# ROLE OF RADIOTHERAPY IN THE ERA OF TARGETED THERAPY AND PRECISION ONCOLOGY

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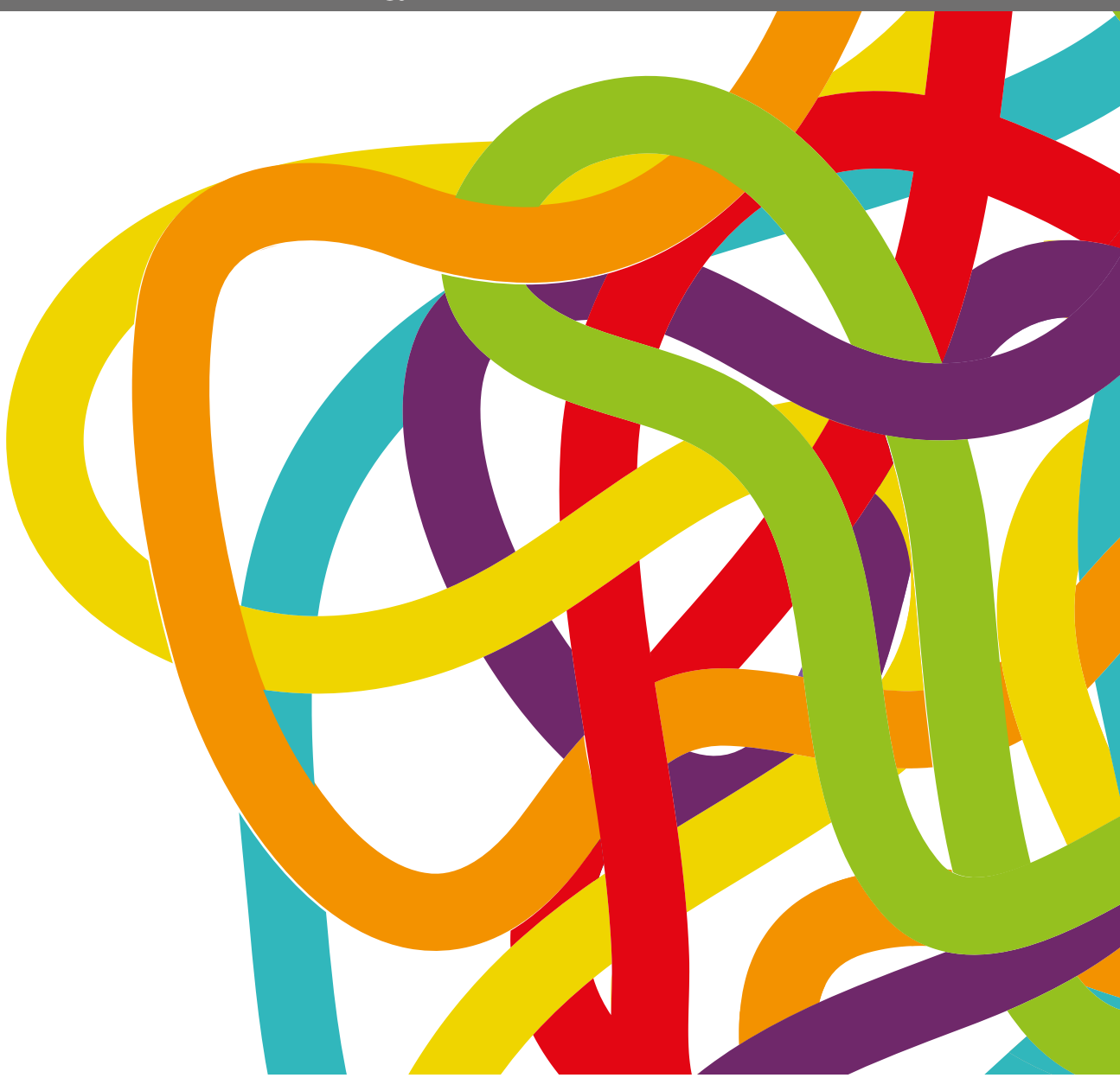

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# ROLE OF RADIOTHERAPY IN THE ERA OF TARGETED THERAPY AND PRECISION ONCOLOGY

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CASE REPORT
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# Anti-PD-1 Immunotherapy Combined With Stereotactic Body Radiation Therapy and GM-CSF as Salvage Therapy in a PD-L1-Negative Patient With Refractory Metastatic Esophageal Squamous Cell Carcinoma: A Case Report and Literature Review

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Esophageal squamous cell carcinoma (ESCC) is a malignancy with poor prognosis, which is often diagnosed at a late stage. Effective treatment options are limited when patients fail standard systemic therapy. The application of PD-1 inhibitors have led to a paradigm shift in the treatment of ESCC, but its efficacy as monotherapy is limited. Previous studies have shown that the antitumor effects may be reinforced when a PD-1 inhibitor is combined with radiotherapy or GM-CSF. This study aimed to report a case of a patient about advanced unresectable ESCC negative expression of PD-L1, who experienced tumor progression after chemoradiotherapy and targeted therapy.A significant systemic effect was seen after PD-1 inhibitor combined with GM-CSF and stereotactic body radiotherapy (SBRT) for metastatic lesions, however, severe pneumonia occurred after the triple-combination therapy. This study also reviewed several reports about the efficacy and safety of combination therapy.

**Keywords: immunotherapy, radiotherapy, PD-L1, esophageal squamous cell carcinoma, GM-CSF**

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## INTRODUCTION

Esophageal carcinoma is the sixth leading cause of cancer-related death in the world, with high malignancy and poor prognosis (1). More than half of patients with ESCC were initially diagnosed in an advanced or metastatic stage and treated with platinum-based chemotherapy regimens, commonly combined with fluoropyrimidine or taxane, as the main treatment. However, the long-term survival of these regimens remains poor, and the overall survival (OS) is as short as 7.7–15.5 months (2–4). The treatment options are more limited if ESCC progresses during or after standard first-line chemotherapy. Single-agent second-line chemotherapy, such as irinotecan, is recommended, resulting in poor OS of approximately 5 months. In addition, the incidence of adverse events caused by chemotherapy is high, seriously affecting the quality of life of patients.

Targeted therapy, such as apatinib or anlotinib, was approved as backline treatment in China, but it had no obvious breakthrough in efficacy, with a median OS of only 6 months (5, 6). Hence, it was believed that the treatment of advanced esophageal cancer had entered the bottleneck.

In 2019, pembrolizumab was officially approved for second-line and above treatment of PD-L1 positive combined positive score(CPS) $\geq$ 10 patients with advanced ESCC, which led to longer OS compared with chemotherapy (9.3 and 6.7 months, respectively) with statistical significance (7). However, the objective response rate (ORR) was only 6.4% in PD-L1-negative patients (7). Previous studies showed that the antitumor effects might be reinforced when a PD-1 inhibitor was combined with radiotherapy or GM-CSF. Recently, a substantial amount of data has emerged showing that stereotactic ablative radiotherapy (SABR), also known as SBRT, can enhance the immune system to kill tumors and achieve better tumor control. This reaction can be strengthened by the use of a PD-1 inhibitor or GM-CSF (8, 9). In addition, PD-L1-negative patients can benefit more from SBRT combined with a PD-1 inhibitor compared with PD-L1-positive ones (10). GM-CSF can promote the proliferation, maturation and migration of dendritic cells. Dendritic cells are antigen-presenting cells and play important roles in the anti-tumor effect of T cells (11). GM-CSF has also shown encouraging results in combination with a PD-1 inhibitor or radiotherapy in cancer treatment (12–14). The combination of PD-1 inhibitors with GM-CSF or radiotherapy induced remarkable antitumor immune effects (14) and produced objective abscopal effects in some patients with metastatic solid tumors (15).

In the present case, the tumor burden was significantly reduced by the triple-combination treatment, the suggested mechanisms might involve radio-sensitization of immunotherapy.

## CASE PRESENTATION

In 2018, a 57-year-old non-smoker male patient was diagnosed with 90-mm-long ESCC with multiple lymph nodes and lung metastases. Immunohistochemical staining of the tumor tissue showed that the PD-L1 expression was <1% (**Supplementary Figure 1**). The patient received intensity-modulated radiotherapy (IMRT) from February 28 to April 11, 2018, with doses of 60 Gy/28 f in primary esophageal tumor area and 56 Gy/28 f in metastatic lymph nodes and 50.4 gy/28 f in mediastinal lymphatic drainage with tumor involvement area. At the same time, chemotherapy with six cycles of nedaplatin (35 mg/m$^2$ on d1$^{-2}$) and paclitaxel (135 mg/m$^2$ on d1) at 3-week intervals was administered. Unfortunately, the patient's lung lesions progressed 2 months after the end of chemotherapy, indicating primary resistance to first-line chemoradiotherapy. Considering the poor condition, the patient was treated with apatinib, but lung metastases progressed in the 3-month evaluation. The patient was then rechallenged with 3 months of anlotinib, but the lung lesions continued to progress. Consequently, the treatment plan was changed, and the PD-1 inhibitor was combined with radiotherapy and GM-CSF from March 2019. The patient received the PD-1 inhibitor (sintilimab 200 mg) on the first day and then was treated with SBRT (3 doses of 8 Gy, daily) for one metastatic lesion in the right lung. On the second day after radiotherapy, GM-CSF 200 μg daily was subcutaneously injected for 2 weeks. This course was repeated every 3 weeks. Three courses of triple-combination therapy were administrated in total and every course was targeted different metastases with SBRT (**Figure 1A**, **Supplementary Figures 3–5**). Imaging assessment was performed after three cycles of triple-combination treatment, which revealed remarkable tumor regression at both the irradiated sites and distant unirradiated sites (**Figure 2**). However, only the mediastinal lymph nodes enlarged (**Supplementary Figures 2B,F**). Given the significant reduction of tumor burden (**Figure 3**), the patient continued to receive two cycles of sintilimab (200 mg/q3w) after triple-combination therapy. Later, the enlarged lymph nodes shrunk, indicating that the prior change in the lesion was pseudo-progression (**Supplementary Figures 2D,H**). In July 2019, the patient was diagnosed with new brain metastases with a Response Evaluation Criteria in Solid Tumors (RECIST1.1) score of PD (progressive disease) and progression-free survival (PFS) of 4 months (**Figure 1B**).

Grade 1–2 adverse events based on the Common Toxicity Criteria for Adverse Events (version4.0) include fatigue, poor appetite, hypothyroidism, and abnormal liver function. However, these adverse events did not significantly reduce the patient's quality of life. After five cycles of sintilimab, the patient began to have symptoms such as fever, cough, and dyspnea, which gradually aggravated. Chest computed tomography showed inflammatory changes in the lungs and partial lung consolidation (**Figure 4A**) and sputum culture suggested Acinetobacter epidermidis infection, considering radiation pneumonia combined with bacterial infection, immune-related pneumonia cannot be ruled out. The antitumor therapy was stopped when the patient was diagnosed with grade 3–4 pneumonia. Intravenous methylprednisolone 40 mg every 12 h and antibiotics were administrated. Methylprednisolone was reduced to 40 mg once a day 3 days later. After taking a sufficient amount of steroids and antibiotics for 2 weeks, the patient's symptoms improved significantly and methylprednisolone was reduced to oral 20 mg daily. The chest CT showed that pulmonary infiltration was absorbed and the patient was discharged (**Figures 4B,C**). However, he had a "flare" of symptoms of pneumonia because he did not follow doctor's advice to slowly tapered off steroids but direct deactivation. We followed up the patient's chest CT (**Figure 4D**) and continued to administrate steroids and antibiotics. Eventually, the patient and family members refused ventilator-assisted ventilation and died of respiratory failure in August 2019 (**Figure 1B**).

## DISCUSSION

In recent years, immune checkpoint inhibitors have shown encouraging results in the treatment of metastatic esophageal cancer (16–18). In the KEYNOTE-180 study, a clinically meaningful antitumor activity was observed, the ORR of ESCC

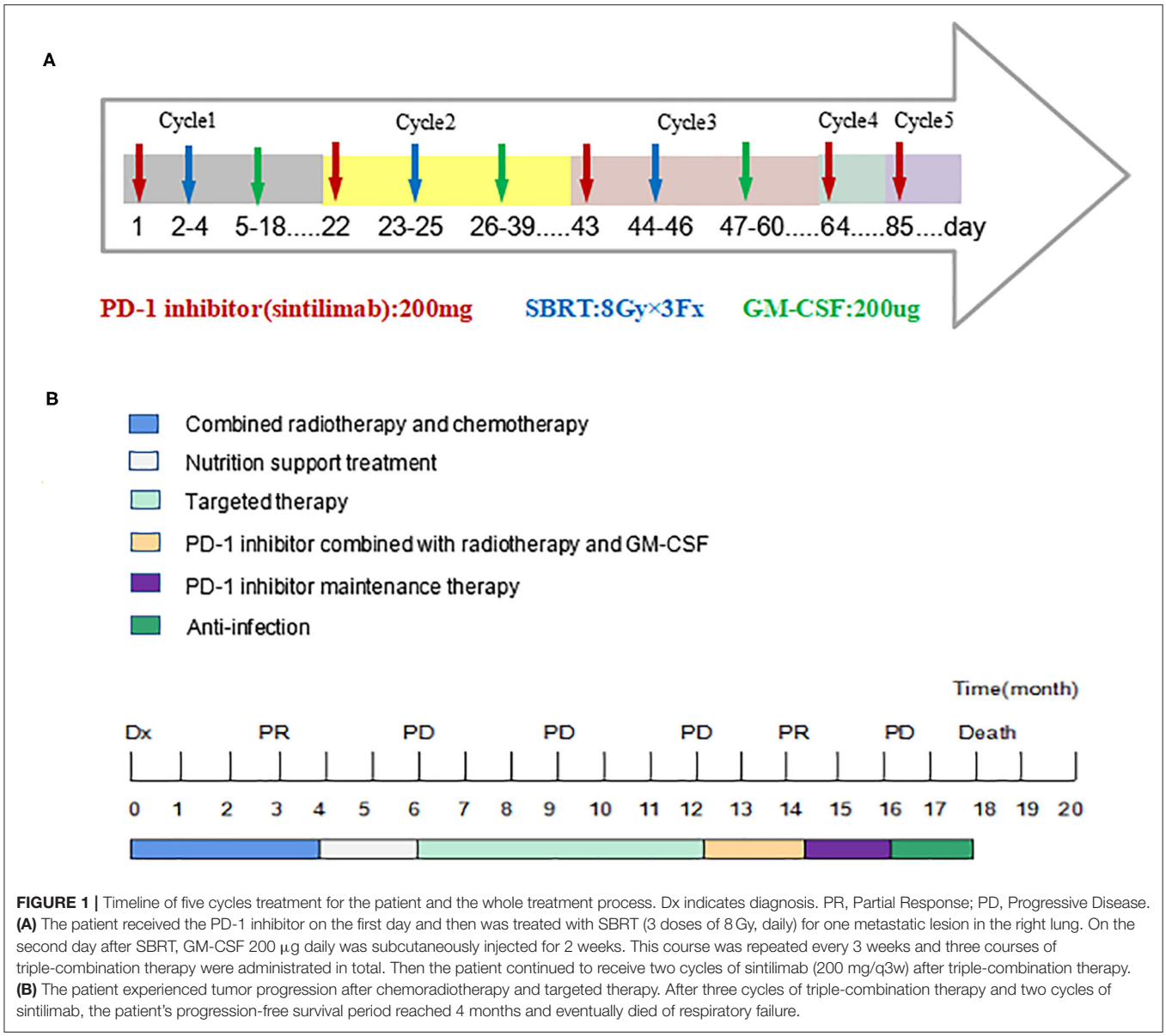


**FIGURE 1 |** Timeline of five cycles treatment for the patient and the whole treatment process. Dx indicates diagnosis. PR, Partial Response; PD, Progressive Disease. **(A)** The patient received the PD-1 inhibitor on the first day and then was treated with SBRT (3 doses of 8 Gy, daily) for one metastatic lesion in the right lung. On the second day after SBRT, GM-CSF 200 μg daily was subcutaneously injected for 2 weeks. This course was repeated every 3 weeks and three courses of triple-combination therapy were administrated in total. Then the patient continued to receive two cycles of sintilimab (200 mg/q3w) after triple-combination therapy. **(B)** The patient experienced tumor progression after chemoradiotherapy and targeted therapy. After three cycles of triple-combination therapy and two cycles of sintilimab, the patient's progression-free survival period reached 4 months and eventually died of respiratory failure.

patients (14.3%) was higher than that of adenocarcinoma patients (5.2%). The ORR of PD-L1-positive (CPS $\geq$ 10) population was higher than that of PD-L1-negative (CPS $<$ 10) population (14 vs. 6%) (17). The KEYNOTE-181 was a phase 3 trial where pembrolizumab was used in the second line of therapy in patients with advanced or metastatic ESCC or adenocarcinoma/Siewert-type gastroesophageal junction(GEJ) tumors. In the subgroup of PD-L1 CPS $\geq$ 10, pembrolizumab treatment showed significant benefits in mOS compared with chemotherapy (9.3 and 6.7 months, respectively) (7). In the ATTRACTION-3 study, patients with advanced ESCC refractory or intolerant to previous chemotherapy treatment with nivolumab had achieved a significant improvement in OS and safety profile vs. chemotherapy. It was also showed that the survival benefit of nivolumab was not related to tumor PD-L1 expression, but the patients with PD-L1 expression $\geq$1% had a 15% lower risk of death than the ones with PD-L1 expression $<$1% (18).

A phase I study investigated the efficacy and safety of camrelizumab in $\geq$2 line treatment of ESCC. The treatment with camrelizumab resulted in an ORR of 33.3%, a disease control rate (DCR) of 56.7%, and median PFS of 3.6 months. The incidence of treatment-related adverse events (TRAEs) and grade 3 TRAEs was 83.3 and 10%, respectively. Notably, the disease control rate was 33.3% in PD-L1-negative tumors and 66.7% in PD-L1-positive tumors (19). ESCORT was a phase III trial that evaluated the efficacy and safety of camrelizumab vs. chemotherapy for locally advanced or metastatic ESCC that progressed after first-line treatment, regardless of PD-L1 expression (NCT03099382). The final results of this study were reported at the 15th OESO World Conference. Camrelizumab provided a better survival

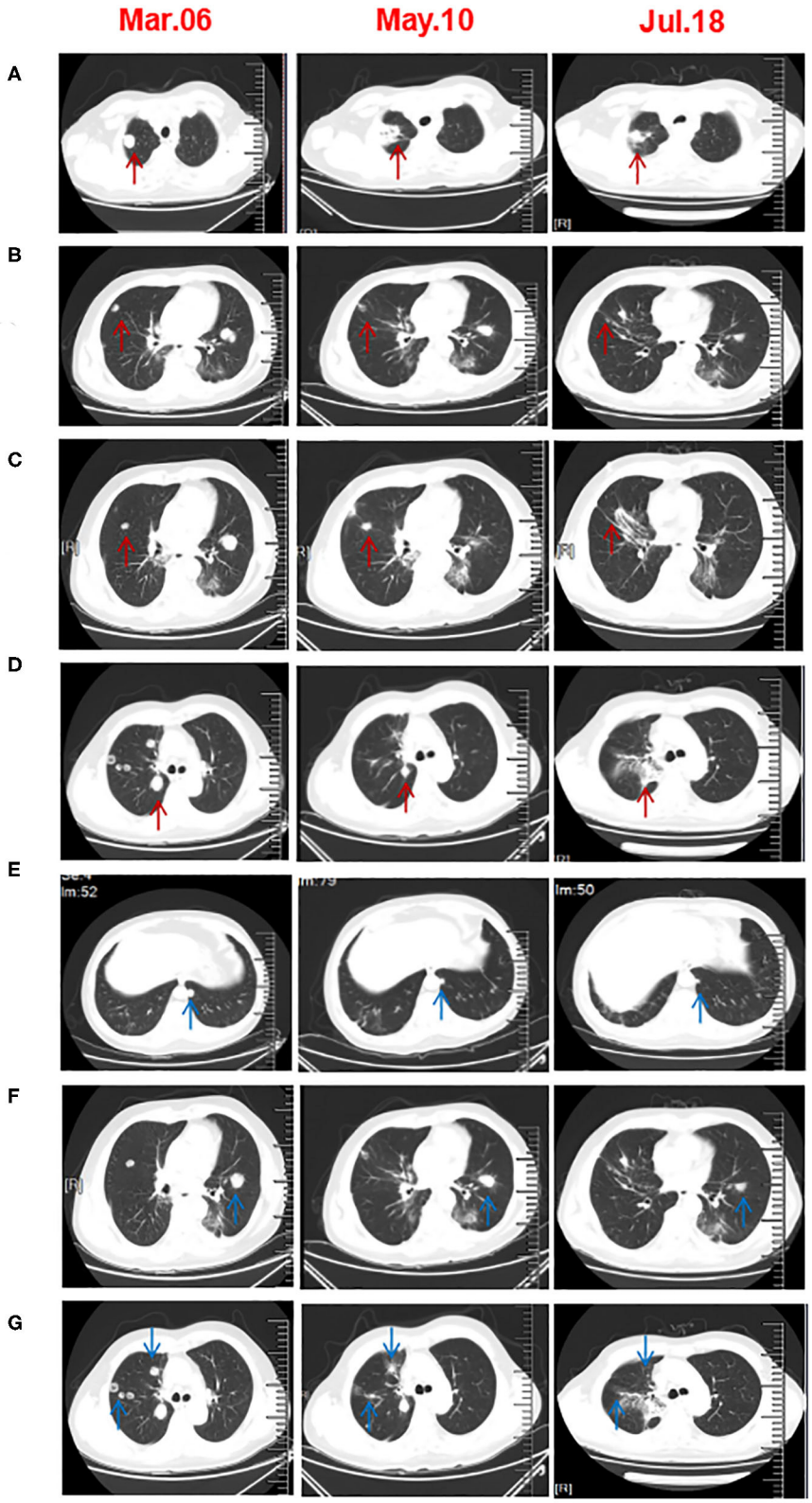


**FIGURE 2 |** Chest CT scans before and after three cycles of triple-combination therapy and two cycles of sintilimab treatment. **(A–D)** CT revealed that the irradiated right lung lesions shrunk or even disappeared. The arrow in A is the first lesion of the right lung SBRT. The arrow in **(B,C)** are the second lesions of the right lung SBRT. The arrow in **(D)** is the third lesion of the right lung SBRT. **(E,F)** The unirradiated metastatic lesion of the lung was significantly smaller than that before treatment. **(G)** After five cycles of treatment, the CT scan showed that the unirradiated lesions in right lung had disappeared.

