkpoint inhibitors targeting the programmed cell death protein 1 (PD-1) was introduced soon afterwards (2).

Metastatic NSCLC patients typically have a relatively poor response rate to standard cytotoxic chemotherapy. Targeted therapies against epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) gene rearrangements have improved the survival of a small

subset of patients (3,4). However, immunotherapy has recently become the most promising emerging therapy for subsets of patients with advanced-stage disease. In the 2015 phase I KEYNOTE-001 trial, patients with locally advanced or metastatic NSCLC showed an overall response rate of 19.4% to pembrolizumab, with a 45.2% response rate in the PD-L1 ligand high-expressing population (5). Shortly afterwards, pembrolizumab was approved by the U.S. Food and Drug Administration (FDA) as second line therapy for patients with locally advanced or metastatic NSCLC and high tumor expression of PD-L1.

Immunotherapy provides a step forward, but response rates still need improvement

The phase III randomized KEYNOTE-024 trial in 2016 would go on to demonstrate a significant survival benefit in previously untreated patients with metastatic PD-L1 positive NSCLC receiving pembrolizumab versus standard chemotherapy (6). However, even with PD-L1 positivity, overall response rate was 44.8%, with majority of treated patients remaining non-responders.

Two trials went on to compare a second antibody against PD-1, nivolumab, with docetaxel in the second line for the treatment of metastatic squamous (CheckMate-017) and non-squamous lung cancer (CheckMate-057) (7,8). For both trials, 2-year overall survival was higher in the nivolumab arm. Thus, nivolumab was approved by the FDA in March of 2015 for second line treatment of advanced squamous cell NSCLC. However, with 2-year overall survival of 25–30% in the nivolumab arms of these trials, it is clear that improvement is needed to convert non-responders.

Current strategies to improve the response rate

Most of the current strategies to improve response rate to immunotherapy involve combining multiple immunotherapy agents. Targeting PD-1 and CTLA-4 concurrently, found to be successful in melanoma, was adapted to the treatment of NSCLC. The CheckMate-012 trial demonstrated an overall response rate of 38–47% in recurrent stage IIIB or IV, chemotherapy-naïve NSCLC with the combination approach depending on the dosing schedule (9). Some studies are also testing combining immunotherapy with chemotherapy. A randomized phase II study investigated carboplatin and pemetrexed with or without pembrolizumab in advanced non-squamous NSCLC patients, and found a significant improvement in progression-free survival (PFS) for the chemo-immunotherapy combination (10). However, the driving factor of the beneficial results may have been the efficacy of pembrolizumab in the high PD-L1-expressing subset of patients.

The ability to achieve 2-year plus survival in a subset of patients with PD-1 and CTLA-4 blockade spurred investigation of the use of immunotherapy with curative intent. The recent phase 3 PACIFIC trial using durvalumab demonstrated a landmark improved PFS for patients with unresectable, locally advanced stage III NSCLC. Following chemoradiotherapy, patients received either 1 year of durvalumab or placebo. Median PFS was substantially improved in the durvalumab arm (16.8 *vs.* 5.6 months) (11). Ongoing trials are attempting to improve outcomes and increase the response rate, including CheckMate-227 (NCT02477826, nivolumab *vs.* nivolumab/ipilimumab *vs.* nivolumab/platinum-based doublet *vs.* platinum-based doublet), and Impower-111 (NCT02409355, atezolizumab *vs.* gemcitabine with cisplatin or carboplatin). However, even in combination trials, there remains a subset of patients who do not respond to treatment, and most often these are groups of patients without high expression of molecules such as PD-L1. In these cases, RT is a tool that may be able to circumvent resistance patterns and expand the efficacy of immunotherapies.