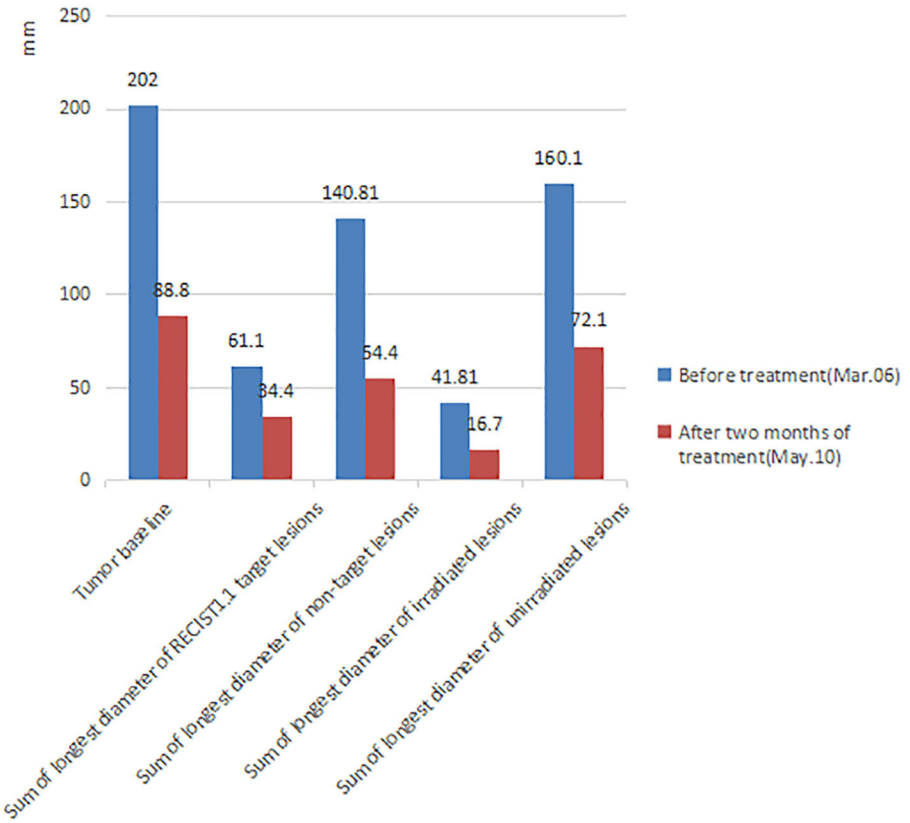


**FIGURE 3 |** Comparison of tumor burden before treatment and after 2 months of triple-combination therapy. After 2 months of treatment, the patient's tumor burden significantly reduced (The tumor volume data was measured by two doctors, the measurement error of each lesion is <2 mm and we finally took the average value). **Tumor baseline**: Sum of longest diameter of all measurable lesions. **RECIST1.1 Target lesions**: According to the standard of RECIST1.1, each organ can select at most two lesions as target lesions, so we randomly selected two lung metastatic lesions as target lesions before treatment. **Non-target lesions**: All measurable metastatic lesions except target lesions. **Irradiated lesions**: All the SBRT lesions. Considering that the regression of the lesion after radiotherapy will affect the real curative effect, the target lesions is not selected as the SBRT lesions. **Unirradiated lesions**: All measurable metastatic lesions except SBRT lesions.

benefit compared with chemotherapy with PD-L1 $\geq$ 1% (mOS: 9.2 vs. 6.3 months). The ORR was 20.2% in the study group and 6.4% in the control group. Currently, a phase 3 trial is ongoing to compare cisplatin combined with paclitaxel(TP) plus sintilimab with TP as the first-line treatment in patients with locally advanced unresectable or metastatic ESCC (CTR20181308). Although a large proportion of patients with ESCC have tumors with PD-L1 expression (18.4–82.8%) (20), how PD-L1-negative patients can benefit from immunotherapy needs to be explored.

Strategies to combine other treatment modalities such as radiotherapy are being investigated as means of improving the response rates to a PD-1/PD-L1 inhibitor (21). SBRT can cause more immunogenic death of tumor cells, promote tumor-associated antigen release and presentation, and induce stronger systemic antitumor effects (22). This response can be augmented by the addition of systemic immune-enhancement measures, such as the use of GM-CSF or PD-1/PD-L1 inhibitors (8, 9). Radiotherapy also significantly increases the infiltration of immune cells, thus changing the "immune desert" tumor microenvironment into "immune-inflamed" one (23, 24). Radiotherapy can not only promote antitumor immunity but also produce an immunosuppressive effect. PD-L1 expression can be significantly upregulated by radiotherapy (25), but this negative effect can be offset when combined with anti-PD-1/PD-L1 therapy. In addition, SBRT avoids lymphopenia, indicating a better combination strategy compared with conventionally fractionated radiotherapy or chemotherapy (26).

Several prospective clinical studies showed the safety and efficiency of SBRT combined with a PD-1 inhibitor. A phase I prospective clinical trial of SBRT combined with pembrolizumab in advanced solid tumors showed that the overall ORR was 13.2% with acceptable toxicity (27). Another phase II trial study (PEMBRO-RT) titled "SBRT (3 doses of 8 Gy) sequential pembrolizumab control single drug pembrolizumab in the treatment of advanced non-small cell lung cancer (NSCLC)," the ORR after 12 weeks was 36% in the study group vs. 18% in the control group. The subgroup analysis showed that PD-L1-negative patients benefited the most from radiotherapy without any increase in toxicity (10).

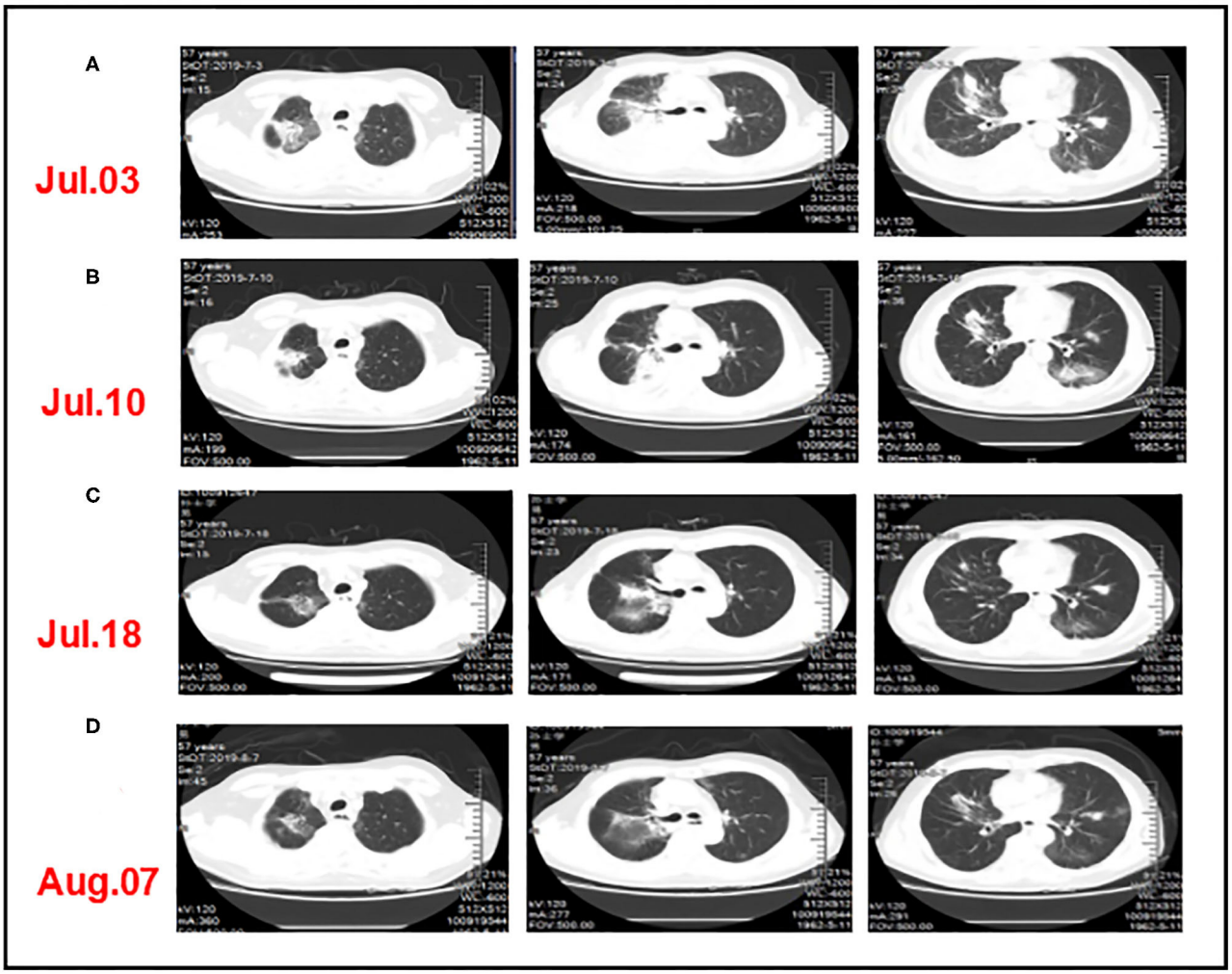


**FIGURE 4 |** CT comparison of the patient's lung inflammation during anti-infective treatment. **(A)** After three cycles of triple-combination therapy and two cycles of sintilimab monotherapy, chest computed tomography showed inflammatory changes in the lungs and partial lung consolidation. **(B,C)** The chest CT showed that pulmonary infiltration was absorbed after taking a sufficient amount of steroids and antibiotics. **(D)** The patient had a "flare" of pneumonitis symptoms when quickly tapered off steroids.

GM-CSF can promote the proliferation of dendritic cells and M1-type macrophages, and enhance antigen presentation to amplify the immune effect of the body (28, 29). The results of a clinical trial of GM-CSF combined with immune checkpoint inhibitors for advanced metastatic melanoma showed that the immune response disease control rate after 24 weeks was 41% and the ORR was 32% (11). The application of a PD-1 inhibitor combined with GM-CSF in the treatment of advanced biliary cancer was found to be safe and effective in a phase II study (14). A prospective study in 2015 showed that local radiotherapy combined with GM-CSF reinforced antitumor effects, inducing tumor regression outside the radiation field, which was called the abscopal effect (13).

In the present case, the lung lesions significantly reduced after three cycles of triple-combination therapy (**Figure 2**), but the mediastinal lymph nodes enlarged after three cycles of triple-combination therapy (**Supplementary Figure 2**). The use of PD-1 inhibitor was continued as maintenance treatment, and the lymph nodes shrunk 3 months later (**Supplementary Figure 2**), which might indicated pseudo-progression in lymph nodes related to T-cell infiltration rather than tumor cell proliferation (30). Unfortunately, we did not perform endoscopic lymph node aspiration for further confirmation. Dynamic efficacy predictors during cancer treatment were important areas of exploration. Some studies confirmed that high levels of tumor-infiltrating lymphocytes were associated with better survival in patients with ESCC (31, 32). Furthermore, several reports indicated that the efficacy of PD-1 inhibitors might be related to peripheral blood lymphocytes. Inflammatory markers such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and lactate dehydrogenase (LDH) may be potential predictive

and prognostic factors related to immunotherapy, as shown in recent studies (33–35). The NLR/PLR was defined as an absolute neutrophil/platelet count divided by an absolute lymphocyte count. However, no consistent cutoff values were obtained (34–36). In the present case, the NLR changes did not respond to the treatment effect (**Supplementary Figure 7**), but the PLR and LDH level decreased during the evaluation after two and three cycles of treatment, indicating the therapeutic effect (**Supplementary Figure 8**).

Although we innovatively used triple-combination therapy and achieved short-term benefits in this case, we do note that this patient had severe pneumonia which led to his death, suggesting that we should pay more attention to the safety of combination therapy. Some evidence has shown that administration of immune checkpoint inhibitors (ICI) after radiotherapy of lung lesions may cause recall effects (37, 38). Study showed that immune-related (IR) pneumonitis was more common in NSCLC patients treated with ICI who received curative-intent chest radiotherapy, but no radiotherapy parameter was significantly associated with IR pneumonitis (39). The PACIFIC study showed the safety of radiotherapy combined with ICI. Compared with the placebo group, the incidence of pneumonia or radiation pneumonitis in the durvalumab group was 33.9 and 24.8%, and that in grades 3 and 4 was 3.4 and 2.6%, respectively (40). PEMBRO-RT study showed that the incidence of pneumonia in the experimental group was more than that in the control group, but there was no significant difference in the grade 3 to 5 pneumonia (10). The sequence of radiotherapy combined with ICI is still controversial and the use of ICI after radiotherapy may reduce severe pneumonia just as PACIFIC and PEMBRO-RT study did. But KEYNOTE-799 indicated that chemoradiotherapy and simultaneous ICI were well tolerated, and the incidence of pneumonia above grade 3 was 3.4% (41).

In this case, the pneumonia was related to radiation dose of the right lung, for the right lung had been irradiated in 2018 and received three times of SBRT in 2019 (**Supplementary Figures 3–6**). It is unclear whether the ICI and GM-CSF can aggravate the initial lung injury caused by radiation. In the clinical course, the corticosteroids played important role in the treatment of pneumonia but rebound effects can occur if incorrect use of corticosteroids.

In summary, the triple-combination therapy was effective in the treatment of chemotherapy-refractory and PD-L1-negative metastatic ESCC, the suggested mechanism might involve the radio-sensitization of anti-PD-1 immunotherapy. Safety should be paid more attention to the combination therapy. Therefore, more clinical researches are needed to explore the efficacy and safety of triple-combination therapy and our related clinical research is ongoing (42) (chictr.org.cn No. ChiCTR 19000 20175).

## DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/**Supplementary Material**.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Second Affiliated Hospital of Soochow University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.01625/full#supplementary-material

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REVIEW
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# Radiotherapy and Immunotherapy for Head and Neck Cancer: Current Evidence and Challenges

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Immune checkpoint inhibitors (ICI) have revolutionized cancer treatment over the past decade. However, although the immune landscape suggests a strong rationale for the use of these agents in patients with head and neck squamous cell carcinoma, the available clinical evidence indicates that most patients currently do not respond to ICI monotherapy. Radiotherapy is a primary treatment modality for many patients with locally advanced head and neck cancer. While ionizing radiation traditionally has been thought to act in a purely cytotoxic fashion, a growing body of preclinical studies have demonstrated additional profound immunomodulatory effects. Consequently, there has been a surge of interest in the potential synergy between radiotherapy and immunotherapy, both the potential for radiotherapy to augment the systemic anti-tumor immune response and the potential for immunotherapy to improve in-field tumor response to radiation. In this review, we summarize the current preclinical and clinical evidence for radioimmunotherapy, with a particular focus on studies directly relevant to head and neck squamous cell carcinoma, as well as existing challenges and future directions for this emerging field.

**Keywords: anti-PD-1, immunotherapy, radiation therapy, head and neck cancer, anti-PD-L1**

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## INTRODUCTION

Head and neck cancers comprise a significant portion of the global cancer burden; when aggregating subsites, they are the 8$^{th}$ most common cancer worldwide by both incidence and mortality (1). Although the vast majority of head and neck cancers are squamous cell carcinomas (HNSCC) and have traditionally been associated with tobacco and alcohol use, HPV-associated oropharyngeal squamous cell carcinoma (SCC) has emerged as a new disease entity with markedly different biological behavior (2).

Ever since the foundational work of Henri Coutard, who was the first to use X-rays to treat laryngeal cancer almost 100 years ago (3), radiation therapy has played a key role in the treatment of HNSCC. Radiation continues to be used extensively both in the curative as well as palliative setting, although the distinction between the two is now sometimes blurred with growing recognition of the oligometastatic state, where patients with limited numbers of metastases can achieve prolonged survival, or even cure (4, 5). Technological advancements, both in imaging as well as treatment delivery, have enabled more precise radiation treatment that has reduced treatment-related

morbidity and improved patient outcomes. However, even with the use of modern radiation techniques, there are still opportunities for further improvement (4).

The immune system has a critical role in tumor development, and the development of immune evasion by tumors is a key step in carcinogenesis (6, 7). Attempts to reinvigorate an anti-tumor immune response have been widely integrated into practice following the development of the immune checkpoint inhibitors (ICIs) targeted against the immune checkpoint receptors cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed death-ligand 1 (PD-L1). Since the initial FDA approval of ipilimumab (a CTLA-4 inhibitor) in 2011 for the treatment of metastatic melanoma based on a proven overall survival advantage (8), antibodies blocking CTLA-4 and PD-1/PD-L1 have been tested and approved across a wide spectrum of malignancies. In HNSCC, both pembrolizumab and nivolumab (PD-1 inhibitors) have gained FDA approval for use in recurrent/metastatic HNSCC after progression through platinum-based chemotherapy (9–11). Pembrolizumab additionally has been approved in the US for use in the first line setting in patients with recurrent/metastatic HNSCC, either in combination with chemotherapy or alone as monotherapy depending on tumor/tumor microenvironment PD-L1 expression (12).

Unfortunately, overall response rates to PD-1 inhibitors in unselected patients with HNSCC remain low at approximately 10–20% (9–12), although patients who do respond can have long-lasting, durable remissions, as has been the case with other solid tumor patients who respond to PD-1 blockade (13). The possibility of durable long-term response has been a driver of the rapid uptake in clinical practice and has invigorated efforts to develop predictive biomarkers. Tumor mutational burden, a potential surrogate for tumor neoantigens that can be recognized by the immune system, is one such biomarker, leading to the first ever histology-agnostic FDA approval of the PD-1 inhibitor pembrolizumab for mismatch repair deficient tumors of any histology (14, 15), though there is increasing recognition that the types and functional nature of mutations may be as important as the number of mutations present (16). PD-L1 expression on both tumor cells and infiltrated immune cells has also been explored as a biomarker across several histologies with varying results; in HNSCC, subgroup analyses of Checkmate 141, KEYNOTE-040, and KEYNOTE-048 all suggest that higher PD-L1 expression does correlate with the likelihood of survival benefit (10–12). It is less clear whether patients with low or no PD-L1 expression still benefit from PD-1 directed therapy; analyses of Checkmate 141 and KEYNOTE-048 show questionable benefit for the PD-L1 negative subgroup when comparing the treatment and control arms (11, 17). Finally, for HNSCC patients, HPV-associated malignancies with relatively fewer tumor mutations as compared to tobacco-associated malignancies may also respond to immune checkpoint blockade as novel viral-associated neoantigens might be recognized by the immune system. Indeed, subgroup analyses of the Checkmate 141 and KEYNOTE-040 trials did not show any clear differences in response or clinical benefit based on p16 expression status (a surrogate for HPV-associated tumors) (10, 11).

In addition to better patient selection through the use of predictive biomarkers, augmenting the anti-tumor immune response with other therapies could also improve immunotherapy response rates. Radiation therapy increasingly has been recognized to have diverse immunomodulatory effects, and there has consequently been intense interest in possible synergism between radiation therapy and immunotherapy. In this review, we will summarize the preclinical data that illustrate the immune effects of radiation therapy, review the unique immune landscape of HNSCC, and finally discuss both current preclinical and clinical data relevant to the combination of radiation therapy and immunotherapy specifically in HNSCC (**Figure 1**).