Radiation and the immune system

Molecular impact of radiation on the immune system

Although radiation has historically been considered to mediate tumor cell death through its DNA-damaging cytotoxic effect, X-ray irradiation has been shown to induce immunostimulatory effects within the tumor that can trigger an antitumor immune response against a now *in situ* vaccine (12). The immunoadjuvant effects of radiation are based on the principles of immunogenic cell death (ICD) or phenotypic shifts within a tumor, as well as reprogramming of the tumor microenvironment. Radiation induces irradiated cells to induce release of tumor antigens or damage-associated molecular patterns (DAMPs), triggering a cascade that leads to activation of antigen presenting cells (APCs)/dendritic cells (DCs). Danger signals, such as HMGB1, prime $CD8^+$ T-cells through activation of toll-like receptors on APCs (13). These T-cells can then develop memory responses against the tumor. Radiation can also lead to the increase of both MHC class I expression on tumor cells for antigen presentation and release of pro-inflammatory chemokines that attract other APCs

and cytotoxic T lymphocytes (CTLs) (14-16). RT-induced release of tumor antigens also drives migration of APCs to draining lymph nodes where T-cell priming is augmented to initiate a CTL-dependent systemic response (17,18). Cross-presentation of released antigens by DCs in the tumor microenvironment also occurs as a result of local RT and assists in tumor eradication. This highlights the importance of cross-presentation of tumor antigens by MHC-II expressing APCs in addition to direct presentation via MHC-I on tumor cells in educating CTLs (19). The presence of CTLs before therapy has been correlated with better survival in multiple tumor types, including NSCLC (20,21).

Clinical reports of abscopal effects in lung cancer

The majority of clinical reports documenting systemic abscopal responses, in which out-of-RT field tumors regress after localized therapy, are in patients with melanoma. However, in 2013, a patient with metastatic NSCLC received conventionally fractionated RT (60 Gy) to a left upper lobe primary adenocarcinoma, and stereotactic body radiation therapy (SBRT) (26 Gy ×1) to a right lower lobe primary adenocarcinoma. The patient seemingly progressed over the next 2 months with FDG avid metastases in the adrenal gland and humerus, but by 1 year after radiation these lesions had achieved a complete metabolic response. The patient ultimately progressed but this demonstrated the occurrence of abscopal responses in NSCLC (22).

A promising method of inducing greater rates of abscopal responses is combining the immunostimulatory effects of radiation and immunotherapy. In a murine melanoma model, dual PD-1 and CTLA-4 blockade combined with radiation was associated with T-cell receptor diversification and resulted in greater control of non-irradiated tumors (23). A similar result was seen in peripheral blood samples of patients with metastatic melanoma who received combination anti-CTLA-4 therapy and hypofractionated high-dose RT. Seventeen percent of patients experienced responses in non-irradiated lesions, which was higher than the expected response rate for CTLA-4 blockade monotherapy. Trials have yet to show improvement in disease outcomes for patients receiving CTLA-4 blockade alone for NSCLC. However, in a case report of a patient with metastatic lung adenocarcinoma who had progressed on multiple systemic therapies, the patient experienced a clinical response in multiple metastatic lesions after receiving RT concurrently with CTLA-4 blockade (24). A clinical series of 69 patients who received a novel metronomic chemotherapy regimen with dose-fractioned cisplatin, oral etoposide and bevacizumab had 45 patients who also received palliative radiation to one or more metastatic sites (25). Median survival was longer in the group of patients who received RT [12.1±2.5 (95% CI: 3.35–8.6) *vs.* 22.12±4.3 (95% CI: 11.9–26.087) months; P=0.015]. Survival correlated with the chemotherapy regimen's ability to induce activated DCs and central-memory T-cells, suggest that tumor irradiation may prolong survival by eliciting an immune-mediated effect.

Ongoing trials

Many of the currently ongoing studies of combination radioimmunotherapy focus on anti-PD-1 therapy given their better safety profile over anti-CTLA-4 agents. Trials are ongoing in all stages of lung cancer, such as a phase I study of atezolizumab and SBRT in early stage NSCLC (NCT02599454) and another phase I study of pembrolizumab and dose escalated RT in the metastatic NSCLC setting (NCT02587455).

Some trials focus on immunotherapies other than immune checkpoint blockade, such as cancer vaccines against telomerase and MUC-1, or antigens including NY-ESO-1 and MAGE-A3 (26,27). Various immunomodulating molecules outside of PD-1 and CTLA-4 blockade are also being investigated in the settings of metastatic and recurrent NSCLC. At one institution SBRT is administered with concurrent FLT3 ligand, which is thought to enhance antigen presentation, in patients with metastatic refractory NSCLC (NCT02839265). Another trial is testing hypofractionated RT delivered with PD-1 blockade and nelfinavir, an agent thought to inhibit PI3Kinase-dependent DNA repair and myeloid-derived suppressor cell (MDSC) proliferation (NCT03050060).