## IMMUNE EFFECTS OF RADIATION THERAPY

Traditionally, the anti-tumor effects of radiation therapy have been attributed to direct cytotoxicity secondary to the induction of DNA damage, and while it was known over 40 years ago that radiation therapy also depends on an intact immune system to exert its full anti-tumor effect (18), the interaction between the immune system and radiation therapy has garnered more interest in the past two decades. It is now recognized that the immune effects of radiation may contribute significantly to an anti-tumor response; however, these immune effects are also quite complex and can be both immunostimulatory and immunosuppressive.

Radiation can induce immunogenic cell death, which gives rise to adaptive immune responses (19, 20). Many mechanisms can be involved in this process, and a full detailed review is beyond the scope of this discussion. However, recent studies have shown radiation can promote release of danger-associated molecular patterns such as calreticulin, ATP, and HMGB (20, 21). Radiation also induces release of cytosolic DNA, which triggers the cGAS/STING pathway to upregulate production of type-I interferon (22, 23). Type-I interferon is crucial for the activation of dendritic cells, which ultimately recruit and prime T-cells. These signals together are critical for the initial development of an immune response specific to tumor neoantigens.

Radiation can promote anti-tumor immunity through additional mechanisms. Radiation can diversify antigen presentation by tumor cells through promotion of intracellular peptide degradation as well as upregulation of MHC expression (24, 25). This ultimately can enhance recognition and tumor cell killing by cytotoxic T-cells (26). Radiation has also been associated with increased production of other immune stimulating cytokines and chemokines, which together can promote the infiltration of T-cells into tumors and modulate the function of these T-cells, as well as dendritic cells and macrophages (21).

Radiation also has immunosuppressive effects that could be detrimental to an anti-tumor immune response. Lymphocytes

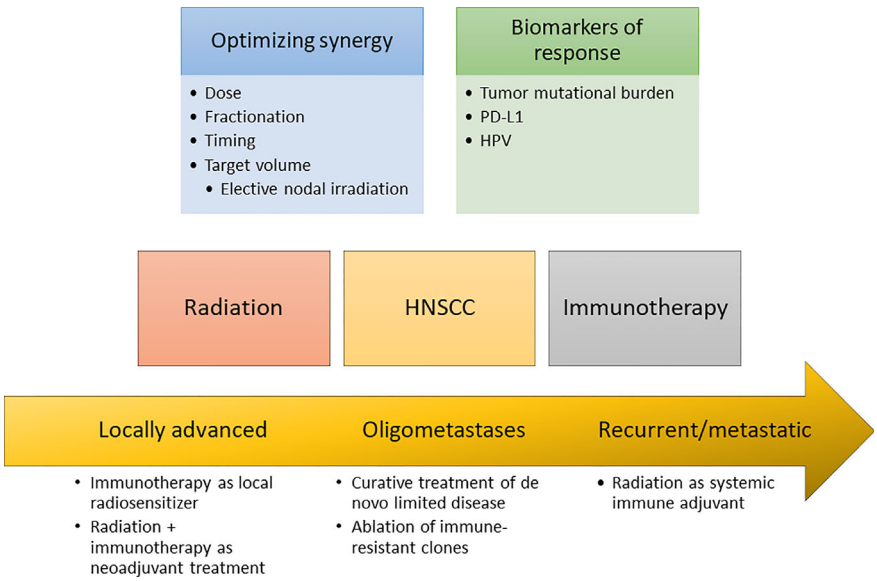


**FIGURE 1** | Opportunities for radioimmunotherapy in HNSCC.

are radiosensitive, with *in vitro* studies demonstrating that 3 Gy of radiation is enough to deplete 90% of human lymphocytes (27). This may be overly simplistic, however, as more recent work suggests differential radiosensitivity of T-cell subtypes. Pre-existing intra-tumoral T-cells in particular appear to be potentially more radioresistant than either circulating T-cells or lymphoid tissue T-cells. These intra-tumoral T cells survive even high doses (20 Gy) of radiation in preclinical studies and can develop a similar transcriptomic profile to tissue-resident memory T-cells, which are also thought to be radioresistant (28, 29). These intra-tumoral T-cells can mediate some of the anti-tumor immune effects of high dose radiation. Regardless, clinical data suggest that radiation-induced lymphopenia may be a negative prognostic factor in patients treated with PD-1 and CTLA-4 inhibitors (30).

Within the local tumor microenvironment, a variety of inhibitory immune cells, such as T-regulatory cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs, and specifically M2 macrophages), are often already present. In several studies, radiation increases recruitment of these inhibitory immune cells and can also modulate their function towards an even more immunosuppressive phenotype (21). There may also be dose-dependent effects of radiation; for instance, Vanpouille-Box et al. demonstrated that as radiation doses were escalated to 12–18 Gy, there was induction of Trex1, a DNA exonuclease which degrades cytosolic DNA and thus prevents activation of the cGAS/STING pathway (23). The balance between competing activating and inhibitory immune responses, then, likely plays a key role in the probability of a successful anti-tumor immune response and provides opportunity for therapeutic intervention.

## IMMUNE LANDSCAPE OF HNSCC

Work over the past decade has helped characterize the immune landscape of HNSCC. As noted above, HPV-associated oropharyngeal SCC is a distinct disease entity from other non-HPV-driven, tobacco-associated HNSCC, with a distinct immune profile. Using data from The Cancer Genome Atlas, Mandal et al. showed that HPV-positive tumors were significantly more immune infiltrated than HPV-negative tumors (31). However, both HPV-positive and HPV-negative HNSCC had the highest rate of immunosuppressive Treg infiltration among 10 different cancer types. There was a correlation between the molecular smoking signature of HNSCC tumors and increased tumor mutational burden, but also conversely an inverse association between the molecular smoking signature and immune infiltration, despite this higher tumor mutation burden (and therefore presumably increased neoantigen load). This suggests that tobacco-associated tumors can still be immunologically cold despite their higher mutational load. Further work has demonstrated that HPV-positive tumors are associated with increased T-cell receptor diversity, higher levels of immune cytolytic activity, and an overall enriched inflammatory response (32, 33). The anatomic subsite where head and neck cancer develops likely plays a key role in tumor immunity as well; the oropharynx contains particularly lymphoid-rich tissue, and this unique immune environment may explain why the improved prognosis for HPV-driven HNSCC is largely limited to oropharyngeal tumors (34). Additional work on oropharyngeal SCC has confirmed a higher degree of infiltration of CD8+ T-cells in HPV-positive *vs* HPV-negative tumors (35). Overall, these studies suggest that the increased sensitivity of HPV-associated oropharyngeal SCC to chemotherapy and radiation therapy may

at least in part be mediated through immune mechanisms (36, 37), and that differing immunotherapeutic approaches may be optimal for HPV-positive and HPV-negative HNSCC.

HNSCC also appears to be uniquely associated with high levels of natural killer (NK) cell infiltration, even when compared to other highly-immune infiltrated cancer types (31, 35). Patients with high levels of NK cell infiltration were also found to have improved survival compared to those with low levels of infiltration (31). The potential anti-tumor effects of NK cells is an emerging area of research and has been reviewed elsewhere (38); currently, there is limited clinical data on their role in HNSCC, or whether opportunities for synergy between NK-directed therapies and radiation exist.

# PRECLINICAL EVIDENCE FOR RADIOIMMUNOTHERAPY IN HNSCC MODELS

## Augmenting Anti-tumor Cellular Immunity

Preclinical work in HNSCC models has demonstrated synergy between radiation and immunotherapy. In a poorly immunogenic orthotopic HNSCC mouse model, Oweida et al. demonstrated effective tumor cell killing when both 10 Gy of radiation and an anti PD-L1 antibody were administered together, but not for either treatment individually (39). Tumor control was correlated with increased tumor T-cell infiltration and was abrogated when CD4+ and CD8+ T-cells were depleted. In addition, although much of research on anti-tumor immunity has focused on the role of T-cells, work from Kim et al. in a mouse model of HPV-associated HNSCC suggests that the combination of radiation and PD-1 inhibition also promotes maturation and activation of B-cells, leading to the development of memory B-cells, plasma cells, and antigen-specific B-cells, as well as increasing formation of B-cell germinal centers in tumor draining lymph nodes (40). Finally, there is growing interest in harnessing additional molecular pathways to promote anti-tumor immunity. For instance, in a mouse model of HPV-driven carcinoma, Dillon et al. demonstrated that inhibitors of ATR, a key protein in the DNA damage response pathway, significantly sensitized tumors to radiation, and this effect was correlated with upregulation of interferon-stimulated genes and a significant increase in innate immune cell infiltration into the tumor microenvironment (41). Xiao et al. showed that ASTX600, an inhibitor of IAP1/2 and XIAP, proteins that modulate apoptosis and the tumor necrosis factor signaling pathway, significantly enhanced T-cell mediated tumor cell killing when combined with radiation and PD-1 inhibition in a mouse model of oral cavity carcinoma (42).

## Decreasing an Immunosuppressive Microenvironment

The immunosuppressive microenvironment remains a challenge even with combined radiation and immunotherapy. In a follow-up study, Oweida et al. demonstrated that the anti-tumor immune responses to combined radiation and PD-1 inhibition in their HNSCC mouse model were ultimately transient, as compensatory mechanisms of immune evasion were activated, including upregulation of another immune checkpoint, TIM-3, as well as increased tumor infiltration of Tregs (39, 43). Adding an anti-TIM-3 antibody further delayed tumor growth, but the response was still not durable; only targeted depletion of Tregs was able to induce durable immunologic memory. Another group has explored the use of cyclophosphamide and an inhibitor of inducible nitric oxide synthase (iNOS) as immunomodulatory agents in a mouse model of HPV-associated HNSCC. When combined with traditional chemoradiation, addition of these two agents increased the CD8+ T-cell/Treg ratio and decreased immunosuppression (44). In this particular model system the combination of radiation with PD-1 and CTLA-4 inhibition only minimally altered the immunologically cold tumor microenvironment, but the addition of cyclophosphamide and the iNOS inhibitor shifted the balance of infiltrated immune cells away from immunosuppressive types (such as MDSCs) to those more associated with anti-tumor immunity (such as dendritic cells and anti-tumor M1 macrophages). This led to an increased CD8+ T-cell-dependent response and complete tumor rejection in more than 70% of the treated mice (45). This is now being investigated in a clinical trial, NCT03844763, which explores the use of cyclophosphamide, avelumab (a PD-L1 inhibitor), and radiation therapy in the treatment of recurrent/metastatic HNSCC.

## Radiation Dose and Fractionation Effects

Additional studies have demonstrated the importance of radiation dose and fractionation in generating an effective anti-tumor immune response. Consistent with work in other diseases (46), Morisada et al. showed in a syngeneic mouse oral cavity carcinoma model that hypofractionated radiation (16 Gy in two fractions) was associated with preservation of both peripheral and tumor-infiltrating lymphocytes, reduction of both peripheral and tumor-associated MDSCs, and increased expression of interferon genes, when compared to conventionally fractionated radiation (20 Gy in 10 fractions) (47). Moreover, analysis of the draining lymph nodes (which notably were included within the radiation fields) suggested that 20 Gy in 10 fractions suppressed local tumor-specific T-cell responses. Consequently, only 16 Gy in two fractions demonstrated synergy with an anti-PD-1 antibody in these mice. Additional work by this group suggests a dose-dependent effect of radiation on both antigen release and T-cell priming, with 8 Gy in a single fraction enhancing these pathways compared to 2 Gy in a single fraction, resulting in increased tumor cell susceptibility to T-cell mediated killing (48). However, the doses used in these preclinical models differ from those used in clinical practice, as do the size of the treated tumors, and so it is uncertain how these findings might translate to the treatment of patients.

# CLINICAL EVIDENCE FOR RADIOIMMUNOTHERAPY IN HNSCC

## Recurrent/Metastatic Setting

Despite the widespread use of ICIs in advanced malignancies, prospective clinical data on their combination with radiation

therapy remain scarce, particularly in HNSCC. The unique immune-related adverse effects (irAEs) that have been observed with ICIs are now well established (49) and there have been concerns that the pro-inflammatory effects of radiation could enhance toxicities when combined with ICIs. Reassuringly, however, most of the available clinical data to date suggests that the combination of radiation and ICIs is generally well tolerated (50). For instance, in a cohort of 133 patients with metastatic melanoma, non-small cell lung cancer (NSCLC), or renal cell cancer who received palliative radiation to a wide range of anatomic sites, Bang et al. demonstrated numerically higher rates of irAEs when radiation was given within 14 days of immunotherapy, but the toxicities were generally mild with rates of grade 3+ toxicity less than 10% (51). Similarly, a prospective phase I trial of pembrolizumab and stereotactic body radiotherapy (SBRT) in patients with a variety of metastatic solid tumors also demonstrated a grade 3+ toxicity rate of less than 10% (52). Notably, this study did include four patients with HNSCC, and radiation was delivered to two distinct anatomic sites in more than 60% of the cohort. Finally, a phase 2 trial which randomized 62 patients with metastatic HNSCC to nivolumab with or without SBRT to a single metastatic site did not find a significant difference in either grade 3–5 adverse events (13% for nivolumab alone *vs* 10% for nivolumab with SBRT, p = 0.70) or any grade adverse events (70% for nivolumab alone *vs* 87% for nivolumab with SBRT, p = 0.12) with the addition of SBRT (53).

Nevertheless, a few key issues must be considered when interpreting these and other safety data. Just as dose and fractionation likely affect potential anti-tumor immunity induced by radiation (as demonstrated in preclinical work), it is probable that these parameters influence potential toxicities when combined with ICIs. The relative timing of radiation and immunotherapy is likely to be important as well; notably, radiation recall, a relatively rare, unpredictable, and poorly understood phenomenon wherein an inflammatory reaction can develop in previously irradiated tissue following administration of a new systemic agent (54), has now been reported following ICI administration (55, 56). Additionally, the anatomic site treated with radiation could influence the side effect profile of combination treatment; for instance, the landmark PACIFIC trial, which demonstrated a significant overall survival benefit to adjuvant durvalumab (an anti-PD-L1 antibody) after definitive chemoradiation for stage III NSCLC, also showed an increase in any-grade pneumonitis with the addition of durvalumab (although rates of clinically relevant pneumonitis, i.e. grade 3+, were similar between treatment groups and low overall) (57). Within the brain, there is a potential increased risk of developing radiation necrosis after treatment of brain metastases with combined ICIs and radiation (58, 59). Finally, as discussed earlier, in certain settings radiation can induce lymphopenia, which could ultimately interfere with the efficacy of ICIs (30). These data highlight the importance of collecting robust radiation treatment and toxicity data to facilitate future analyses as we study combination radiation and immunotherapy treatments.

There are very few efficacy data relevant to the addition of radiation to ICIs in patients with recurrent or metastatic HNSCC. In general, the primary rationale for radiation in this setting is to help stimulate a systemic anti-tumor immune response, or abscopal effect. This is particularly difficult to study retrospectively, as disentangling a true abscopal effect from a delayed response to immunotherapy is challenging (60). The only available prospective data for HNSCC comes from the randomized phase 2 trial noted above, in which 62 patients with metastatic HNSCC were randomized to nivolumab with or without SBRT to a single metastatic site (9 Gy ×3 fractions, between the first and second doses of nivolumab). Ultimately, there was no improvement in overall response rate (34.5% for nivolumab alone *vs* 29.0% for nivolumab with SBRT, p = 0.86) (53). In NSCLC, a similarly designed phase 2 trial of pembrolizumab with or without SBRT to a single metastatic site in patients with advanced NSCLC also failed to meet its primary endpoint, although it did demonstrate a doubling of overall response rate with the addition of SBRT that was not statistically significant (18% for pembrolizumab alone *vs* 36% for pembrolizumab with SBRT, p = 0.07) (61). Differences between the designs of these two studies include the anti-PD-1 agent used (nivolumab *vs* pembrolizumab), the type of cancer (HNSCC *vs* NSCLC), timing of SBRT (between first and second dose of nivolumab *vs* prior to starting pembrolizumab), and dose of SBRT (9 Gy ×3 fractions *vs* 8 Gy ×3 fractions). Given the results of these trials, further research is clearly needed; **Table 1** summarizes ongoing trials that will help address these questions specifically in patients with recurrent/metastatic HNSCC. Notably, however, only a few of these studies are randomized, and so any efficacy data will require confirmation in larger, phase 3 trials.

Finally, as noted above, there is growing recognition of an oligometastatic disease state. Contrary to previous conceptualization of metastatic disease as inevitably widespread and thus incurable, the oligometastatic hypothesis suggests that there is a wide range of metastatic potential that varies among different cancers and from patient to patient, and that an intermediate state likely exists between purely localized disease and widely metastatic disease, wherein a limited number of metastases might develop with limited further metastatic potential (62). Aggressive local treatment of patients with limited metastases would thus potentially offer a significant survival benefit. Results from several randomized phase 2 trials have supported this hypothesis (though notably HNSCC was not represented in any of these studies) (63–67). Consequently, there is interest in the addition of ICIs to radiation in this population of patients to improve outcomes (68). In this setting, radiation would be administered at ablative doses to all metastatic sites, and so the addition of ICIs would also be intended to augment the local effects of radiation at each treatment site. To our knowledge, no prospective clinical data has yet been published on the combination of radiation and ICIs in patients with oligometastatic HNSCC, though there is at least one ongoing clinical trial (NCT03283605, which examines the use of durvalumab, tremelimumab [a CTLA-4 inhibitor], and SBRT in patients with HNSCC with fewer than 10 metastases).

**TABLE 1 |** Ongoing trials evaluating combinations of ICIs and radiation in the management of recurrent/metastatic HNSCC.

| NCT# | Title | Inclusion criteria | Treatment arms | Timing | Phase |
|---|---|---|---|---|---|
| NCT03539198 | Study of Proton SBRT and Immunotherapy for Recurrent/Progressive Locoregional or Metastatic Head and Neck Cancer | Recurrent/metastatic HNSCC, ≥2 metastatic sites | 1: nivolumab given every 2 weeks, with proton SBRT to one metastatic site administered with cycle 3 | concurrent | n/a |
| NCT03283605 | Immunotherapy and SBRT for Metastatic Head and Neck Carcinomas | Metastatic HNSCC, ≥2 metastatic sites | 1: durvalumab + tremelimumab for four cycles (4 weeks each), SBRT between cycles 2 and 3 | concurrent | 1/2 |
| NCT03844763 | CONFRONT: Targeting the Tumor Microenvironment in R/M SCCHN | Recurrent/metastatic HNSCC | 1: avelumab, cyclophosphamide, and radiation (8 Gy/1 fx) to a single site 1 week after first dose of avelumab | concurrent | 1/2 |
| NCT03522584 | Durvalumab, Tremelimumab and Hypofractionated Radiation Therapy in Treating Patients With Recurrent or Metastatic Head and Neck Squamous Cell Carcinoma | Recurrent/metastatic HNSCC; progression through prior PD-1/PD-L1 inhibitor | 1: durvalumab + tremelimumab for four cycles (4 weeks each) followed by durvalumab alone for nine cycles; SBRT during week 3 in three fractions, every other day | concurrent | 1/2 |
| NCT03474497 | UCDCC#272: IL-2, Radiotherapy, and Pembrolizumab in Patients Refractory to Checkpoint Blockade | Recurrent/metastatic HNSCC; progression through prior PD-1/PD-L1 inhibitor | 1: one cycle of pembrolizumab, then SBRT (24 Gy/3 fx) and intratumoral injection of interleukin-2 during cycle 2, then additional pembrolizumab | concurrent | 1/2 |
| NCT03317327 | REPORT: REirradiation and Programmed Cell Death Protein 1 (PD-1) Blockade on Recurrent Squamous Cell Head and Neck Tumors | Recurrent HNSCC after prior radiation or second primary HNSCC | 1: nivolumab with re-irradiation to 60 Gy (in 1.5 Gy bid fx), followed by nivolumab for up to 12 months | concurrent | 1/2 |
| NCT04340258 | Trial Combining Pembrolizumab and Cesium 131 Brachytherapy With Salvage Surgery in HNSCC | Resectable recurrent HNSCC after prior surgery or radiation | 1: one dose of pembrolizumab, then salvage surgery with implantation of Cesium-131 brachytherapy seeds (60–70 Gy), followed by adjuvant pembrolizumab for 6 months | concurrent | 1/2 |
| NCT04454489 | Quad Shot Radiotherapy in Combination With Immune Checkpoint Inhibition | Recurrent/metastatic HNSCC | 1: pembrolizumab given every 3 weeks; quad-shot radiation (14.8 Gy in 4 bid fx) between cycles 2 and 3 | concurrent | 2 |
| NCT03313804 | Priming Immunotherapy in Advanced Disease With Radiation | Recurrent/metastatic HNSCC | 1: nivolumab, pembrolizumab, or atezolimuab, with either SBRT (BED > 100 Gy) or 30 Gy fractionated RT | concurrent | 2 |
| NCT03386357 | Radiotherapy With Pembrolizumab in Metastatic HNSCC | Recurrent/metastatic HNSCC, ≥2 metastatic sites, progression through platinum-based therapy | 1: radiation to 1–3 metastases (36 Gy/12 fx), with pembrolizumab starting between fraction 3 and 4<br>2: pembrolizumab alone | concurrent | 2 |
| NCT03511391 | CHEERS: CHEckpoint Inhibition in Combination With an Immunoboost of External Body Radiotherapy in Solid Tumors | Recurrent/metastatic HNSCC, progression through platinum-based therapy | 1: 2 cycles of nivolumab, then SBRT to 1–3 metastases (24 Gy/3 fx) prior to cycle 3<br>2: nivolumab alone | concurrent | 2 |
| NCT03085719 | Targeting PD-1 Therapy Resistance With Focused High or High and Low Dose Radiation in SCCHN | Metastatic HNSCC, progression through prior PD-1 inhibition, ≥3 metastatic sites | 1: pembrolizumab and high dose SBRT (3 fx) to one metastatic site<br>2: pembrolizumab and high dose SBRT (3 fx) to one metastatic site, and low dose radiation (2 fx) to another site | concurrent | 2 |
| NCT03546582 | KEYSTROKE: SBRT +/– Pembrolizumab in Patients With Local-Regionally Recurrent or Second Primary Head and Neck Carcinoma | Recurrent HNSCC after prior radiation or second primary HNSCC | 1: reirradiation with SBRT over 2 weeks, then pembrolizumab every 3 weeks for up to 2 years<br>2: reirradiation with SBRT over 2 weeks | sequential | 2 |
| NCT03521570 | Intensity-Modulated Radiation Therapy & Nivolumab for Recurrent or Second Primary Head & Neck Squamous Cell Cancer | Recurrent HNSCC after prior radiation or second primary HNSCC | 1: one dose of nivolumab, then radiation with concurrent nivolumab, then adjuvant nivolumab for 5 months | concurrent + sequential | 2 |
| NCT02289209 | Reirradiation With Pembrolizumab in Locoregional Inoperable Recurrence or Second Primary Squamous Cell CA of the Head and Neck | Unresectable recurrent HNSCC after prior radiation or second primary HNSCC | 1: pembrolizumab with re-irradiation to 60 Gy (in 1.2 Gy bid fx), followed by pembrolizumab for 3 months | concurrent + sequential | 2 |
| NCT02684253 | Screening Trial of Nivolumab With Image Guided, Stereotactic Body Radiotherapy (SBRT) Versus Nivolumab Alone in Patients With Metastatic Head and Neck Squamous Cell Carcinoma (HNSCC) | Metastatic HNSCC, ≥2 metastatic sites | 1: one cycle of nivolumab, then SBRT (27 Gy/3 fx) with the 2nd cycle, followed by additional nivolumab<br>2: nivolumab alone | concurrent | 2 |