Proton radiation, more immunogenicity with less immunosuppression?

Physics of protons

Radiotherapy can kill cancer cells by either directly causing DNA damage pushing the cell to undergo apoptosis or necrosis, or by creating oxygen free-radicals that then indirectly lead to DNA damage. Photon, or X-ray, radiation is highly penetrating and although some energy is deposited in tissues in the beam path, much of the radiation traverses the entire body and exits the other side, causing exit dose.

PBT uses a charged particle that deposits most of its dose at the Bragg peak, which occurs at a depth that can be controlled by calibrating the beam energy, eliminating exit dose. This difference between proton and photon radiation means proton treatment plans could improve sparing of normal tissues and organs-at-risk (28-31).

Data on immunosuppressive effects of photon radiation

Although RT induces immunoactivation through multiple mechanisms, immune cells are very sensitive to radiation and can be eradicated at much lower doses than required to kill cancer cells. The tumor microenvironment includes various inhibitory immune cells that may be upregulated as well including T_{reg} cells, MDSCs, and tumor-associated macrophages (TAMs) (32). T_{reg} cells are $CD4^+$ T-cells characterized by expression of the transcription factor forkhead box P3 (FOXP3). These cells can accumulate in the tumor microenvironment and secrete inhibitory cytokines, namely TGFB and IL-10, which both suppress CTL activation and stimulate MDSCs (33,34). Multiple studies have demonstrated an increase in number of T_{reg} cells in response to localized or whole body radiation, indicating that T_{reg} cells may be more radioresistant than other immune cells or regenerate more quickly (35-37). MDSCs contribute to tumor progression by both suppressing CTL function and promoting tumor angiogenesis (38,39). They are rapidly recruited to tumor stroma following localized RT within 3 days (40,41), with a local and systemic decrease in numbers 7–14 days after a single high dose of radiation (42,43). TAMs can be triggered by radiation to alter expression levels of chemokines, altering the regulation of T-cell infiltration (44). Depletion of all TAM subtypes, including M1 tumor-killing TAMs as well as M2 tumor-promoting TAMs before irradiation was shown to increase the antitumor effects of RT, indicating that TAM populations may be predominantly immunosuppressive M2 cells (45). In contrast to this, low dose irradiation delivered to certain tumors may also be able to normalize aberrant vasculature and induce TAMs to undergo a M1 phenotypic switch, which is required for CTL recruitment and function (46). Clearly there is a need for precision RT techniques that maximize the immunogenic properties of therapy while avoiding the immunosuppressive ones. PBT is an attractive option with its dose-distribution advantages, allowing clinicians to minimize unnecessary radiation of normal tissues that may trigger immunosuppressive components of the body's response to RT.

Lymphopenia and impact on clinical outcome

T lymphocytes are exquisitely sensitive to radiation and die at low doses of RT (47). This presents an issue when considering the goal of systemic immune responses and the fact that photon-based plans often involve significant areas of low dose bath due to the many overlapping beams used. This can expose large circulating blood volumes to radiation. Degree of lymphopenia in NSCLC patients receiving definitive RT has been associated with gross tumor volume and the volume of lung receiving 5–10 Gy. Furthermore, low nadir lymphopenia was shown to be associated with a worse overall survival (48). Dosimetrically, proton therapy provides a clear advantage in terms of the size of the low dose region and has been shown to provide a significant benefit in RT induced lymphopenia as well (49,50).

An issue with standard conventionally fractionated treatment plans for large tumors is that they may deliver potentially lymphotoxic radiation doses to the entire circulating blood pool (51). Although tumor radiation causes immunostimulation and chemokine secretion leading to recruitment of CTLs, any recruited cells may be depleted by conventional fractionation patterns used in most radiation oncology centers today. One study demonstrated that an ablative dose of 30 Gy ×1 induced a strong CTL infiltration of the tumor microenvironment with concurrent loss of MDSCs. However, when this 30 Gy ×1 was followed by 3 Gy ×10 to mimic conventional fractionation delivered in clinics today, the CTLs were lost and MDSC numbers began to increase (43). Historically RT has been delivered in multiple low dose fractions to spare normal tissues, counting on the lower fidelity of repair mechanisms by tumor cells to maximize tumor cell kill while minimizing normal tissue damage. With the advent of techniques such as SBRT, we have entered an era in which hypofractionation can be used to sculpt not only dose but the immune responses they generate. However, there are conflicting data on what doses and fractionation schemes have the greatest potential for abscopal responses after RT. Fractionated regimens (8 Gy ×3 and 6 Gy ×5) were found to be superior to an ablative dose (20 Gy ×1) with concurrent CTLA-4 blockade in triggering abscopal responses in murine breast and colon carcinoma lines (52). Doses above 12 Gy were also found to be associated with increase in expression of Trex, a DNA exonuclease that attenuates cancer cell immunogenicity by degrading DNA that accumulates in the cytosol after radiation (53). This removes an important signal for expression of a protein called stimulator of