*BED, biologically effective dose; bid, twice a day; fx, fraction; HNSCC, head and neck squamous cell carcinoma; ICIs, immune checkpoint inhibitors; SBRT, stereotactic body radiotherapy.*

Related to the overall concept of oligometastases is oligoprogression, or the development of a limited number of progressive metastatic lesions after a period of stability on systemic therapy (69). In the context of ICIs, oligoprogression may herald general immune escape in patients who had previously been responding to treatment. However, in certain cases oligoprogression may develop as the result of resistant tumor clones that lack particular tumor antigens or antigen presentation, or because of differences in the underlying immune microenvironment of the anatomic site that permit localized immune escape (e.g. brain) (70, 71). If this is the case, local treatment such as radiation to these oligoprogressive sites may enable the patient to continue to derive benefit from ICIs (72–74). This paradigm is being tested prospectively in SCCHN (NCT03085719).

## Locally Advanced/Definitive Setting

ICIs are being investigated in the setting of curative treatment of earlier stages of disease across all cancer types, including HNSCC. Addition of ICIs to radiation in this setting would be intended to potentially augment the local effects of radiation (i.e. as a radiosensitizer) and address micrometastatic disease. Several possible combinations are under investigation—immunotherapy added to a chemoradiation regimen to intensify therapy (for patients with currently poor outcomes), immunotherapy given concurrently with radiation instead of chemotherapy or with a lower dose of radiation (potentially as a way to reduce treatment morbidity while maintaining overall efficacy), or immunotherapy administered adjuvantly and/or as induction (i.e. sequential therapy). To date adjuvant immunotherapy has proven successful in NSCLC; as noted earlier, the PACIFIC trial demonstrated a significant and meaningful overall survival benefit for adjuvant durvalumab starting within 6 weeks of completing standard chemoradiation for unresectable stage III NSCLC, with an increase in 2-year overall survival from 55.6 to 66.3% (75). Of note, the magnitude of benefit was greater patients who were randomized within 2 weeks of completing chemoradiation. Adjuvant immunotherapy also has newly demonstrated success in esophagogastric cancer; Checkmate-577 demonstrated improved disease-free survival with the administration of adjuvant nivolumab following neoadjuvant chemoradiation and surgical resection in patients with esophageal and gastroesophageal cancer, though full trial results have yet to be presented (76).

As shown in **Table 2**, ongoing trials are evaluating various combinations of radiation and ICIs for HNSCC in the definitive setting, and several have now reported safety data. In general, combinations of PD-1/PD-L1 inhibitors with definitive radiation appear well tolerated with no unexpected toxicities. KEYCHAIN is a randomized phase 2 study of radiation combined with concurrent and adjuvant pembrolizumab compared with radiation and concurrent cisplatin in intermediate-risk p16-positive HNSCC; the safety lead-in phase of the study found only one dose-limiting toxicity (grade 4 adrenal insufficiency) among eight patients in the pembrolizumab arm, and so the trial has proceeded to its phase 2 component (77). A single arm phase 2 trial of radiation administered with concurrent and adjuvant pembrolizumab in cisplatin-ineligible patients with locally advanced HNSCC similarly demonstrated relatively low toxicity in the first 12 enrolled patients, and 11 of 12 patients received all planned cycles of pembrolizumab (78). Finally, PembroRad is a randomized phase 2 trial of radiation combined with concurrent pembrolizumab *versus* radiation combined with concurrent cetuximab, again in cisplatin-ineligible patients with locally advanced HNSCC. There have been 133 patients randomized in a 1:1 fashion, and the pembrolizumab arm was found to have significantly less mucositis or dermatitis within the radiation field than the cetuximab arm (79).

Early results also suggest that intensification of existing chemoradiation regimens with the addition of ICIs is reasonably safe. In a small phase 1 trial of concurrent and adjuvant avelumab added to standard cetuximab/radiation in 10 cisplatin-ineligible patients with locally advanced HNSCC, no grade 4–5 toxicities were observed, and only one of eight evaluable patients discontinued avelumab for toxicity (80). REACH is a phase 3 trial that is also comparing concurrent avelumab, cetuximab, and radiation, followed by 12 months of adjuvant avelumab, against either standard bolus cisplatin with radiation or cetuximab with radiation (depending on if the patient is judged to be fit for cisplatin or not) in patients with locally advanced HNSCC; results for the 82 patients randomized during the safety phase of the trial suggested that addition of avelumab was tolerable, with 88% of patients completing concurrent avelumab as per protocol, and rates of grade 4+ events similar between control and experimental arms (81). Similarly, a single arm phase 1b study of the addition of concurrent and adjuvant pembrolizumab to standard radiation and weekly cisplatin in patients with locally advanced HNSCC demonstrated in 59 patients that concurrent pembrolizumab did not prevent patients from completing chemoradiation, and only 5 of 59 patients ultimately discontinued treatment because of irAEs (82). Finally, RTOG 3504 is a four-arm phase 1 trial in patients with intermediate or high risk HNSCC that is examining the addition of concurrent and adjuvant nivolumab to either radiation alone or radiation with weekly cisplatin, bolus cisplatin, or cetuximab; safety results from the latter three arms again demonstrated that nivolumab did not prevent timely completion of chemoradiation, and rates of dose-limiting toxicities were low (83).

Efficacy data, however, have not yet been reported from most of these or other ongoing trials. One of the single arm phase 2 trials noted above (78) of radiation with concurrent and adjuvant pembrolizumab in cisplatin-ineligible patients with locally advanced HNSCC ultimately enrolled 29 patients, and reported 1-yr progression-free survival and overall survival of 76 and 86%, respectively (84). Notably, the phase 3 Javelin 100 study is a double-blind, placebo-controlled trial that randomized 697 patients with locally advanced HNSCC to standard of care cisplatin-based chemoradiation with or without concurrent and adjuvant (for 12 months) avelumab, with progression-free survival as the primary endpoint. Unfortunately, this trial was recently terminated for likely futility after a preplanned interim

**TABLE 2 |** Ongoing trials evaluating combinations of ICIs and radiation in the definitive management of locally advanced HNSCC.

| NCT# | Title | Inclusion criteria | Treatment arms | Timing | Phase |
|---|---|---|---|---|---|
| NCT02819752 | PEmbrolizumab Combined With Chemoradiotherapy in Squamous Cell Carcinoma of the Head and Neck (PEACH) | LA HNSCC | 1: pembrolizumab added to standard chemoradiation, three doses concurrently, four doses adjuvantly | concurrent + sequential | 1 |
| NCT04477759 | Dose-Escalated Hypofractionated Adaptive Radiotherapy for Head and Neck Cancer (DEHART) | LA HNSCC, cisplatin-ineligible, or primary metastatic HNSCC | 1: MR-guided hypofractionated radiation (50–60 Gy/15 fx); atezolizumab given with fraction 1 and 11 of radiation, then every 4 weeks for up to 1 year | concurrent + sequential | 1 |
| NCT03509012 | CLOVER: Immunotherapy in Combination With Chemoradiation in Patients With Advanced Solid Tumors | LA HNSCC | 1: durvalumab concurrent with standard radiation and cisplatin | concurrent | 1 |
| NCT02764593 | RTOG 3504: Safety Testing of Adding Nivolumab to Chemotherapy in Patients With Intermediate and High-Risk Local-Regionally Advanced Head and Neck Cancer | LA HNSCC, intermediate or high risk | 1: one dose of nivolumab as induction, then radiation (70 Gy/35 fx) and nivolumab with weekly cisplatin, then adjuvant nivolumab for seven doses<br>2: one dose of nivolumab as induction, then radiation (70 Gy/35 fx) and nivolumab with bolus cisplatin, then adjuvant nivolumab for seven doses<br>3: one dose of nivolumab as induction, then radiation (70 Gy/35 fx) and nivolumab with weekly cetuximab, then adjuvant nivolumab for seven doses<br>4: one dose of nivolumab as induction, then radiation (70 Gy/35 fx) with nivolumab, then adjuvant nivolumab for seven doses | concurrent + sequential | 1 |
| NCT03051906 | DUCRO-HN: Durvalumab, Cetuximab and Radiotherapy in Head Neck Cancer | LA HNSCC | 1: durvalumab every 4 weeks, cetuximab weekly, and radiation to 69.96 Gy/33 fx, followed by adjuvant durvalumab for 6 months | concurrent + sequential | 1/2 |
| NCT03247712 | Neoadjuvant Immunoradiotherapy in Head & Neck Cancer | Resectable LA HNSCC | 1: neoadjuvant SBRT (24–40 Gy/3–5 fx) and nivolumab, followed by surgery, followed by adjuvant nivolumab | concurrent + sequential | 1/2 |
| NCT02296684 | Immunotherapy With MK-3475 in Surgically Resectable Head and Neck Squamous Cell Carcinoma | Resectable LA HNSCC, except p16-positive oropharyngeal SCC | 1: two doses of pembrolizumab neoadjuvantly followed by surgery and standard risk-adapted adjuvant (chemo)radiation<br>2: one dose of pembrolizumab neoadjuvantly, followed by surgery and standard risk-adapted adjuvant (chemo)radiation, followed by adjuvant pembrolizumab for up to six doses for patients with ENE or positive margins | sequential | 2 |
| NCT03894891 | Induction TPN Followed by Nivolumab With Radiation in Locoregionally Advanced Laryngeal and Hypopharyngeal Cancer | LA p16-negative SCC of larynx or hypopharynx | 1: induction cisplatin, docetaxel, and nivolumab, followed by concurrent radiation and nivolumab | concurrent + sequential | 2 |
| NCT03708224 | Phase II Study of Perioperative Immunotherapy in Patients With Advanced Non-Virally Associated Squamous Cell Carcinoma | Resectable LA HNSCC, except p16-positive oropharyngeal SCC | 1: one dose of atezolizumab neoadjuvantly, followed by surgery and standard risk-adapted adjuvant (chemo)radiation, followed by atezolizumab every 3 weeks for up to 12 cycles<br>2: one dose of atezolizumab and tocilizumab neoadjuvantly, followed by surgery and standard risk-adapted adjuvant (chemo)radiation, followed by atezolizumab every 3 weeks for up to 12 cycles | sequential | 2 |
| NCT03426657 | Radiotherapy With Double Checkpoint Blockade of Locally Advanced HNSCC | LA HNSCC | 1: one cycle of induction cisplatin, docetaxel, durvalumab, and tremelimumab; patients with increased CD8+ T-cell infiltration on interval biopsy then receive durvalumab, tremelimumab, and radiation, followed by adjuvant durvalumab for 8 months | concurrent + sequential | 2 |
| NCT03532737 | Concomitant Immune Check Point Inhibitor With Radiochemotherapy in Head And Neck Cancer | LA HNSCC, non-nasopharynx | 1: pembrolizumab for six cycles (3 weeks each), and chemoradiation starting with cycle 2, with either bolus cisplatin or cetuximab, and radiation to 66–70 Gy/30–35 fx | concurrent + sequential | 2 |
| NCT02892201 | Pembrolizumab in HNSCC With Residual Disease After Radiation | LA HNSCC with residual disease after definitive radiation | 1: pembrolizumab for four cycles, followed by evaluation for salvage surgery; unresectable patients continue pembrolizumab for up to 1 year | sequential | 2 |
| NCT03721757 | CA209-891: Neoadjuvant and Adjuvant Nivolumab as Immune Checkpoint Inhibition in Oral Cavity Cancer (NICO) | LA oral cavity SCC | 1: one dose of neoadjuvant nivolumab followed by surgery, then one dose of nivolumab, then standard post-operative radiation or | sequential | 2 |

*(Continued)*

**TABLE 2** | Continued

| NCT# | Title | Inclusion criteria | Treatment arms | Timing | Phase |
|---|---|---|---|---|---|
| | | | chemoradiation (60 Gy/30 fx), then 6 months of adjuvant nivolumab | | |
| NCT03944915 | De-Escalation Therapy for Human Papillomavirus Negative Disease (DEPEND) | LA p16-negative HNSCC | 1: induction carboplatin, paclitaxel, and nivolumab, followed by response-adapted chemoradiation (66–75 Gy) | sequential | 2 |
| NCT04405154 | A Study of Concomitant Camrelizumab With Chemoradiation for Locally Advanced Head and Neck Cancer | LA HNSCC | 1: camrelizumab for eight cycles (2 weeks each), with standard chemoradiation (bolus cisplatin and radiation [66 Gy/33 fx]) starting with cycle 2 | concurrent + sequential | 2 |
| NCT02777385 | Pembrolizumab in Combination With Cisplatin and Intensity Modulated Radiotherapy (IMRT) in Head and Neck Cancer | LA HNSCC, intermediate or high risk | 1: pembrolizumab for one initial dose, then concurrent with radiation and weekly cisplatin, then adjuvant pembrolizumab for a total of eight doses<br>2: radiation and weekly cisplatin, followed by adjuvant pembrolizumab for eight doses | concurrent + sequential<br>sequential | 2 |
| NCT03383094 | KEYCHAIN: Chemoradiation vs Immunotherapy and Radiation for Head and Neck Cancer | LA HNSCC, p16-positive, intermediate risk | 1: pembrolizumab and standard radiation to 70 Gy/33–35 fx, followed by adjuvant pembrolizumab for up to 20 cycles (3 weeks each)<br>2: standard chemoradiation to 70 Gy/33–35 fx with bolus cisplatin | concurrent | 2 |
| NCT02707588 | PembroRad: Tolerance and Efficacy of Pembrolizumab or Cetuximab Combined With RT in Patients With Locally Advanced HNSCC | LA HNSCC | 1: radiation (69.96 Gy/33 fx) with concurrent pembrolizumab<br>2: radiation (69.96 Gy/33 fx) with concurrent cetuximab | concurrent | 2 |
| NCT02609503 | Pembrolizumab + Radiation for Locally Adv SCC of the Head and Neck (SCCHN) Not Eligible Cisplatin | LA HNSCC, cisplatin-ineligible | 1: radiation (70 Gy/35 fx) with three concurrent cycles of pembrolizumab, then three adjuvant cycles | concurrent + sequential | 2 |
| NCT03258554 | NRG-HN004: Radiation Therapy With Durvalumab or Cetuximab in Treating Patients With Locoregionally Advanced Head and Neck Cancer Who Cannot Take Cisplatin | LA HNSCC, cisplatin-ineligible | 1: durvalumab for seven cycles (4 weeks each); radiation to 70 Gy/35 fx starting week 2<br>2: cetuximab for eight cycles (weekly); radiation to 70 Gy/35 fx starting week 2 | concurrent + sequential | 2/3 |
| NCT01810913 | RTOG 1216: Testing Docetaxel-Cetuximab or the Addition of an Immunotherapy Drug, Atezolizumab, to the Usual Chemotherapy and Radiation Therapy in High-Risk Head and Neck Cancer | Resected LA HNSCC, except p16-positive oropharyngeal SCC, with pathologic ENE or positive margins | 1: atezolizumab for eight cycles (3 weeks each) following surgery, with standard chemoradiation (to 60 Gy/30 fx with weekly cisplatin) starting week 2 | concurrent + sequential | 2/3 |
| NCT03811015 | EA3161: Testing Immunotherapy Versus Observation in Patients With HPV Throat Cancer | p16-positive oropharyngeal SCC, intermediate risk | 1: radiation (70 Gy/35 fx) and concurrent weekly cisplatin, then adjuvant nivolumab for 12 months<br>2: radiation (70 Gy/35 fx) and concurrent weekly cisplatin, then observation | sequential | 2/3 |
| NCT03452137 | IMvoke010: A Study of Atezolizumab (Anti–Pd-L1 Antibody) as Adjuvant Therapy After Definitive Local Therapy in Patients With High-Risk Locally Advanced Squamous Cell Carcinoma of the Head and Neck | LA HNSCC after definitive local therapy (chemoradiation or surgery + [chemo] radiation) | 1: adjuvant atezolizumab for 1 year<br>2: placebo for 1 year | sequential | 3 |
| NCT03576417 | NIVOPOSTOP: A Trial Evaluating the Addition of Nivolumab to Cisplatin-RT for Treatment of Cancers of the Head and Neck | Resected LA HNSCC, with ENE, positive margins, or multiple positive nodes | 1: one dose of nivolumab, then nivolumab concurrent with radiation (66 Gy/33 fx) and bolus cisplatin<br>2: radiation (66 Gy/33 fx) with bolus cisplatin | concurrent + sequential | 3 |
| NCT03673735 | Maintenance Immune Check-point Inhibitor Following Post-operative Chemo-radiation in Subjects With HPV-negative HNSCC (ADHERE) | Surgically resected p16-negative HNSCC with pathologic ENE or positive margins | 1: one dose of induction durvalumab followed by standard chemoradiation (bolus cisplatin and radiation [66 Gy/33 fx]), followed by 6 months of adjuvant durvalumab<br>2: standard chemoradiation (bolus cisplatin and radiation [66 Gy/33 fx]) | sequential | 3 |
| NCT03700905 | IMSTAR-HN: Study of Nivolumab Alone or in Combination With Ipilimumab as Immunotherapy vs Standard Follow-up in Surgical Resectable HNSCC After Adjuvant Therapy | Resectable LA HNSCC, except p16-positive oropharyngeal SCC | 1: one dose of neoadjuvant nivolumab followed by surgery, followed by standard risk adapted adjuvant (chemo)radiation, followed by either adjuvant nivolumab or adjuvant nivolumab+ ipilimumab for 6 months<br>2: surgical resection followed by standard risk adapted adjuvant (chemo)radiation | sequential | 3 |

*(Continued)*

**TABLE 2** | Continued

| NCT# | Title | Inclusion criteria | Treatment arms | Timing | Phase |
|---|---|---|---|---|---|
| NCT03765918 | Study of Pembrolizumab Given Prior to Surgery and in Combination With Radiotherapy Given Post-surgery for Advanced Head and Neck Squamous Cell Carcinoma (MK-3475-689) | Resectable LA HNSCC | 1: two doses of neoadjuvant pembrolizumab, then surgery, then pembrolizumab with adjuvant radiation or chemoradiation, then adjuvant pembrolizumab for 12 additional doses<br>2: surgery followed by adjuvant radiation or chemoradiation | concurrent + sequential | 3 |
| NCT03673735 | ADHERE: Maintenance Immune Check-point Inhibitor Following Post-operative Chemo-radiation in Subjects With HPV-negative HNSCC | Resected LA HNSCC, except p16-positive oropharyngeal SCC, with pathologic ENE or positive margins | 1: following surgery, one dose of durvalumab, then standard radiation (66 Gy/33 fx) with bolus cisplatin, then adjuvant durvalumab for six doses<br>2: following surgery, standard radiation (66 Gy/33 fx) with bolus cisplatin | sequential | 3 |
| NCT03040999 | KEYNOTE-412: Study of Pembrolizumab (MK-3475) or Placebo With Chemoradiation in Participants With Locally Advanced Head and Neck Squamous Cell Carcinoma | LA HNSCC | 1: one dose of induction pembrolizumab, then pembrolizumab with radiation (70 Gy/35 fx) and bolus cisplatin, then adjuvant pembrolizumab for a total of 17 doses<br>2: standard radiation (70 Gy/35 fx) with bolus cisplatin | concurrent + sequential | 3 |
| NCT02999087 | REACH: Randomized Trial of Avelumab-cetuximab-radiotherapy Versus SOCs in LA SCCHN | LA HNSCC, both cisplatin eligible and ineligible | 1: cetuximab and avelumab, one dose prior to radiation, then concurrent during radiation (69.96 Gy/33 fx), then adjuvant avelumab for 12 months<br>2: standard radiation (69.96 Gy/33 fx) with concurrent bolus cisplatin for cisplatin-eligible patients<br>3: standard radiation (69.96 Gy/33 fx) with concurrent cetuximab for cisplatin-ineligible patients | concurrent + sequential | 3 |
| NCT02952586 | Javelin 100: Study To Compare Avelumab In Combination With Standard of Care Chemoradiotherapy (SoC CRT) Versus SoC CRT for Definitive Treatment In Patients With Locally Advanced Squamous Cell Carcinoma Of The Head And Neck | LA HNSCC | 1: one dose of induction avelumab, then avelumab with radiation (70 Gy/35 fx) and bolus cisplatin, then adjuvant avelumab for 12 months<br>2: radiation (70 Gy/35 fx) and bolus cisplatin | concurrent + sequential | 3 |

*ENE, extranodal extension; fx, fraction; HNSCC, head and neck squamous cell carcinoma; ICIs, immune checkpoint inhibitors; LA, locally advanced; SBRT, stereotactic body radiotherapy.*

analysis performed by their independent data monitoring committee (85).