interferon genes (STING) and ultimately downstream interferon beta secretion, which have been tied to antitumor immunity in murine tumor models (54,55). However, clinical reports have indicated that SBRT is associated with significantly less severe radiation induced lymphopenia than conventional RT at 1 month (56). Thus, further research is needed to elucidate the relative importance of preserving circulating lymphocyte pools versus fractionating RT regimens. The dosimetric advantages of proton therapy provide an additional tool in the search for the optimal radiation dose, fractions, and fields to maximize an anti-tumor immunogenic response.

Photon radiation versus charged particle radiation—a different biological effect?

In addition to its different dose deposition profile, proton beams also have a higher linear energy transfer (LET) than photon radiation which translate to a different biologic effect. LET is defined as the amount of energy per particle transferred per unit distance, and the increased number of ionization events delivered in a shorter distance increases the probability for double strand DNA breaks in addition to other effects in a tumor cell. This is related to the biological damage delivered per unit dose by calculated comparison to an equivalent photon dose, and is described by the term relative biological effectiveness (RBE). *In vitro* work has suggested that higher radiation-induced immunogenicity may be correlated with higher LET (57,58). Much of the pioneering work in particle radiotherapy has been performed in Japan and Germany using carbon ions, a form of particle therapy with dose distributive effects similar to protons but with higher LET (59).

A major question is the degree to which these differences in biological effect may translate to a clinical benefit. In combination, the dose-distribution benefit and increased RBE 2 to 3 times that of photon irradiation (for carbon ions) act through a predominantly direct DNA damage mechanism that is relatively cell-cycle and oxygenation independent compared to conventional X-ray therapy. This may have applications in radioresistant and hypoxic tumors (60). Clinically, although proton therapy dose is converted to photon therapy dose by simple multiplication of an RBE of 1.1, it is known that the actual RBE of a proton beam varies with beam depth and increases nonlinearly beyond the Bragg peak, leading to a small region with increased RBE at the end of the beam. Preclinical work supports the immunogenic potential of proton therapy and suggests that it may in fact have broader immunogenic applications than photons. For example *in vitro* studies suggest that protons may mediate calreticulin translocation to cell surfaces at higher levels than photons, increasing cross-priming and sensitivity to CTLs (61,62). *In vitro* data has also shown that PBT and X-ray irradiation achieves similar levels of survival of radiated melanoma cells, but only PBT induces long-term inhibition of migration (63). Anti-metastatic potential was also demonstrated by PBT in human breast cancer cells and NSCLC cells (64,65). A study in murine breast tumor (EMT6) cells and human salivary gland tumor cells showed that sublethal damage recovery was suppressed more after PBT than after X-ray irradiation (66). However, low energy proton beams induce tumor cell apoptosis through reactive oxygen species formation and activation of caspases, a process that may not be expected to prime CTLs through ICD (67). Like the contrasting data on hypo *vs.* hyperfractionation and ablative dose *vs.* low dose in generating immunostimulatory effects, many of the biological effects of protons compared to photons and their corresponding clinical relevance have yet to be elucidated.

In vivo and clinical data for systemic tumor responses resulting from protons is limited, but preliminary *in vivo* work with carbon ions has shown significant reductions in the number of lung metastases in murine osteosarcoma and squamous cell carcinoma models even without concurrent immunotherapy (68,69). Studies have also linked DC injection immunotherapy alongside carbon-ion beam therapy as a promising method for anti-tumor immune responses, with photon RT requiring a higher dose to suppress metastasis (70). Clinically, two cases of patients experiencing abscopal responses following carbon ion RT without immunotherapy for recurrent colorectal cancer have been reported. A 75-year-old patient received 73.6 Gy (RBE) in 16 fractions to a painful recurrence in his left flank, with resolution of a para-iliac artery mass on FDG PET/CT 1 month following treatment. An 85-year-old patient with recurrence in a lymph node near the abdominal aorta received 50.4 Gy (RBE) in 12 fractions, with mediastinal lymph node metastases resolving 6 months following RT. The question remains whether these abscopal responses were due to ablative dose delivery afforded by particle therapy, an immunogenic effect secondary to high-LET radiation, or both (71). Taken together, the body of preclinical work with protons and other charged particles brings up the following questions: can protons

produce greater ICD in tumor cells? Can differences in LET change antigen release or MDSC/T_{reg} induction? Can other particles improve ICD? These questions will be the subject of ongoing investigations regarding the relationship between immunotherapy and particle beam radiotherapies such as PBT.

Conclusions

In an era of cancer treatment that is becoming more focused on activating the immune system against tumor cells, radiotherapy is and will continue to be an essential multifunctional tool.

PBT is becoming an increasingly common option for the 50% of cancer patients undergoing RT with over 20 proton centers now operating within the United States and over 75 worldwide (72). Potential radiobiological differences due to the LET of protons compared to photons may be able to further enhance the immunoactivating properties of conventional RT. Furthermore, pre-clinical and clinical data have shown potential immunosuppressive mechanisms associated with conventional RT that PBT with its dose distribution advantages may be able to mitigate while still provoking proimmunogenic effects. In this vein, an area of potential fruitfulness may be to investigate the efficacy of using proton-like dosimetry to spare important immune organs in RT plans such as large sections of bone marrow, the spleen, or even circulating blood volume.

The potential clinical benefits for protons in facilitating immune responses are abundant. For example, the PACIFIC trial was able to demonstrate a landmark PFS benefit in advanced NSCLC patients using a trial design in which patients received multiple lymphocyte-depleting interventions: chemotherapy, fractionated dosing at 2 Gy per fraction which poorly activates STING and may deplete newly primed T-cells, and irradiation of lymph nodes surrounding tumors where T-cells may need to be educated. If outcomes such as this can be obtained even with non-ideal treatment delivery from an immunological standpoint, the potential benefits might be even more pronounced with the dose-sparing effects of protons aimed toward avoidance of triggering immunosuppressive effects.

In this review we have demonstrated an overview of major proimmunogenic and immunosuppressive events occurring in tumor cells and the tumor microenvironment after RT. However, with the myriad competing components of these two sides, and the heterogeneity in treatment and tumor characteristics in published clinical abscopal cases to date, it may be useful to view immunogenicity and immunosuppression as two sides of the same scale as proposed by Drs. Formenti and Demaria (73). In the balance between the proimmunogenic and immunosuppressive effects of radiation on the immune system, proton therapy is a promising modality that can potentially remove components from the immunosuppressive side while adding to the proimmunogenic side. We eagerly await the results of numerous studies that may inform clinicians how to tip that balance and convert the non-responders of current clinical trials into patients with durable systemic immune responses.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus Dacarbazine for Previously Untreated Metastatic Melanoma. N Engl J Med 2011;364:2517-26.
2. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer. N Engl J Med 2012;366:2443-54.
3. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or Carboplatin–Paclitaxel in Pulmonary Adenocarcinoma. N Engl J Med 2009;361:947-57.
4. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693-703.
5. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. N Engl J Med 2015;372:2018-28.
6. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. N Engl J Med 2016;375:1823-33.
7. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non–Small-Cell Lung Cancer. N Engl J Med 2015;373:123-35.
8. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non–Small-Cell Lung Cancer. N Engl J Med 2015;373:1627-39.