Possible reasons for the failure of Javelin 100 to achieve its primary endpoint may be revealed when more complete data are available. However, in the interim, it is interesting to highlight distinctions from the successful incorporation of PD-L1 blockade into the treatment of locally advanced NSCLC as evidenced by the PACIFIC study. A predominant mode of failure in locally advanced HNSCC is locoregional recurrence (4), whereas distant metastases are more common in locally advanced NSCLC (86). Thus, examining patterns of failure in the Javelin 100 study and comparing these to patterns of failure in the PACIFIC study may inform whether ICIs in this setting are mainly eradicating systemic micrometastatic disease versus also improving local disease control. Unfortunately, PACIFIC did not collect data distinguishing intrathoracic failures within versus outside of the radiation field, highlighting the importance of thorough radiation data collection to tease out these types of questions (87). Given the high risk of lymph node metastases in patients with locally advanced HNSCC, standard radiation generally entails elective treatment of the draining cervical lymph node chains (in contrast to NSCLC, where elective lymph nodes are not intentionally irradiated). These draining lymph nodes are precisely where antigen-presenting cells migrate to for T-cell priming, following radiation to the primary tumor (21, 25). Correlative positron emission tomography–computed tomography (PET-CT) studies from a recently published clinical trial of neoadjuvant ICIs (nivolumab or nivolumab and ipilimumab) prior to surgery in patients with oral cavity SCC provides further support for the importance of the draining lymph nodes; following initiation of neoadjuvant ICIs, there was a high rate of increased fluorodeoxyglucose (FDG) uptake in the draining cervical lymph nodes on an interval PET-CT, which ultimately on surgical pathology demonstrated only reactive findings without any evidence of cancer. This observed increase in FDG uptake may therefore represent radiographic evidence of a mounting immune response (88). Given the radiosensitivity of lymphocytes, then, it seems possible that radiation (particularly longer conventionally fractionated regimens) that electively treats the draining lymph nodes following the receipt of ICI could actually hinder T-cell priming. Indeed, as noted above, there is some preclinical data to support this, as Morisada et al. demonstrated in an syngeneic mouse model of oral cavity cancer that 20 Gy in 10 fractions compared to 16 Gy in 2 fractions to both the primary tumor and the draining lymph nodes blunted tumor-specific CD8+ T-cell responses within those draining lymph nodes (although notably tumors were implanted in the mice legs and thus this is not a perfect model for head and neck lymphatics) (47). The phase 2 trial

reported by Weiss et al. also noted a rate of grade 3+ lymphopenia of 58.6% (84). Another notable issue is that the design of Javelin 100, as well as many of the other trials described above, incorporated both concurrent and adjuvant ICIs in the experimental arm, whereas PACIFIC (and Checkmate-577) only tested the value of adjuvant immunotherapy. Timing and sequencing of ICIs and radiation remains a critical issue that requires further study, although the concerns regarding radiation-induced T-cell death may be particularly problematic when ICI is administered concurrently as compared with sequentially (89). Finally, as demonstrated in the preclinical work above, radiation dose and fractionation are also likely critical to successful synergy between radiation and ICIs; however, the hypofractionated regimens that appear to have the greatest immunologic potential in preclinical models differ tremendously from the long conventionally fractionated regimens (1.8–2 Gy/fraction) used in the current standard management of HNSCC. PACIFIC did also employ conventional fractionation, though standard total doses for NSCLC are somewhat lower than for HNSCC (54–66 Gy *versus* 70 Gy). Overall, given the years of experience supporting the current standard radiation regimen and fields used in the definitive management of HNSCC, careful studies will be required to determine what kinds of modifications to elective nodal irradiation, timing/sequencing, dose, and/or fractionation are required to maximize synergy with ICIs and ultimately improve patient outcomes. There is already significant heterogeneity amongst the ongoing trials in **Tables 1** and **2** with regard to these parameters, and so examining the results collectively will hopefully be informative.

## CONCLUSIONS/FUTURE DIRECTIONS

There remains excitement for the possibility of combining radiation therapy and immunotherapy to improve outcomes for patients with HNSCC. Ongoing trials will help advance this emerging field, and the developing paradigm of oligometastatic disease provides further opportunity to integrate improving systemic and local therapies. Biomarker studies conducted in parallel will also inform optimal patient selection for combined treatment approaches. Moreover, while this review has largely focused on ICIs (and PD-1/PD-L1 targeted therapies in particular) given their widespread use, immunotherapeutic agents targeting other checkpoints and pathways are in development as well (90), as are trials testing their combination with radiation (e.g. NCT04220775). Nevertheless, significant work remains to be done in both the preclinical and clinical space to determine the dose, fractionation, timing, target, and field size of radiation that will be the most synergistic with immunotherapies. Finding the optimal balance between the immunostimulatory and immunosuppressive effects of radiation is key and hopefully will herald continued improvement in outcomes for patients with HNSCC.

## AUTHOR CONTRIBUTIONS

JQ: conceptualization; writing, original draft; writing, review and editing. JS: conceptualization; writing, review and editing. All authors contributed to the article and approved the submitted version.

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# Survival After Combining Stereotactic Body Radiation Therapy and Tyrosine Kinase Inhibitors in Patients With Metastatic Renal Cell Carcinoma

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**Background:** Stereotactic body radiation therapy (SBRT) and tyrosine kinase inhibitors (TKIs) are effective treatments for metastatic renal cell carcinoma, but data on combining these two modalities are scarce. We aimed to investigate the survival outcomes of SBRT plus TKIs.

**Methods:** Data of patients treated with TKIs from December 2007 to June 2019 were collected. Patients received SBRT plus TKIs (TKI + SBRT group) or TKIs alone (TKI alone group). Local control (LC), time to change of systemic therapy (TTS), and overall survival (OS) were assessed.

**Results:** A total of 190 patients were included, and 85 patients received TKI + SBRT. The 2-year LC rate was 92.8%. The median OS in the TKI + SBRT group was significantly longer than that of the TKI alone group (63.2 vs 29.8 months; $P < 0.001$). In multivariate analysis, IMDC intermediate (HR 1.96; 95% CI 1.10–3.48; $P = 0.022$) and poor risk (HR 2.43; 95% CI 1.25–4.75; $P = 0.009$), oligometastasis (HR 0.41; 95% CI 0.26–0.65; $P < 0.001$), and the addition of SBRT (HR 0.48; 95% CI 0.31–0.75; $P = 0.001$) were prognostic factors for OS. Patients with oligometastasis ($P = 0.009$) and those with IMDC favorable ($P = 0.044$) or intermediate ($P = 0.002$) risk had significantly longer OS with TKI + SBRT. The median TTS were 21.5, 6.4, and 9.0 months in patients receiving SBRT before first-line TKI failure, SBRT after first-line TKI failure, and first-line TKI alone ($P < 0.001$). Five patients (5.9%) experienced SBRT-related grade 3 toxicities.

**Conclusions:** Combining SBRT with TKIs is tolerable and associated with longer OS in selected patients, such as those with oligometastasis and favorable or intermediate risk.

**Keywords: renal cell carcinoma, stereotactic body radiotherapy, tyrosine kinase inhibitors, survival, metastasis**

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## INTRODUCTION

Renal cell carcinoma accounted for 403,262 new cases worldwide in 2018 (1). Approximately 30%–40% of patients present with metastatic renal cell carcinoma (mRCC) (2). Targeted therapy has prolonged the survival of mRCC patients, yet the objective response rate (ORR) is low, ranging from 10%–30% (3, 4). Although the combination of immune checkpoint inhibitors and targeted therapy has substantially raised the ORR to about 40%–60% and prolonged the progression-free survival (PFS) to 12–15 months, complete response remains low, at less than 10% (5–7). In most cases, resistance to systemic agents is inevitable, and the depletion of effective systemic agents is merely a matter of time. Thus, systemic therapy requires other complementary treatment modalities to make additional gains in survival.

Metastasis-directed local therapy represents an indispensable component of mRCC treatment. Evidence on metastasis-directed surgery has demonstrated that the overall survival (OS) after complete metastasectomy is about 40.8 months, compared with 14.8 months after incomplete or no metastasectomy (8). In the era of targeted therapy, the importance of metastasectomy has somewhat decreased (9). On the one hand, perioperative targeted therapy application is associated with an increase in surgical complications (10). On the other hand, perioperative interruption of targeted therapy can result in rapid angiogenesis, which stimulates tumor growth (11).

Stereotactic body radiation therapy (SBRT) enables the delivery of intensified radiation doses in a highly conformal way, which could overcome the inherent radioresistance of renal cell carcinoma. The local control (LC) rate is around 90% after SBRT in mRCC (12), and deferred use or even permanent discontinuation of systemic therapy has been observed in oligometastasis patients receiving SBRT to all metastases (13, 14). Given the favorable therapeutic ratio of SBRT in mRCC, the current National Comprehensive Cancer Network guidelines have recommended it as an effective treatment option for oligometastases.

Current studies on patients with mRCC have predominantly focused on the role of SBRT alone in oligometastatic or oligoprogressive settings (13, 15). A few studies investigating the combined use of tyrosine kinase inhibitors (TKIs) and SBRT have only reported the results of response rates and local control (16, 17). Considering the lack of evidence regarding the survival gains obtained by adding SBRT to TKI treatment in patients with mRCC, our study aimed to compare the survival outcomes of patients receiving SBRT plus TKIs versus TKIs alone.

## MATERIALS AND METHODS

### Patients

This study was approved by our institutional review board (ID: B2020-057-01), and informed consent was waived. We retrospectively reviewed the medical records of patients with mRCC treated in our center between December 2007 and June 2019. Eligible patients were aged ≥ 18 years who received TKI treatment for mRCC. Those who were followed up for less than three months, were treated with conventionally fractionated radiotherapy, or received immunotherapy as first-line treatment were excluded.

### Treatment

Usually, patients were recommended to initiate TKI treatment shortly after the diagnosis of mRCC. The TKIs were administered at their usual dosage regimens in accordance with current guidelines. No interruption or dose reduction of TKI was required during SBRT, except for serious treatment-related toxicities.

SBRT was delivered to all lesions in oligometastasis, to the major tumor burden or oligoprogressive lesions as cytoreductive therapy, and to the symptomatic lesions with palliative intent. Oligometastasis and oligoprogression were defined as the presence of no more than five metastatic and progressive sites, respectively, without brain or liver involvement. Major tumor burden was defined as the largest lesion accounting for at least 50% of the tumor burden, which was calculated as the sum of the longest unidimensional diameter of the target lesions as per Response Evaluation and Criteria in Solid Tumours (RECIST) version 1.1.

All patients underwent computed tomography (CT) with or without magnetic resonance imaging simulation scanning with site-specific immobilization as previously described. Four-dimensional CT was applied to the lungs and used for some upper abdominal lesions. In all patients, SBRT was implemented with volumetric intensity modulated arc therapy planning. SBRT was predominantly delivered in five fractions, either once daily or every other day. The biologically effective dose (BED) was calculated using the linear-quadratic model, with (18). Cone beam CT was performed before every treatment.

### Outcomes

Clinical examination and follow-up scans were recommended every three months for the first two years. The response of bone metastases to SBRT was evaluated according to the University of Texas MD Anderson Cancer Center criteria (19). Otherwise, response was evaluated according to RECIST version 1.1. OS was defined from the time of metastasis detection to the last follow-up or death. Time to change of systemic therapy (TTS) was calculated from the start of first-line TKIs to the initiation of second-line therapy. PFS after SBRT was calculated from the start of SBRT to disease progression or death. LC was defined as freedom from progression at the treated sites after SBRT. Toxicities were graded according to the Common Terminology Criteria for Adverse Events 4.0 rating scale.

### Statistical Analysis

Categorical data were compared using the chi-squared test, and continuous variables were compared by Mann-Whitney tests. The Kaplan–Meier method and log-rank test were used to estimate and compare survival among the groups, respectively. The Cox regression method was used to analyze the hazard ratios (HRs) and associated 95% confidence intervals (CIs) for OS. Univariate and multivariate analyses were performed, and only the factors evaluated as significant in the univariate analyses were included in the multivariate model. A two-sided P-value of $< 0.05$ was considered statistically significant. SPSS version 23 (IBM Corp., Armonk, NY, USA) was used for statistical analyses.

# RESULTS

## Patient and Treatment Characteristics

In total, 190 mRCC patients treated with TKIs were identified. Eighty-five patients (44.7%) received SBRT in addition to TKIs (TKI + SBRT group), while 105 patients (55.3%) were treated with TKIs alone (TKI alone group). At the time of metastasis detection, 82 patients (43.2%) had oligometastasis. One-hundred and forty-nine patients (78.4%) had intermediate or poor risk, according to the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) criteria. Baseline characteristics were similar between the TKI + SBRT and TKI alone groups, except that patients in the TKI + SBRT group were older and were more likely to have bone metastases (**Table 1**).

Sunitinib was the most common first-line systemic therapy, accounting for 57.9% of the cases. Fifteen patients (7.9%) discontinued first-line TKI because of intolerable toxicities despite dose-schedule adjustments, leaving 175 patients treated with first-line TKI regularly. A total of 144 lesions were treated with SBRT. SBRT was indicated for oligometastasis in 28 patients (32.9%), oligoprogression in eight patients (9.4%), major tumor burden in 16 patients (18.8%), and palliation in 33 patients (38.8%). Nearly 70% of the irradiated sites were located in the bones. One-hundred and eighteen lesions (81.9%) received 35–45 Gy in five fractions, and the median $BED_3$ of all irradiated sites was 146.7 Gy (65.6–237.5 Gy) (**Supplementary Table 1**).

## Response to SBRT

Complete response, partial response, stable disease, and progressive disease (PD) were recorded in 30 (20.8%), 89 (61.8%), 22 (15.2%), and 3 (2.1%) sites after SBRT, resulting in an ORR of 82.6%. With a median follow-up of 13.6 months after SBRT, PD was observed in three lesions after SBRT. The 1-year and 2-year LC rates were 99.2% and 92.8%, respectively.

## Survival and Prognostic Factors

At a median follow-up of 25.8 months (range, 4.8–122.7 months), nine patients (4.7%) were lost to follow-up and 86 patients (45.3%) were still alive. The median PFS after SBRT was 9.0 months. For the entire cohort, the median OS was 36.3 months. The median OS was significantly longer in the TKI + SBRT group than in the TKI alone group (63.2 vs 29.8 months; $P < 0.001$). The OS rates at 2 and 5 years were 74.4% and 53.8% in the TKI + SBRT group and 61.2% and 24.6% in the TKI alone group, respectively (**Figure 1**).

**Table 2** summarizes the results of univariate and multivariate analyses. In the multivariate analysis, intermediate (HR 1.96; 95% CI 1.10–3.48; $P = 0.022$) and poor IMDC risk groups (HR 2.43; 95% CI 1.25–4.75; $P = 0.009$) were associated with inferior OS, whereas oligometastasis (HR 0.41; 95% CI 0.26–0.65; $P < 0.001$) was correlated with good prognosis. The addition of SBRT was associated with a 52% decreased hazard of death (HR 0.48; 95% CI 0.31–0.75; $P = 0.001$).

In the subgroup analysis, patients with clear cell histology ($P = 0.001$), IMDC favorable ($P = 0.044$) and IMDC intermediate risk group ($P = 0.002$), and oligometastasis ($P = 0.009$) had significant improvement in OS after adding SBRT (**Figure 2**). Patients with oligometastasis who received TKI + SBRT treatment have the most favorable outcome, with median OS not reached ($P < 0.001$; **Figure 3A**). As for subgroups stratified by the IMDC criteria, the median OS in the TKI + SBRT and TKI alone groups were 70.0 months and 33.3 months in the favorable

**TABLE 1** | Baseline characteristics (N=190).

| Characteristics, N (%) | Total (N=190) | TKI Alone (N=105) | TKI + SBRT (N=85) | *P* |
|---|---|---|---|---|
| Age, median (range), years | 54 (18–86) | 54 (18–83) | 55 (21–86) | 0.049 |
| Gender | | | | 0.666 |
| Male | 147 (77.4) | 80 (76.2) | 67 (78.8) | |
| Female | 43 (22.6) | 25 (23.8) | 18 (21.2) | |
| Pathology | | | | 0.125 |
| Clear cell | 140 (73.7) | 82 (78.1) | 58 (68.2) | |
| Non-clear cell | 50 (26.3) | 23 (21.9) | 27 (31.8) | |
| IMDC risk group | | | | 0.412 |
| Favorable | 41 (21.6) | 23 (21.9) | 18 (21.2) | |
| Intermediate | 110 (57.9) | 57 (54.3) | 53 (62.3) | |
| Poor | 39 (20.5) | 25 (24.8) | 14 (16.5) | |
| Metastatic sites | | | | |
| Lung | 90 (47.4) | 53 (50.5) | 37 (43.5) | 0.340 |
| Bone | 66 (34.7) | 20 (19.0) | 46 (54.1) | <0.001 |
| Liver | 18 (9.5) | 13 (12.3) | 5 (5.9) | 0.128 |
| Brain | 8 (4.2) | 6 (5.7) | 2 (2.4) | 0.433 |
| Synchronous metastasis | | | | 0.485 |
| Yes | 97 (51.1) | 56 (53.3) | 41 (48.2) | |
| No | 93 (48.9) | 49 (46.7) | 44 (51.8) | |
| Oligometastasis | | | | 0.204 |
| Yes | 82 (43.2) | 41 (39.0) | 41 (48.2) | |
| No | 108 (56.8) | 64 (61.0) | 44 (51.8) | |
| Nephrectomy | | | | 0.103 |
| Yes | 159 (83.7) | 92 (87.6) | 67 (78.8) | |
| No | 31 (16.3) | 13 (12.4) | 18 (21.2) | |

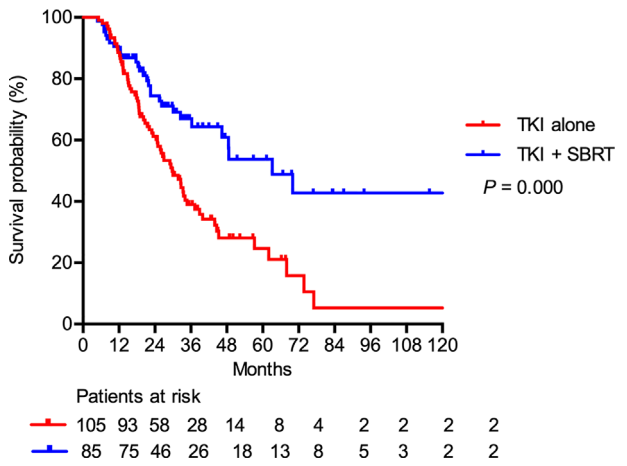


**FIGURE 1** | Overall survival in patients treated with stereotactic body radiation therapy (SBRT) in addition to tyrosine kinase inhibitors (TKI) (TKI + SBRT group) and TKI alone.

or intermediate risk group and 21.9 months and 25.0 months in the poor risk group, respectively (P < 0.001; **Figure 3B**).