9. Hellmann MD, Rizvi NA, Goldman JW, et al. Nivolumab plus ipilimumab as first-line treatment for advanced non-small-cell lung cancer (CheckMate 012): results of an open-label, phase 1, multicohort study. Lancet Oncol 2017;18:31-41.
10. Langer CJ, Gadgeel SM, Borghaei H, et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. Lancet Oncol 2016;17:1497-508.
11. Antonia SJ, Villegas A, Daniel D, et al. Durvalumab after Chemoradiotherapy in Stage III Non–Small-Cell Lung Cancer. N Engl J Med 2017;377:1919-29.
12. Crittenden M, Kohrt H, Levy R, et al. Current Clinical Trials Testing Combinations of Immunotherapy and Radiation. Semin Radiat Oncol 2015;25:54-64.
13. Apetoh L, Ghiringhelli F, Tesniere A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 2007;13:1050-9.
14. Reits EA, Hodge JW, Herberts CA, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. J Exp Med 2006;203:1259-71.
15. Matsumura S, Wang B, Kawashima N, et al. Radiation-Induced CXCL16 Release by Breast Cancer Cells Attracts Effector T Cells. J Immunol 2008;181:3099-107.
16. Meng Y, Mauceri HJ, Khodarev NN, et al. Ad.Egr-TNF and local ionizing radiation suppress metastases by Interferon-B-Dependent Activation of Antigen-specific CD8 T Cells. Mol Ther 2010;18:912-20.
17. Lugade AA, Moran JP, Gerber SA, et al. Local Radiation Therapy of B16 Melanoma Tumors Increases the Generation of Tumor Antigen-Specific Effector Cells That Traffic to the Tumor. J Immunol 2005;174:7516-23.
18. Lee Y, Auh SL, Wang Y, et al. Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment. Blood 2009;114:589-95.
19. Sharabi AB, Nirschl CJ, Kochel CM, et al. Stereotactic Radiation Therapy Augments Antigen-Specific PD-1-Mediated Antitumor Immune Responses via Cross-Presentation of Tumor Antigen. Cancer Immunol Res 2015;3:345-55.
20. Characiejus D, Jacobs JJ, Pašukonienė V, et al. Prediction of response in cancer immunotherapy. Anticancer Res 2011;31:639-47.
21. Kawai O, Ishii G, Kubota K, et al. Predominant infiltration of macrophages and CD8+ T cells in cancer nests is a significant predictor of survival in stage IV nonsmall cell lung cancer. Cancer 2008;113:1387-95.
22. Siva S, Callahan J, MacManus MP, et al. Abscopal Effects after Conventional and Stereotactic Lung Irradiation of Non–Small-Cell Lung Cancer. J Thorac Oncol 2013;8:e71-2.
23. Twyman-Saint Victor C, Rech AJ, Maity A, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature 2015;520:373-7.
24. Golden EB, Demaria S, Schiff PB, et al. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. Cancer Immunol Res 2013;1:365-72.
25. Pastina P, Nardone V, Botta C, et al. Radiotherapy prolongs the survival of advanced non-small-cell lung cancer patients undergone to an immune-modulating treatment with dose-fractioned cisplatin and metronomic etoposide and bevacizumab (mPEBev). Oncotarget 2017;8:75904-13.
26. Brunsvig PF, Kyte JA, Kersten C, et al. Telomerase peptide vaccination in NSCLC: A phase II trial in stage III patients vaccinated after chemoradiotherapy and an 8-year update on a phase I/II trial. Clin Cancer Res 2011;17:6847-57.
27. Butts C, Socinski MA, Mitchell PL, et al. Tecemotide (L-BLP25) versus placebo after chemoradiotherapy for stage III non-small-cell lung cancer (START): A randomised, double-blind, phase 3 trial. Lancet Oncol 2014;15:59-68.
28. Parikh RR, Rhome R, Hug E, et al. Adjuvant Proton Beam Therapy in the Management of Thymoma: A Dosimetric Comparison and Acute Toxicities. Clin Lung Cancer 2016;17:362-6.
29. Ohno T, Oshiro Y, Mizumoto M, et al. Comparison of dose-volume histograms between proton beam and X-ray conformal radiotherapy for locally advanced non-small-cell lung cancer. J Radiat Res 2015;56:128-33.
30. Berman AT, Teo BK, Dolney D, et al. An in-silico comparison of proton beam and IMRT for postoperative radiotherapy in completely resected stage IIIA non-small cell lung cancer. Radiat Oncol 2013;8:144.
31. Roelofs E, Engelsman M, Rasch C, et al. Results of a Multicentric In Silico Clinical Trial (ROCOCO): Comparing Radiotherapy with Photons and Protons for Non-small Cell Lung Cancer. J Thorac Oncol 2012;7:165-76.
32. Fridman WH, Zitvogel L, Sautès–Fridman C, et al. The immune contexture in cancer prognosis and treatment. Nat Rev Clin Oncol 2017;14:717-34.