## SBRT Delivered With First-Line TKI

In the 175 patients receiving regular first-line TKI treatment, SBRT was delivered concomitantly with first-line TKI treatment to 38 patients (21.7%). Among them, 23 patients (60.5%) underwent irradiation before first-line TKI failure (pre-PD SBRT group), and the remaining 15 patients (39.5%) received SBRT after first-line TKI failure (post-PD SBRT group). For the entire subgroup, the median TTS after first-line TKIs was 9.0 months. The median TTS after first-line TKIs was similar in patients treated with first-line TKI with or without SBRT (12.4 vs 9.0 months; P = 0.139). However, the median TTS were 21.5 months, 6.4 months, and 9.0 months in the pre-PD SBRT, post-PD SBRT, and first-line TKI alone groups (P < 0.001; **Figure 4A**). The OS was longer in the pre-PD SBRT group than in the post-PD SBRT or first-line TKI alone groups (median OS not reached vs 11.2 vs 39.3 months, 2-year OS 89.3% vs 19.4% vs 70.0%; P < 0.001; **Figure 4B**).

## Toxicities After SBRT

SBRT combined with TKI was generally well tolerated. No grade 4 or 5 toxicities occurred. Grade 3 and grade 2 toxicities were reported in five patients (5.9%) and 24 patients (28.2%), respectively. There were 10 events of grade 3 toxicities, eight (80.0%) of which were hematological toxicities that were later resolved (**Table 3**). The number of SBRT-related toxicities were similar between the pre-PD SBRT group and the post-PD SBRT group. The number of grade 1, 2, and 3 SBRT-related events were 8, 1, and 2 in the pre-PD SBRT group, and 3, 3, and 2 in the post-PD SBRT, respectively.

# DISCUSSION

Although a couple of studies have validated the safety of combining SBRT with TKIs (16, 17, 20), the impact of SBRT on survival remains unknown. Our study demonstrated that the integration of SBRT and TKIs was associated with improved survival compared with that with TKIs alone in patients with mRCC. To our knowledge, this study represents the largest report on patients' survival after SBRT plus TKIs in the general mRCC patient population.

In our study, the median OS of patients in the TKI alone group was similar to that reported in the studies of sequential targeted therapies (median OS, 18–30 months) (21). The addition of SBRT to TKI was associated with significant reduction in the hazard of death. Preclinical studies suggest that combining SBRT and TKIs might yield superior anti-tumor activity. TKIs may enhance the tumor response to SBRT through several mechanisms, including the reversal of hypoxia in the tumor microenvironment, the facilitation of apoptosis, and the prevention of SBRT-induced re-vascularisation (22, 23). SBRT could potentiate the effect of TKIs by eradicating resistant clones, destroying the tumor microvasculature, inhibiting growth factors and inducing an anti-tumor immune response (12, 24). As observed in some clinical

**TABLE 2** | Prognostic factors of overall survival (N=190).

| Variables | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | HR (95% CI) | *P* | HR (95% CI) | *P* |
| Oligometastasis | | | | |
| No | Reference | | Reference | |
| <Yes | 0.36 (0.23, 0.57) | <0.001 | 0.41 (0.26, 0.65) | <0.001 |
| Nephrectomy | | | | |
| No | Reference | | Reference | |
| Yes | 0.49 (0.29, 0.84) | 0.009 | 0.65 (0.37, 1.12) | 0.120 |
| Treatment | | | | |
| TKI alone | Reference | | Reference | |
| TKI + SBRT | 0.46 (0.30, 0.72) | 0.001 | 0.48 (0.31, 0.75) | 0.001 |
| IMDC score | | | | |
| Favorable | Reference | | Reference | |
| Intermediate | 2.08 (1.17, 3.69) | 0.012 | 1.96 (1.10, 3.48) | 0.022 |
| Poor | 3.59 (1.87, 6.89) | <0.001 | 2.43 (1.25, 4.75) | 0.009 |

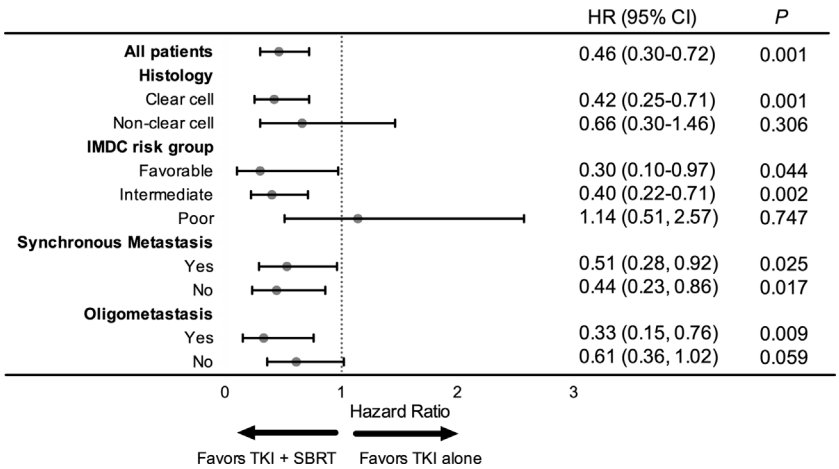


**FIGURE 2** | Forest plot of the association between tyrosine kinase inhibitors (TKI) + stereotactic body radiation therapy (SBRT) and overall survival by subgroup. HR, hazard ratio; CI, confidential interval.

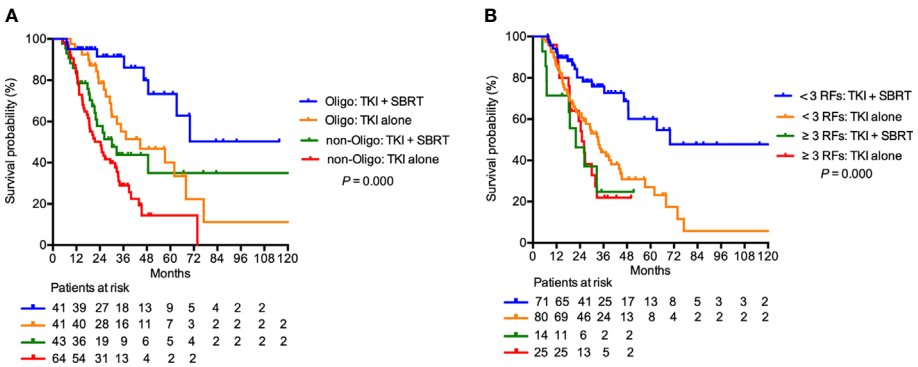


**FIGURE 3** | Overall survival in patients treated with stereotactic body radiation therapy (SBRT) in addition to tyrosine kinase inhibitors (TKI) (TKI + SBRT group) and TKI alone stratified by **(A)** oligometastasis and **(B)** International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) risk factors. IMDC favorable or intermediate risk group were illustrated as < 3 RFs, and poor risk group was illustrated as ≥ 3 RFs. RFs, risk factors; Oligo, oligometastasis.

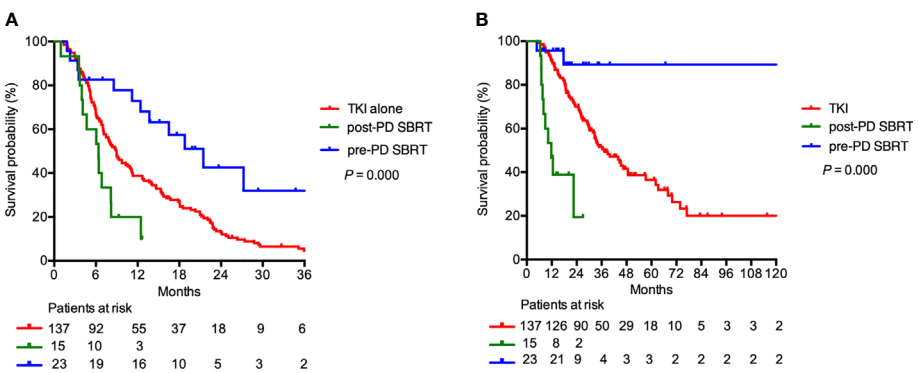


**FIGURE 4** | **(A)** Time to change of systemic therapy and **(B)** overall survival in patients receiving regular first-line TKI treatment (N=175). Patients may receive stereotactic body radiation therapy (SBRT) before first-line tyrosine kinase inhibitors (TKI) failure (pre-PD SBRT), SBRT after first-line TKI failure (post-PD SBRT), or first-line TKI alone.

**TABLE 3** | Radiotherapy-related toxicity.

| | Grade 1 | Grade 2 | Grade 3 |
|---|---|---|---|
| Dermatitis radiation | 4 | 2 | 1 |
| Alopecia | 1 | | |
| Skin induration | 1 | | |
| Nausea/Vomiting | 16 | 4 | |
| Colonic hemorrhage | | 1 | |
| Neuropathy | 3 | 3 | 1 |
| Pneumonitis | 6 | | |
| Bronchopleural fistula | | 2 | |
| Neutropenia | 13 | 9 | 2 |
| Anemia | 9 | 2 | 6 |
| Thrombocytopenia | 3 | 1 | |
| Fracture | 7 | 2 | |

studies, concurrent TKI treatment and SBRT is safe might achieve a superior tumor response (16, 17, 20). The results of our study imply that beyond tumor response improvement, SBRT may be associated with improved survival in some patients. However, whether the survival benefit is truly realized by the addition of SBRT needs to be verified in prospective trials.

In addition to the reports of survival, our study provided potential insights into patient selection for combined modality therapy. Our cohort observed that the addition of SBRT was associated with better survival among patients with oligometastasis or those with favorable or intermediate risk. Oligometastasis has been generally accepted as an indicator for local therapy. In studies that included oligometastatic mRCC patients, the median OS of patients treated with SBRT was remarkable (median OS, 34–51 months) (14, 25, 26), with some not even reaching the median OS (13, 27). The patient's IMDC risk group may also be an indicator for treatment selection. In the update on the CARMENA trial, patients with more than one risk factor according to the IMDC criteria did not benefit from cytoreductive nephrectomy (28), which was similar to our findings. In the favorable or intermediate risk groups, however, we observed a significantly longer OS in patients in the TKI + SBRT group than in the TKI alone group. These results suggest that the addition of local therapy may be beneficial for the subgroups of patients with favorable prognosis, such as those with oligometastasis, and IMDC favorable or intermediate risk.

Finally, our study may provide some clues as to the sequence in which the systemic and local therapies should be administered. Our results showed that mRCC patients treated with SBRT before first-line TKI failure had better survival than those who received SBRT after first-line TKI failure. The traditional concept of upfront cytoreductive nephrectomy has been reshaped in the era of targeted therapy. In the CARMENA trial and the SURTIME trial, upfront cytoreductive nephrectomy failed to demonstrate survival gains over sunitinib alone, but survival benefit was observed in the deferred nephrectomy arm (28, 29). Besides, patients with early disease progression during first-line sunitinib had a similarly poor prognosis, regardless of when nephrectomy was implemented (30). These results imply that local therapy may still have a role in mRCC management, and attention should be paid to the sequence of different therapies (9). Targeted therapy followed by local therapy may be a more effective strategy, as initial targeted therapy may be able to screen out patient tumors with aggressive biological behavior that demand intensification of systemic therapy instead of local therapy.

Our study has several limitations. Firstly, it is retrospective. Patients included in the SBRT group may represent a selected cohort with indolent disease that could not be fully elucidated by current clinical parameters. Secondly, SBRT was delivered at various timepoints for different purposes. Thirdly, we cannot control for the type and sequence of targeted regimens. Finally, our study was conducted in a high-volume cancer center, and these results might be difficult to replicate in smaller centers. Future studies with multiple centers involved could reduce this selection bias, especially when standardized data collection and retrieval project has been designed (31).

## CONCLUSIONS

Our study suggests that the use of SBRT on top of current TKI treatment is tolerable and may be associated with survival improvement. Patients with oligometastasis and with favorable or intermediate risk as per the IMDC criteria may be potential candidates for this combined modality treatment. The value of local therapy may be diminished in patients who progress during first-line systemic therapy. Prospective studies are needed to confirm our findings and determine the candidates, the timing of implementation, and the optimal combining strategy of the two treatments.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee of Sun Yat-Sen University Cancer Center. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

YL, PD, and LH contributed to the conception and design. ZZ, HH, SG, ZL, ML, FZ, and PD contributed to data collection. YL performed data analysis and interpretation. YL and ZZ contributed to manuscript preparation. LH and PD revised the manuscript. All authors contributed to the article and approved the submitted version.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.607595/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Emerging Role of Consolidative Radiotherapy After Complete Remission Following R-CHOP Immunochemotherapy in Stage III–IV Diffuse Large B-Cell Lymphoma: A Single Institutional and Case-Matched Control Study

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**Purpose:** The role of consolidative radiotherapy (RT) after complete-remission (CR) following rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) in advanced-stage diffuse large B-cell lymphoma (DLBCL) remains unclear. We retrospectively analyzed the survival outcomes and patterns of failure with our institutional experience.

**Material and Methods:** Between 2009 and 2018, 206 patients with stage III-IV DLBCL achieved CR after receiving R-CHOP. Propensity-score matching was used to analyze the role of consolidative RT. The consolidative RT group (n = 34) and the R-CHOP alone group (n = 68) were matched at a 1:2 ratio. After propensity-score matching, 102 patients were analyzed.

**Results:** With a median follow-up of 39.7 months, 26 patients (25.5%) showed local recurrence. Only one patient failed at the previous RT field. RT was delivered to bulky sites, head and neck lesions, testes, and bone with median dose of 30.6 Gy. The most common site of failure was head and neck lesions followed by bulky sites. The 5-year overall survival (OS), progression-free survival (PFS), and isolated-local recurrence free survival (LRFS) were 73.5, 64.0, and 79.9%. In univariate and multivariate analysis, bone marrow

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involvement and consolidative RT were associated with isolated LRFS (p = 0.006 and 0.032) significantly.

**Conclusion:** Consolidative RT improved isolated local control. Based on the pattern of failure, we carefully suggest to radiate on initially involved bulky sites or head and neck lesions. Further studies need to be done to find out the optimal radiation dose and selection of RT site.

**Keywords: consolidation, radiotherapy, advanced-stage, diffuse large B-cell lymphoma, rituximab, complete remission**

## INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid neoplasm in adults (1) and the most common non-Hodgkin's lymphoma (NHL) subtype (2, 3). With heterogeneous pathologic features, it generally has an aggressive clinical course. Approximately 60–70% of patients with DLBCL are initially diagnosed with advanced-stage disease. Although the addition of rituximab, a monoclonal antibody against CD20, to cytotoxic chemotherapy has substantially improved DLBCL survival (4), outcomes remain poor in advanced disease, with a 10-year overall survival (OS) of 43% (5).

In the pre-rituximab era, the role of consolidative radiotherapy (RT) after chemotherapy has been studied in several randomized trials, including the Southwest Oncology Group (SWOG) 8736 trial, the Eastern Cooperative Oncology Group (ECOG) 1484 study, the Groupe d 'Etudes des Lymphomes de I'Adulte (GELA LNH) 93-1 trial, and the GELA LNH 93-4 trial (6–8). Although these landmark randomized trials aimed to show the potential benefits of RT, consolidative RT did not show significant improvement in survival outcomes. However, in the rituximab era, several single institutional series (9, 10) showed the benefit of consolidative RT. The role of consolidative RT remains unclear but the results of several studies, including the Italian lymphoma study group, Ricover-60 trial, and Min T trial (11, 12) support its beneficial role in early-stage DLBCL, with better local control (LC), progression-free survival (PFS), and OS.

Although consolidative RT is often recommended for early-stage DLBCL, the role of consolidative RT in advanced disease remains unclear (13, 14). Furthermore, patients with stage III or IV DLBCL tend to have treatment failure more often than those with early-stage DLBCL (13). Although several studies have assessed consolidative RT after chemotherapy (15), few studies have evaluated the addition of rituximab for advanced-stage DLBCL (9). This study retrospectively analyzed the survival outcomes and patterns of failure of advanced-stage DLBCL.

## MATERIALS AND METHODS

### Patients

Between December 2009 and November 2018, 639 patients with histologically proven DLBCL of clinical stage III–IV were reviewed. Patients who were aged <19 years (n = 3); did not receive chemotherapy (n = 28); received fewer than four cycles of rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) (n = 59); received chemotherapy without rituximab (n = 120); had other malignancies (n = 57); were under immunosuppressive conditions with human immunodeficiency virus infection (n = 3); and underwent organ transplantations, such as kidney or liver (n = 8) were excluded. Among the 361 patients with stage III–IV DLBCL, only 206 patients who achieved complete response (CR) after receiving more than four cycles of R-CHOP and did not undergo bone marrow transplantation were included. Of these, 172 patients received R-CHOP alone and 34 patients received R-CHOP followed by consolidative RT (**Figure 1**).

Patient charts were reviewed, and the following characteristics were extracted: age, sex, pathologic subtype, Ann Arbor stage, Eastern Cooperative Oncology Group (ECOG) performance status (PS) before treatment, lactate dehydrogenase (LDH) level, extranodal disease involvement, bone marrow involvement, International Prognostic Index (IPI) score, number of R-CHOP cycles, and underlying diseases. In the clinical workup, results of bone marrow biopsy, tissue biopsy, and imaging studies, such as computed tomography (CT) and positron emission tomography (PET)-CT, medical history, physical examination results, and blood test findings were evaluated. All tumors were staged using the Ann Arbor staging system.

### Treatment

The administered chemotherapy regimen was R-CHOP. All patients underwent surveillance studies including PET-CT and CT. Response assessments and outcomes were evaluated according to the response criteria for malignant lymphoma (16). Since the Deauville five-point scale was implemented in 2014, there were few PET scans interpreted without a five-point scale (17). They were reviewed *via* medical charts from the Catholic University Lymphoma Group. CR was defined as the disappearance of all diseases on CT or Deauville score 1 to 3 after R-CHOP (17, 18).

Based on decisions from a multidisciplinary team, the Catholic University Lymphoma Group, including radiation and medical oncologists, radiologists, pathologists, and nuclear radiologists, 34 patients were administered consolidative RT as part of the initial therapy.

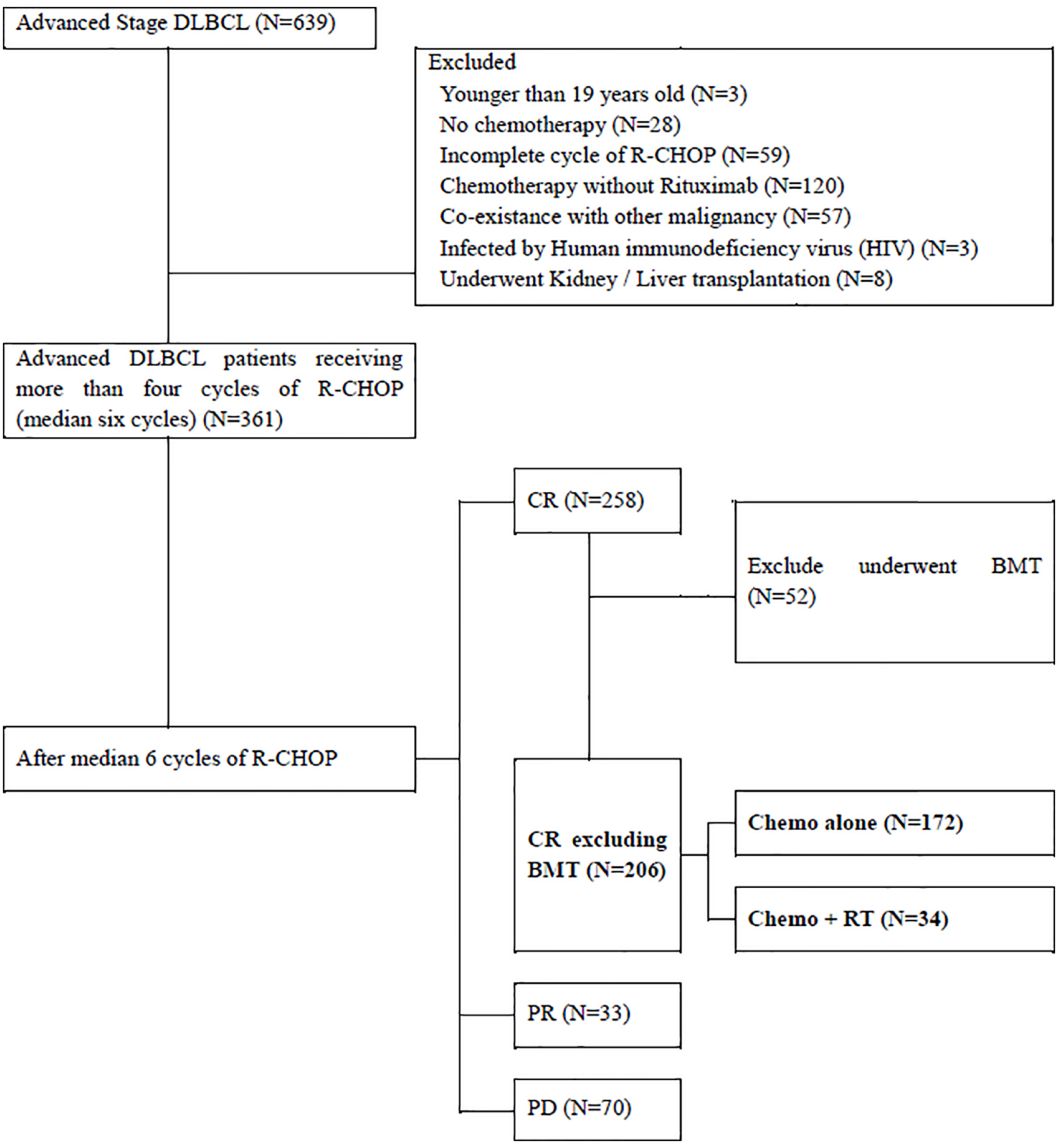


**FIGURE 1** | Inclusion Criteria. *DLBCL*, Diffuse Large B-cell lymphoma; *CNS*, Cranial Nervous System; *R-CHOP*, rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisone; *CR*, complete remission; *BMT*, bone marrow transplantation; *PR*, partial remission; *PD*, progressive disease; *RT*, radiotherapy.