33. Burnette B, Weichselbaum RR. Radiation as an Immune Modulator. Semin Radiat Oncol 2013;23:273-80.
34. Facciabene A, Motz GT, Coukos G. T-Regulatory cells: Key players in tumor immune escape and angiogenesis. Cancer Res 2012;72:2162-71.
35. Kachikwu EL, Iwamoto KS, Liao YP, et al. Radiation enhances regulatory T cell representation. Int J Radiat Oncol Biol Phys 2011;81:1128-35.
36. Balogh A, Persa E, Bogdándi EN, et al. The effect of ionizing radiation on the homeostasis and functional integrity of murine splenic regulatory T cells. Inflamm Res 2013;62:201-12.
37. Persa E, Balogh A, Sáfrány G, et al. The effect of ionizing radiation on regulatory T cells in health and disease. Cancer Lett 2015;368:252-61.
38. Condamine T, Ramachandran I, Youn JI, et al. Regulation of Tumor Metastasis by Myeloid-Derived Suppressor Cells. Annu Rev Med 2015;66:97-110.
39. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med 2013;19:1423-37.
40. Crittenden MR, Cottam B, Savage T, et al. Expression of NF-κb p50 in tumor stroma limits the control of tumors by radiation therapy. PLoS One 2012;7:e39295.
41. Xu J, Escamilla J, Mok S, et al. CSF1R signaling blockade stanches tumor-infiltrating myeloid cells and improves the efficacy of radiotherapy in prostate cancer. Cancer Res 2013;73:2782-94.
42. Crittenden MR, Savage T, Cottam B, et al. The Peripheral Myeloid Expansion Driven by Murine Cancer Progression Is Reversed by Radiation Therapy of the Tumor. PLoS One 2013;8:e69527.
43. Filatenkov A, Baker J, Mueller AM, et al. Ablative tumor radiation can change the tumor immune cell microenvironment to induce durable complete remissions. Clin Cancer Res 2015;21:3727-39.
44. Inoue T, Fujishima S, Ikeda E, et al. CCL22 and CCL17 in rat radiation pneumonitis and in human idiopathic pulmonary fibrosis. Eur Respir J 2004;24:49-56.
45. Meng Y, Beckett MA, Liang H, et al. Blockade of tumor necrosis factor α signaling in tumor-associated macrophages as a radiosensitizing strategy. Cancer Res 2010;70:1534-43.
46. Klug F, Prakash H, Huber PE, et al. Low-Dose Irradiation Programs Macrophage Differentiation to an iNOS+/M1 Phenotype that Orchestrates Effective T Cell Immunotherapy. Cancer Cell 2013;24:589-602.
47. Trowell OA. The sensitivity of lymphocytes to ionising radiation. J Pathol Bacteriol 1952;64:687-704.
48. Tang C, Liao Z, Gomez D, et al. Lymphopenia association with gross tumor volume and lung V5 and its effects on non-small cell lung cancer patient outcomes. Int J Radiat Oncol Biol Phys 2014;89:1084-91.
49. Welsh J, Gomez D, Palmer MB, et al. Intensity-modulated proton therapy further reduces normal tissue exposure during definitive therapy for locally advanced distal esophageal tumors: A dosimetric study. Int J Radiat Oncol Biol Phys 2011;81:1336-42.
50. Davuluri R, Jiang W, Fang P, et al. Lymphocyte Nadir and Esophageal Cancer Survival Outcomes After Chemoradiation Therapy. Int J Radiat Oncol Biol Phys 2017;99:128-35.
51. Yovino S, Kleinberg L, Grossman SA, et al. The Etiology of Treatment-related Lymphopenia in Patients with Malignant Gliomas: Modeling Radiation Dose to Circulating Lymphocytes Explains Clinical Observations and Suggests Methods of Modifying the Impact of Radiation on Immune Cells. Cancer Invest 2013;31:140-4.
52. Dewan MZ, Galloway AE, Kawashima N, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. Clin Cancer Res 2009;15:5379-88.
53. Vanpouille-Box C, Alard A, Aryankalayil MJ, et al. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. Nat Commun 2017;8:15618.
54. Woo SR, Fuertes MB, Corrales L, et al. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. Immunity 2014;41:830-42.
55. Deng L, Liang H, Xu M, et al. STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. Immunity 2014;41:843-52.
56. Wild AT, Herman JM, Dholakia AS, et al. Lymphocyte-Sparing Effect of Stereotactic Body Radiation Therapy in Patients with Unresectable Pancreatic Cancer. Int J Radiat Oncol Biol Phys 2016;94:571-9.
57. Elsässer T, Weyrather WK, Friedrich T, et al. Quantification of the relative biological effectiveness for ion beam radiotherapy: Direct experimental comparison of proton and carbon ion beams and a novel approach for treatment planning. Int J Radiat Oncol Biol Phys 2010;78:1177-83.
58. Azzam EI, Jay-Gerin JP, Pain D. Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. Cancer Lett 2012;327:48-60.
59. Kamada T, Tsujii H, Blakely EA, et al. Carbon ion

radiotherapy in Japan: An assessment of 20 years of clinical experience. Lancet Oncol 2015;16:e93-100.
60. Tinganelli W, Durante M, Hirayama R, et al. Kill-painting of hypoxic tumours in charged particle therapy. Sci Rep 2015;5:17016.
61. Gameiro SR, Malamas AS, Bernstein MB, et al. Tumor Cells Surviving Exposure to Proton or Photon Radiation Share a Common Immunogenic Modulation Signature, Rendering Them More Sensitive to T Cell-Mediated Killing. Int J Radiat Oncol Biol Phys 2016;95:120-30.
62. Durante M, Reppingen N, Held KD. Immunologically augmented cancer treatment using modern radiotherapy. Trends Mol Med 2013;19:565-82.
63. Jasińska-Konior K, Pochylczuk K, Czajka E, et al. Proton beam irradiation inhibits the migration of melanoma cells. PLoS One 2017;12:e0186002.
64. Lee KS, Lee DH, Chun SY, et al. Metastatic potential in MDA-MB-231 human breast cancer cells is inhibited by proton beam irradiation via the Akt/nuclear factor-κB signaling pathway. Mol Med Rep 2014;10:1007-12.
65. Akino Y, Teshima T, Kihara A, et al. Carbon-Ion Beam Irradiation Effectively Suppresses Migration and Invasion of Human Non-Small-Cell Lung Cancer Cells. Int J Radiat Oncol Biol Phys 2009;75:475-81.
66. Hashimoto S, Sugie C, Iwata H, et al. Recovery from sublethal damage and potentially lethal damage : Proton beam irradiation vs. X-ray irradiation. Strahlenther Onkol 2018;194:343-51.
67. Lee KB, Lee JS, Park JW, et al. Low energy proton beam induces tumor cell apoptosis through reactive oxygen species and activation of caspases. Exp Mol Med 2008;40:118-29.
68. Ogata T, Teshima T, Kagawa K, et al. Particle Irradiation Suppresses Metastatic Potential of Cancer Cells. Cancer Res 2005;65:113-20.
69. Tamaki T, Iwakawa M, Ohno T, et al. Application of Carbon-Ion Beams or Gamma-Rays on Primary Tumors Does Not Change the Expression Profiles of Metastatic Tumors in an In Vivo Murine Model. Int J Radiat Oncol Biol Phys 2009;74:210-8.
70. Ando K, Fujita H, Hosoi A, et al. Intravenous dendritic cell administration enhances suppression of lung metastasis induced by carbon-ion irradiation. J Radiat Res 2017;58:446-55.
71. Ebner DK, Kamada T, Yamada S. Abscopal effect in recurrent colorectal cancer treated with carbon-ion radiation therapy: 2 case reports. Adv Radiat Oncol 2017;2:333-8.
72. Particle therapy facilities in operation. Particle Therapy Co-Operative Group. 2017. Available online: https://www.ptcog.ch/index.php/facilities-in-operation
73. Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: A paradigm shift. J Natl Cancer Inst 2013;105:256-65.

Cite this article as: Lee HJ Jr, Zeng J, Rengan R. Proton beam therapy and immunotherapy: an emerging partnership for immune activation in non-small cell lung cancer. Transl Lung Cancer Res 2018;7(2):180-188. doi: 10.21037/tlcr.2018.03.28