## Propensity-Score Matching and Statistical Analysis

To reduce selection bias and potential confounding effects of treatment, propensity-score matching with 1:2 matching was performed. The covariates selected for matching were pathologic subtype, Ann Arbor stage, bone marrow involvement, International Prognostic Index (IPI) score, number of R-CHOP cycles, LDH levels, and underlying diseases. Propensity-score matching was performed using "nearest-neighbor matching" without replacement. A total of 34 patients in the R-CHOP followed by consolidative RT group, and 68 patients in the R-CHOP alone group were matched at a 1:2 ratio. After matching, statistical survival rates and failure patterns were analyzed.

Descriptive statistics were used to identify clinical characteristics between the two groups, with and without consolidative RT. Non-continuous values were compared using the Mann Whitney U-test, and continuous variables were presented as medians and compared using the t-tests.

The actuarial 5-year survival rates were calculated. OS was defined as the time from diagnosis until death as a result of any cause or the last follow-up date. PFS was defined as the time from diagnosis until disease progression or death. Local recurrence (LR) was defined as failure at the initial sites with a

Deauville score of 4–5, and distant recurrence (DR) as failure outside the initial sites. Local recurrence free survival (LRFS) and distant recurrence free survival (DRFS) were defined as the time from completion of chemotherapy until local or distant recurrence. Furthermore, in-field failure was defined as recurrence within the previous RT field. Since not every initially involved site was included in the RT field, there is out-field LR, which is recurrence in initially involved sites but outside the RT field. Survival functions were estimated using the Kaplan-Meier method and compared by log-rank tests for univariate analysis. The Cox proportional hazards model was used in the multivariate analysis. All tests were two-sided, and p-values <0.05 indicated statistical significance. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA) and R version 3.6.3 (R Development Core Team, Vienna, Austria).

# RESULTS

## Patient Characteristics

A total of 102 patients after propensity-score matching were analyzed. Patient characteristics are summarized in **Table 1**. The median age at diagnosis was 57.5 years (range, 19.0–81.0). There were 56 men (54.9%) and 46 women (45.1%). According to the Ann Arbor staging system, 19 (18.6%) and 83 (81.4%) patients had stage III and stage IV diseases, respectively.

A comparison of characteristics between patients who received R-CHOP alone and those who received consolidative

**TABLE 1** | Patients' characteristics.

| Characteristics | All (n=102) | R-CHOP alone (n=68) | R-CHOP + RT (n=34) | P value |
|---|---|---|---|---|
| Age at diagnosis | | | | 0.989 |
| Median | 57.5 | 57 | 58 | |
| Range | 19.0–81.0 | 21.0–81.0 | 19.0–79.0 | |
| Gender | | | | 1.000 |
| Male | 56 (54.9%) | 37 (54.4%) | 19 (55.9%) | |
| Female | 46 (45.1%) | 31 (45.6%) | 15 (44.1%) | |
| Pathologic subtype | | | | 0.859 |
| ABC | 63 (61.8%) | 43 (63.2%) | 20 (58.8%) | |
| GCB | 27 (26.5%) | 18 (26.5%) | 9 (26.5%) | |
| T cell rich | 1 (1.0%) | 1 (1.5%) | 0 (0.0%) | |
| NOS | 11 (10.8%) | 6 (8.8%) | 5 (14.7%) | |
| Ann arbor stage | | | | 1.000 |
| Stage III | 19 (18.6%) | 13 (19.1%) | 6 (17.6%) | |
| Stage IV | 83 (81.4%) | 55 (80.9%) | 28 (82.4%) | |
| Performance status (ECOG-PS) | | | | 1.000 |
| 0–1 | 71 (69.6%) | 47 (69.1%) | 24 (70.6%) | |
| 2–4 | 32 (31.4%) | 21 (30.9%) | 10 (29.4%) | |
| LDH level | | | | **0.015** |
| Normal (250–450 IU/L) | 28 (27.5%) | 15 (22.1%) | 13 (38.2%) | |
| Elevated | 74 (72.5%) | 53 (77.9%) | 21 (61.8%) | |
| Extranodal disease involvement | | | | 0.935 |
| <2 | 25 (24.5%) | 16 (23.5%) | 9 (26.5%) | |
| ≥2 | 77 (75.5%) | 52 (76.5%) | 25 (73.5%) | |
| Bone marrow involvement | | | | 0.688 |
| No | 76 (74.5%) | 52 (76.5%) | 24 (70.6) | |
| Yes | 26 (25.5%) | 16 (23.5%) | 10 (29.4%) | |
| IPI score | | | | 0.327 |
| Low 0–1 | 6 (5.9%) | 4 (5.9%) | 2 (5.9%) | |
| Low-intermediate 2 | 21 (20.6%) | 11 (16.2%) | 10 (29.4%) | |
| High-intermediate 3 | 30 (29.4%) | 20 (29.4%) | 10 (29.4%) | |
| High 4–5 | 45 (44.1%) | 33 (48.5%) | 12 (35.3%) | |
| Number of R-CHOP cycles | | | | 1.000 |
| 6 cycle | 58 (56.9%) | 39 (57.4%) | 19 (55.9%) | |
| 7 cycle | 5 (4.9%) | 3 (4.4%) | 2 (5.9%) | |
| 8 cycle | 39 (38.2%) | 26 (38.2%) | 13 (38.2%) | |
| Follow-up | | | | 0.699 |
| Median | 39.7 | 39.9 | 39.2 | |
| Range | 6.8–125.1 | 6.8–119.2 | 9.6–125.1 | |

*ABC, activated B-cell; GCB, germinal center B-cell; NOS, Not otherwise specified; ECOG PS, Eastern Cooperative Oncology Group Performance Status; LDH, lactate dehydrogenase; IPI, International Prognostic Index; R-CHOP, rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisone; RT, radiotherapy.*
*In bold, p value with less than 0.05.*

RT after achieving CR is also shown in **Table 1**. A significant difference in LDH level (p = 0.015) was observed between the two groups. Characteristics such as patient age (p = 0.989), gender (p = 1.000), pathologic subtype (p = 0.859), Ann arbor stage (p = 1.000), performance status (p = 1.000), extranodal disease involvement (p = 0.935), bone marrow (BM) involvement (p = 0.688), IPI score (p = 0.327), and number of R-CHOP cycles (p = 1.000) did not significantly differ between groups.

All 102 patients received more than six cycles (range, 6–8) of R-CHOP. After receiving immunochemotherapy, each patient was evaluated by PET-CT. Details on RT are described in **Table 2**. RT was administered at a median of 5.0 weeks (range, 2.6–13.1) after completion of R-CHOP. A total of 32 patients (94.1%) started RT within 8 weeks after completion of R-CHOP, except two patients who had to recover from previous treatment. The median dose of consolidative RT was 30.6 Gy (range, 30.0–50.4 Gy), and the median fraction size was 180 cGy (range, 180–300 cGy). Moreover, 27 patients (79.4%) received ≤30.6Gy. RT was administered to initially bulky sites (≥5 cm), head and neck lesions, testes, and bony lesions using 3D RT (n = 23, 67.6%) or intensity-modulated RT (n = 11, 32.4%). No patients received RT at all involved sites. Involved-field radiotherapy (IFRT) was administered to 18 (52.9%) patients, and involved-site radiotherapy (ISRT), which delivers radiation only to the initially involved sites, to 16 (47.1%) patients.

**TABLE 2** | Characteristics of Radiation Therapy.

| RT character | Number of Patients (%) |
|---|---|
| Timing of RT (interval of RT start date and R-CHOP end date) | |
| ≤6 week | 25 (73.5%) |
| >6 week | 9 (26.5%) |
| Radiation Dose, Gy | |
| ≤30.6 | 27 (79.4%) |
| >30.6 | 7 (20.6%) |
| RT technique | |
| 3DRT | 23 (67.6%) |
| IMRT | 11 (32.4%) |
| RT field | |
| ISRT | 16 (47.1%) |
| IFRT | 18 (52.9%) |
| RT duration | |
| ≤4 weeks | 28 (82.4%) |
| >4 weeks | 6 (17.6%) |
| RT sites | |
| Bony sites | 6 (17.6%) |
| Bulky sites (≥5 cm) | 17 (20.6%) |
| Head and neck lesions | 8 (23.5%) |
| Testes | 3 (8.8%) |

*RT, radiotherapy; R-CHOP, rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisone; 3DRT, 3-demensional radiotherapy; IMRT, intensity-modulated radiotherapy; ISRT, involved-site radiotherapy; IFRT, involved-field radiotherapy.*

**TABLE 3** | Patterns of failure.

| | All (n = 102) | | R-CHOP alone (n = 68) | | R-CHOP+RT (n = 34) | | P value |
|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | |
| Any recurrence | 33 | 32.4 | 25 | 36.8 | 8 | 23.5 | 0.180 |
| LR only | 16 | 15.7 | 14 | 20.6 | 2 | 5.9 | 0.055 |
| DR only | 7 | 6.9 | 5 | 7.4 | 2 | 5.9 | 0.783 |
| Both LR and DR | 10 | 9.8 | 6 | 8.8 | 4 | 11.8 | 0.639 |

*LR, local recurrence; DR, distant recurrence; R-CHOP, rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisone; RT, radiotherapy.*

## Patterns of Failure

With a median follow-up of 39.7 months (range, 6.8–125.1), 33 patients (32.4%) showed recurrence (**Table 3**). LR occurred in 26 patients (25.5%) with and without DR, and DR alone occurred in 7 patients (6.9%). Ten patients (9.8%) showed both LR and DR.

Of 68 patients who received R-CHOP alone, 14 (20.6%) showed isolated LR, 5 (7.4%) showed isolated DR, and 6 (8.8%) showed both LR and DR. Isolated LR was defined as LR without DR, and isolated DR was defined as DR without LR. Twenty patients showed LRs. The most common site of LR was head and neck lesions, which was observed in 10 patients (50.0%). Of the 14 patients with progression to isolated LR, 6 (42.9%) developed LR in head and neck lesions. The second most common site of LR was lymph nodes with initially bulky sizes (>5 cm) which was observed in 7 patients (35%).

Of 34 patients who received R-CHOP with consolidative RT, 2 (5.9%) showed isolated LR, 2 (5.9%) showed isolated DR, and 4 (11.8%) showed both LR and DR. Although the difference was marginally significant, only two patients who received consolidative RT showed isolated LR (5.9%). Furthermore, in-field failure after consolidative RT occurred in only one patient, which suggested that local control was related to consolidative RT.

## Survival Outcomes

The estimated actuarial 5-year OS, PFS, LRFS, DRFS, and isolated-LRFS rates were 73.5%, 64.0%, 68.4%, 80.1%, and 79.9%, respectively. Consolidative RT significantly improved the 5-year isolated-LRFS (73.6 *vs.* 92.9%, p = 0.049) compared to R-CHOP alone (**Figure 2C**). However, consolidative RT did not show significant improvement in OS (**Figure 2A**) or PFS (**Figure 2B**).

Univariate analysis (**Table 4**) showed that elevated LDH level (p = 0.015), extranodal disease involvement (p = 0.040), and high intermediate to high IPI score (p = 0.009) significantly decreased the 5-year OS. Elevated LDH levels were also associated with significantly decreased 5-year PFS (p = 0.042). Patients with Ann Arbor stage IV and elevated LDH levels showed significantly decreased 5-year DRFS (p = 0.046 and p = 0.028), while age, gender, pathologic subtype, stage, LDH level, extranodal disease involvement, bone marrow involvement, IPI score, and consolidative RT did not show any significance for LRFS. In the multivariate analyses, IPI score was a significant factor for OS (hazard ratio, 9.033, p = 0.031), and LDH level was a significant factor for PFS (hazard ratio, 3.175, p = 0.019).

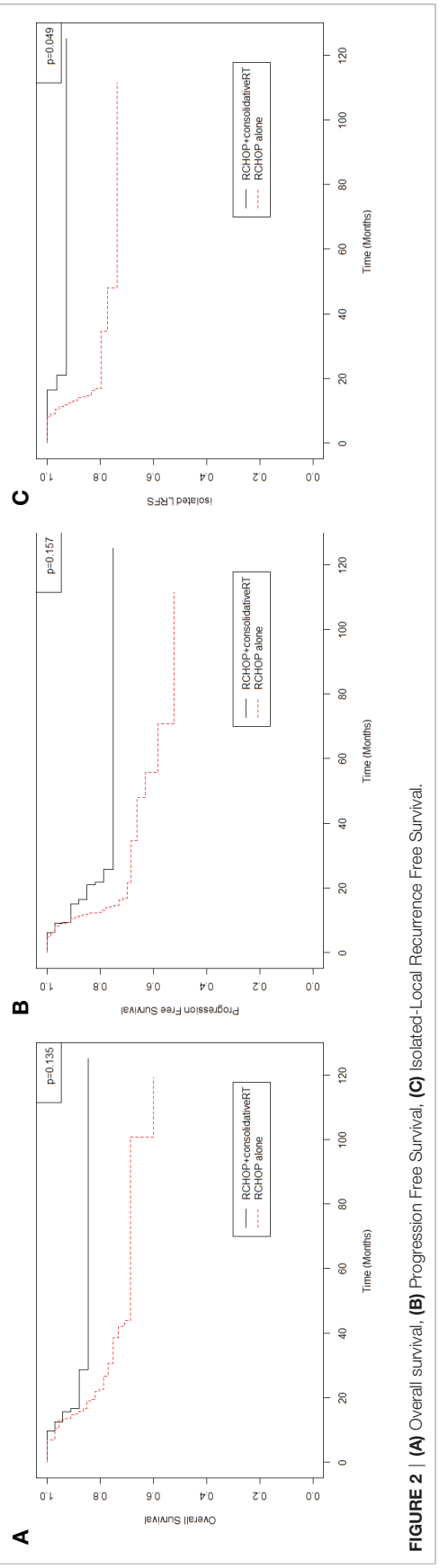


**FIGURE 2** | **(A)** Overall survival, **(B)** Progression Free Survival, **(C)** Isolated-Local Recurrence Free Survival.

In the univariate analysis of isolated-LRFS (**Table 5**), bone marrow involvement and consolidative RT showed significance (p = 0.013 and p = 0.049, respectively). The absence of bone marrow involvement and presence of consolidative RT also improved isolated-LRFS in the multivariate analyses (hazard ratio, 3.973, p = 0.006, and hazard ratio, 0.195, p = 0.032, respectively).

Additionally, an analysis based on pathology features was performed. Five patients showed c-MYC protein expression, and all of them showed either BCL 2 or BCL 6 protein expression, while one patient showed c-MYC, BCL 2, and BCL 6 protein expression. Moreover, four patients showed EBV-related DLBCL. Patients with c-MYC and BCL 2 protein expression showed significantly worse DRFS (p = 0.011 and p = 0.026, respectively). EBV-related DLBCL significantly decreased OS (p = 0.011), PFS (p = 0.011), LRFS (p = 0.004), and isolated LRFS (0 = 0.006). However, the number of patients was small, and the statistics should be carefully interpreted with a small number of cases.

In the consolidative RT group, total dose (≤30.6 *vs.* >30.6 Gy), fraction size (≤180 *vs.* >180 cGy), and RT timing (≤6 weeks *vs.* >6 weeks) were not significant factors for OS (p = 0.997, p = 0.237, and p = 0.836, respectively) or PFS (p = 0.758, p = 0.241, and p = 0.387, respectively).

## DISCUSSION

The role of consolidative RT in advanced-stage DLBCL after R-CHOP remains controversial. There is not any randomized controlled trial comparing treatment outcomes between R-CHOP and R-CHOP followed by consolidative RT for complete responders with advanced-stage DLBCL. However, in the GELA LNH 98-5 trial, 24% of patients who achieved CR after R-CHOP showed relapses; among them, 80% had stage III and IV DLBCL. In the era of rituximab, the 5-year survival rate for advanced-stage DLBCL is still approximately 60%, with a disease relapse rate of 50%. Consolidative treatment to reduce relapse and improve survival is needed. Some studies have shown excellent LC after consolidative RT, especially for patients with initially bulky diseases (9, 13).

This study evaluated our experience in administering consolidative RT for advanced-stage DLBCL. We compared the survival outcomes and analyzed failure patterns of patients with R-CHOP alone and R-CHOP followed by consolidative RT. Our analyzed treatment outcomes showed that 26 patients (25.5%) failed at the initially involved sites even after achieving CR following R-CHOP with or without consolidative RT. Among them, 12 (46.2%) and 11 patients (42.3%) had LR at initially bulky lymph nodes >5 cm in size and head and neck lesions, respectively.

In this study, OS did not significantly differ between the R-CHOP alone and R-CHOP followed by the consolidative RT group (p = 0.135, 5-year OS R-CHOP alone *vs.* R-CHOP+RT 68.6 *vs.* 84.7%). The same tendency was observed for PFS (p = 0.175, 5-year PFS R-CHOP alone *vs.* R-CHOP+RT 58.2 *vs.* 75.1%). Although there was no statistical difference in PFS,

**TABLE 4** | Univariate and Multivariate Analysis for Survival Outcomes.

| Characteristics | Overall Survival | | | | Progression Free Survival | | | |
|---|---|---|---|---|---|---|---|---|
| | 5yr OS (%) | Univariate (p) | Hazard Ratio (95% CI) | Multivariate (p) | 5yr PFS (%) | Univariate (p) | Hazard Ratio (95% CI) | Multivariate (p) |
| Age at diagnosis | | 0.109 | | 0.490 | | 0.664 | | 0.699 |
| ≤60 | 83.1 | | Referent | | 67.1 | | Referent | |
| >60 | 62.0 | | 1.361 (0.568–3.263) | | 60.1 | | 1.173 (0.523–2.633) | |
| Gender | | 0.534 | | 0.391 | | 0.983 | | 0.394 |
| Female | 73.7 | | Referent | | 55.5 | | Referent | |
| Male | 72.5 | | 1.498 (0.595–3.769) | | 67.8 | | 1.403 (0.643–3.061) | |
| Pathologic subtype | | 0.084 | | 0.068 | | 0.663 | | 0.582 |
| Non-GCB | 68.3 | | Referent | | 62.4 | | Referent | |
| GCB | 88.6 | | 0.298 (0.081–1.092) | | 69.0 | | 0.788 (0.336–1.844) | |
| Ann arbor stage | | 0.172 | | 0.579 | | 0.129 | | 0.261 |
| Stage III | 89.2 | | Referent | | 57.9 | | Referent | |
| Stage IV | 70.4 | | 1.557 (0.326–7.434) | | 62.2 | | 2.091 (0.577–7.576) | |
| Performance status (ECOG-PS) | | 0.691 | | 0.400 | | 0.067 | | 0.025 |
| 0–1 | 75.4 | | Referent | | 57.3 | | Referent | |
| 2–4 | 69.8 | | 0.683 (0.281–1.660) | | 77.8 | | 0.359 (0.146–0.881) | |
| LDH level | | **0.015** | | 0.107 | | **0.042** | | **0.019** |
| Normal (250–450 IU/L) | 92.0 | | Referent | | 80.5 | | Referent | |
| Elevated | 66.7 | | 4.012 (0.741–21.705) | | 58.3 | | 3.175 (1.214–8.305) | |
| Extranodal disease involvement | | **0.040** | | 0.218 | | 0.786 | | 0.820 |
| <2 | 91.5 | | Referent | | 54.2 | | Referent | |
| ≥2 | 68.1 | | 2.984 (0.523–17.020) | | 68.1 | | 1.136 (0.380–3.396) | |
| Bone marrow involvement | | 0.952 | | 0.647 | | 0.173 | | 0.154 |
| No | 77.0 | | Referent | | 66.6 | | Referent | |
| Yes | 65.3 | | 1.262 (0.465–3.423) | | 57.4 | | 1.751 (0.811–3.782) | |
| IPI score | | **0.009** | | **0.031** | | 0.400 | | 0.846 |
| Low to low intermediate | 96.0 | | Referent | | 60.4 | | Referent | |
| High intermediate to high | 66.2 | | 9.033 (1.221–66.800) | | 64.1 | | 0.860 (0.187–3.946) | |
| Number of R-CHOP cycles | | 0.677 | | 0.642 | | 0.682 | | 0.312 |
| 6 | 70.0 | | Referent | | 57.8 | | Referent | |
| 7–8 | 78.5 | | 0.807 (0.326–1.995) | | 67.6 | | 0.678 (0.320–1.439) | |
| Consolidative RT | | 0.135 | | 0.450 | | 0.157 | | 0.237 |
| No | 68.6 | | Referent | | 58.2 | | Referent | |
| Yes | 84.7 | | 0.676 (0.244–1.870) | | 75.1 | | 0.608 (0.266–1.387) | |

*GCB, germinal center B-cell; ECOG PS, Eastern Cooperative Oncology Group Performance Status; LDH, lactate dehydrogenase; IPI, International Prognostic Index; R-CHOP, rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisone; RT, radiotherapy; OS, overall survival; CI, confidence interval; PFS, progression free survival.*
*In bold, p value with less than 0.05.*

there was a trend that PFS was better in the consolidation RT group. Data from the MDACC (9), Duke (10), and Emory (13) showed that consolidative RT after achieving CR from R-CHOP improved OS, PFS, and LRFS in advanced-stage DLBCL. The MDACC (9) study included all stages, and only 14.0% of patients with advanced-stage DLBCL received consolidative RT. In contrast, the Duke (10) and Emory (13) studies included only patients with stage III–IV. Moreover, 48.1% and 12.7% of patients with advanced-stage DLBCL received RT, respectively. Similar to the MDACC (9) and Emory (13) studies, 16.5% of patients with advanced-stage DLBCL were treated with consolidative RT in this study. In all patients, survival outcomes were relatively comparable with those of other studies. However, for only the consolidative RT group, the 5-year PFS (75.1%) and LRFS (80.1%) were inferior to those of the Duke (10) (82.0 and 92.0%, respectively) and Emory (13) (85.1 and 91.7%, respectively) studies. Unlike the Duke (10) and Emory (13), which included 27.8 and 42.0% of patients with stage III and 72.2 and 58% of patients with stage IV, respectively, this study included 18.6% of patients with stage III and 81.4% of patients with stage IV. With a greater proportion of patients with stage IV, 10 patients (38.5%) with local failure had distant failure. Of note, 5-year isolated-LRFS, which did not include DR, showed better outcomes (in all patients, R-CHOP alone, and consolidative RT group, 79.9, 73.6, and 92.9%, respectively).

In the consolidative RT group, six patients (17.6%) developed LR with four patients showing LR with DR. Furthermore, only one patient showed in-field failure, who also showed DR at the same time. However, in the R-CHOP alone group, 20 patients (29.4%) showed LR. In both the R-CHOP alone group and R-CHOP followed by consolidative RT group, more patients showed LR (29.4 and 17.6%, respectively) than isolated-DR (7.4 and 5.9%, respectively). Seventeen patients showed DR including 9 patients who were initially diagnosed with stage IV disease.

**TABLE 5** | Univariate and Multivariate Analysis for Isolated-LRFS.

| Characteristics | Isolated-Local Recurrence Free Survival. | | | |
|---|---|---|---|---|
| | 5-yr isolated-LRFS (%) | Univariate (p) | Hazard Ratio (95% CI) | Multivariate (p) |
| Age at diagnosis | | 0.146 | | 0.242 |
| ≤60 | 86.9 | | Referent | |
| >60 | 70.9 | | 2.161 (0.594–7.861) | |
| Gender | | 0.468 | | 0.338 |
| Female | 78.3 | | Referent | |
| Male | 79.8 | | 1.853 (0.525–6.537) | |
| Pathologic subtype | | 0.726 | | 0.794 |
| Non-GCB | 80.1 | | Referent | |
| GCB | 80.7 | | 1.195 (0.313–4.568) | |
| Ann arbor stage | | 0.874 | | 0.518 |
| Stage III | 57.9 | | Referent | |
| Stage IV | 81.6 | | 0.595 (0.123–2.880) | |
| Performance status (ECOG-PS) | | 0.081 | | 0.068 |
| 0–1 | 76.8 | | Referent | |
| 2–4 | 89.4 | | 0.195 (0.044–0.866) | |
| LDH level | | 0.505 | | 0.628 |
| Normal (250–450 IU/L) | 83.5 | | Referent | |
| Elevated | 78.8 | | 1.445 (0.326–6.409) | |
| Extranodal disease involvement | | 0.879 | | 0.659 |
| <2 | 75.9 | | Referent | |
| ≥2 | 81.7 | | 0.682 (0.124–3.739) | |
| Bone marrow involvement | | **0.013** | | **0.006** |
| No | 85.0 | | Referent | |
| Yes | 65.7 | | 3.973 (1.476–10.693) | |
| IPI score | | 0.366 | | 0.578 |
| Low to low intermediate | 86.0 | | Referent | |
| High intermediate to high | 77.6 | | 1.860 (0.209–16.531) | |
| Number of R-CHOP cycles | | 0.958 | | 0.534 |
| 6 | 81.3 | | Referent | |
| 7–8 | 79.4 | | 0.684 (0.206–2.266) | |
| Consolidative RT | | **0.049** | | **0.032** |
| No | 73.6 | | Referent | |
| Yes | 92.9 | | 0.195 (0.044–0.866) | |

*GCB, germinal center B-cell; ECOG PS, Eastern Cooperative Oncology Group Performance Status; LDH, lactate dehydrogenase; IPI, International Prognostic Index; R-CHOP, rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisone; RT, radiotherapy; CI, confidence interval; LRFS, local recurrence free survival*
*In bold, p value with less than 0.05.*

Although rituximab improved the survival outcomes of DLBCL, LR was the dominant cause of failure. Particularly, patients who initially had bulky lesions or head and neck lesions need to be aware of LR. In the era of intensity-modulated radiation therapy (IMRT), RT for head and neck lesions became more feasible with less toxicity. Kawk et al. (19) also reported excellent LC of consolidative RT in DLBCL of head and neck lesions. Several studies have also reported that bulky disease is an important prognostic factor for local failure (4, 9, 11, 12, 14, 20). Even though this study did not show statistical significance, the failure pattern indicated the tendency of local failure with bulky disease >5 cm. Some studies have shown that a bulky tumor burden results in a lack of vascular flow that impairs drug delivery (21). With this explanation, the advent of rituximab would lessen this effect. Even though it is difficult to administer RT to all initially involved sites because of concerns on toxicity, the frequency of failure in this study suggests the application of consolidative RT, especially to the initially involved bulky sites and head and neck lesions.

This study has several limitations. First, as a retrospective study, there was selection bias between the two groups. The number of patients between the two groups was imbalanced. There is no consensus regarding the indications for referral of patients with consolidative RT. Usually, in our institution, patients with worse prognostic factors with ABC (activated B-cell) pathologic subtypes, bone involvement, and initially bulky sized lesions tend to receive consolidative RT. However, with propensity-score matching analysis, there was no significant difference in the characteristics of patients. Moreover, patients received combined modality treatment, which made it difficult to compare identical conditions. Second, the follow-up period of 39.7 months is insufficient, which may have affected the accuracy of the statistical analyses. Third, 26.5% of patients showed >6 weeks of interval between the end of chemotherapy and start of RT. These were longer the typical range for consolidative RT, which is 4 to 6 weeks. Finally, the relatively small number of patients might also affect the accuracy of the statistics.

Based on the results of this study and previous studies, consolidative RT is beneficial for local control in advanced-stage DLBCL and is a promising treatment option. Especially, there is only one in-field failure after consolidative RT in our study, which supports the outstanding local control rate of RT. Univariate and multivariate analyses showed that consolidative RT improved 5-year isolated-LRFS ($p = 0.049$ and $p = 0.032$, respectively), while bone marrow involvement statistically significantly decreased 5-year isolated-LRFS ($p = 0.013$ and $p = 0.006$, respectively). Consolidative RT can be considered for improvement in local control, even though it is difficult to insist administrating RT in all patients strongly, since this study is a case-matched control study. Also, it is hard to insist that RT would be more helpful in patients with bone marrow involvement as there is no statistically definitive relationship between RT and bone marrow involvement. However, since consolidative RT showed improved isolated-LRFS, applying consolidative RT might be considered in bone marrow involved patients with worse isolated-LRFS as further local treatment. As the pattern of failure showed, we carefully suggest to radiate on initially involved bulky sites or head and neck lesions. However, further studies on the optimal radiation field and dose evaluation are necessary. Further prospective studies with larger sample sizes are required to validate the role of radiation in advanced-stage DLBCL.

## DATA AVAILABILITY STATEMENT

Datasets are available on request. The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

This study was reviewed and approved by the institutional review board (IRB) of Seoul St. Mary's Hospital. (IRB number: KC19RESI0705) Because of the retrospective nature of the study, patient consent for inclusion was waived.

## AUTHOR CONTRIBUTIONS

Design of the study: SGC, and BOC. Collection of the data: HHL, SEJ, KSP, YWJ and HJH. Analysis and interpretation of the data: BOC and HJH. Writing and drafting of the manuscript: HJH. Revision of the manuscript: All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Survival Benefits of Anti-PD-1 Therapy in Combination With Radiotherapy in Chinese Melanoma Patients With Brain Metastasis

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Limited data reported the synergistic anti-tumor effect of anti-PD-1 (programmed death 1) therapy and radiotherapy on melanoma BM (brain metastasis). And the efficacy in the Chinese population is unclear. This study aimed to evaluate the efficacy of anti-PD-1 therapy and radiotherapy in Chinese melanoma patients with BM. We retrospectively reviewed 96 consecutive melanoma patients with BM treated at Sun Yat-Sen University Cancer Center. Patient demographics, BM characteristics and treatment details were carefully collected. The intracranial PFS (progression free survival) and OS (overall survival) were estimated using the Kaplan-Meier method. Twenty-five patients were treated with anti-PD-1 therapy and radiotherapy. Eighteen (72.0%) patients had SBRT (stereotactic body radiation therapy) or SRS (stereotactic radiosurgery) for BM, 1 (4.0%) patient had WBRT (whole brain radiation therapy), 6 (24.0%) patients had SBRT/SRS and WBRT. The median treatment period of anti-PD-1 therapy was 10.77 months. Objective intracranial response was observed in 15 (60%) patients, and 5 (20%) patients achieved CR (complete response). After a median follow-up of 16 months, 11 (44%) patients experienced intracranial PD (progressive disease), and 15 (60%) patients died. The median intracranial PFS and OS were 10.73 months (range, 1.67–38.83 months) and 15.87 months (range, 2.47–41.50 months), respectively. The 1-year intracranial PFS and OS were 61.9% (95% CI, 44.1–86.9%) and 62.5% (95%CI, 45.8–85.2%), respectively. Patients with BM can benefit from a combination of anti-PD-1 therapy and radiotherapy. It merits further investigation in melanoma patients with BM.

**Keywords: melanoma, anti-PD-1, radiotheapy, brain metastasis, immunotherapy**

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## INTRODUCTION

Malignant melanoma is a commonly reported type of skin cancer in Western countries (1, 2). And it also poses an increasing threat to the health of the Chinese population. Between 1990 and 2017, the annual incidence and prevalence rate of melanoma in China increased significantly, far beyond the global level (3). The clinical and biological characteristics of melanoma differ greatly between Caucasian and Chinese patients (4, 5). Instead of cutaneous melanomas as the major subtype in Caucasian patients, ∼70% of Chinese patients are diagnosed with acral (42.8%) or mucosal melanoma (27.0%) (4, 6). It is generally believed that patients with acral and mucosal melanoma portend a worse prognosis (7). Therefore, it is essential to explore effective treatments for the Chinese population.

Melanoma is the third most common malignant tumor to metastasize to the brain, after lung, and breast cancer. The reported incidence of BM (brain metastasis) in patients with melanoma was 10–40%, even higher (>70%) in the autopsy series (8–10). The prognosis of patients with BM is extremely poor. The median OS (overall survival) from diagnosis of BM was only 4–6 months in unselected patients (10–13). The survival of patients with BM undergoing surgery and/or radiotherapy only slightly improved, with a median OS of 6.4–10.8 months (10, 12–14). Although targeted therapy using BRAF/MEK inhibitors has dramatically changed the prognosis of patients with metastatic melanoma (15), patients with BM who received targeted therapy did not have a significant improvement in survival, with a median OS of 7–9.6 months (16, 17).

More recently, immunotherapy with anti-PD-1 (programmed death-1) antibodies has shown impressive and durable responses in patients with melanoma (18–22). However, the benefit of anti-PD-1 therapy combined with radiotherapy in melanoma patients with BM remains unclear, especially in the Chinese population. Therefore, in the present study, we reported the efficacy of this combined treatment strategy in the Chinese population.

## PATIENTS AND METHODS

### Patients

This retrospective study was approved by our institutional review board, and the requirement to obtain informed consent was waived. Between August 2010 and September 2019, a total of 96 consecutive melanoma patients with BM were treated at Sun Yat-Sen University Cancer Center. Patients who met the following criteria were enrolled for analysis: (1). histologically confirmed melanoma; (2). BM confirmed by MRI or CT scan; (3). KPS (Karnofsky Performance Status) ≥70; (4). received ≥1 dose of anti-PD-1 therapy; (5). received ≥1 course of radiotherapy for BM; (6). at least one follow-up MRI or CT examination after treatment. Patients who had no baseline images were excluded. Finally, 25 melanoma patients with BM were included in the present study (**Figure 1**).

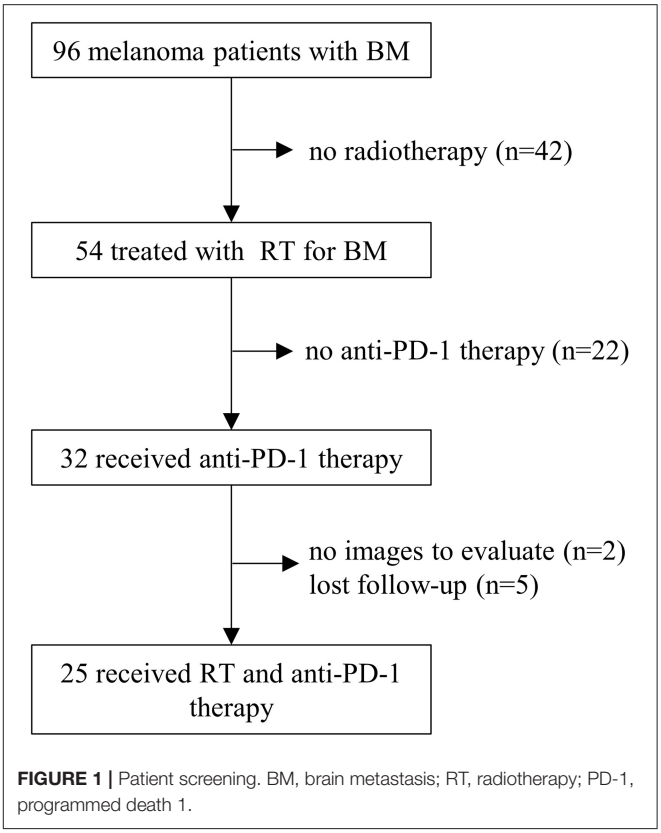


**FIGURE 1 |** Patient screening. BM, brain metastasis; RT, radiotherapy; PD-1, programmed death 1.

### Data Collection

The following data were collected for each patient: baseline demographics, KPS score, initial diagnosis date of melanoma, gene mutational status (BRAF/c-Kit), serum LDH (lactate dehydrogenase) level, stage of disease; initial diagnosis date of BM, the baseline number of BM lesions, the maximum diameter of BM, symptoms of BM, steroids use, number of extracranial metastasis sites; details of anti-PD-1 therapy and radiotherapy, prior systemic treatments, prior local therapy to BMs; time to endpoint data. Endpoints evaluated were intracranial PFS (progression-free survival) and OS.

### Statistics

The follow-up period was counted from the date of diagnosis of BM, which was defined as the first radiological diagnosis date of BM. The treatment efficacy was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (23). The intracranial PFS and OS rates were evaluated using the Kaplan–Meier method. The intracranial PFS was calculated as the time from diagnosis of BM to progression of the intracranial lesions or death due to any cause. The OS was defined as the time from diagnosis of BM to death due to any cause. The melanoma-molGPA score (24) was calculated based on age, KPS score, number of extracranial metastasis sites, number of BMs, and BRAF mutation status for each patient. The association between melanoma molGPA score and the estimated intracranial PFS or OS was tested using the log-rank test. The treatment related toxicities were evaluated according to CTCAE (Common Terminology Criteria for Adverse Events) v4.03. A two-sided $P \leq$

**TABLE 1 |** Baseline patient characteristics.

| Characteristic | Median (range) or *n* (%) |
|---|---|
| Patient number | 25 (100%) |
| Age | 48y (25–77y) |
| Male | 12 (48.0) |
| Female | 13 (52.0) |
| **KPS score** | |
| 70 | 1 (4.0) |
| 80 | 8 (32.0) |
| 90∼100 | 16 (64.0) |
| **Primary tumor type** | |
| Cutaneous melanoma | 8 (32.0) |
| Acral or mucosal melanoma | 17 (68.0) |
| **BRAF V600 mutation** | |
| Yes | 11 (44.0) |
| No | 14 (56.0) |
| **C-kit mutation** | |
| Yes | 2 (8.0) |
| No | 10 (40.0) |
| Unknown | 13 (52.0) |
| **LDH** | |
| <ULN | 20 (80.0) |
| ≥ULN | 3 (12.0) |
| Unknown | 2 (8.0) |
| **Interval between BM and initial diagnosis** | 19.03 m (0.2–74.4 m) |
| **Lines of prior systemic treatment** | |
| 0 | 8 (32.0) |
| 1 | 6 (24.0) |
| 2 | 5 (20.0) |
| ≥3 | 6 (24.0) |

*BM, Brain Metastasis; ULN, Upper Limit of Normal; LDH, Lactate Dehydrogenase.*

**TABLE 2 |** Baseline BM characteristics.

| Characteristic | Median (range) or *n* (%) |
|---|---|
| **Total number of BM** | |
| 1 | 10 (40.0) |
| 2∼4 | 8 (32.0) |
| 5∼10 | 4 (16.0) |
| >10 | 3 (12.0) |
| **Maximum diameter of BM** | 10 mm (2–51 mm) |
| **Number of extracranial metastatic sites** | 3 (0–7) |
| 0 | 2 (8.0) |
| 1∼3 | 12 (48.0) |
| ≥ 4 | 11 (44.0) |
| **BM with CNS symptoms** | |
| Yes | 7 (28.0) |
| No | 18 (72.0) |
| **Steroids for CNS symptoms** | |
| Yes | 3 (12.0) |
| No | 22 (88.0) |
| **Surgery for BM** | |
| Yes | 2 (8.0) |
| No | 23 (92.0) |
| **Radiotherapy for BM** | |
| SBRT or SRS | 18 (72.0) |
| SBRT or SRS + WBRT | 6 (24.0) |
| WBRT | 1 (4.0) |
| **Treatment duration of anti-PD-1 therapy** | 10.77 m (0.7–27.97 m) |
| **Melanoma-molGPA score** | 2 (1–4) |
| 0.0∼2.0 | 15 (60.0) |
| 2.5∼4.0 | 10 (40.0) |

*BM, Brain Metastasis; CNS, Central Nervous System; SBRT, Stereotactic Body Radiation Therapy; SRS, Stereotactic Radiosurgery; WBRT, Whole Brain Radiation Therapy; PD-1, Programmed Death 1; Melanoma-molGPA, Graded Prognostic Assessment for Melanoma Using Molecular Markers.*

0.05 was considered statistically significant. All the analyses were performed using R (version 3.6.0).

# RESULTS

## Patient Demographics

Between August 2010 and September 2019, a total of 25 melanoma patients with BM who received anti-PD-1 therapy and radiotherapy were identified (**Figure 1**). The median follow-up after diagnosis of BM was 16 months (range, 2.5–41.5 months). The baseline patient characteristics are shown in **Table 1**. The median age of patients was 48 years old (range, 25–77 years). Most patients were female (52.0%). All patients were in good conditions with a KPS score ≥70. Seventeen (68.0%) patients were diagnosed with acral or mucosal melanoma, and 8 (32.0%) patients were diagnosed with cutaneous melanoma. Eleven patients (44.0%) were positive for BRAF V600 mutation. C-kit mutations were detected in only two patients (8.0%). At the time of diagnosis of BM, only 3 patients (12.0%) had an elevated LDH level. The median interval from initial diagnosis of melanoma to BM was 19.0 months (range 0.2–74.4 months). Most patients (68.0%) had more than 1 line of systemic treatment before BM.

## BM Characteristics

Baseline BM characteristics are presented in **Table 2**. Most patients (60%) had more than one BM lesion. The median maximum diameter of BM was 10 mm (range, 2–51 mm). Extracranial metastasis was observed in 23 (92%) patients, 11 (44.0%) of them had more than 3 extracranial lesions. At the time of diagnosis, symptomatic BM was present in 7 (28.0%) patients, and 3 of them received steroids for symptom control. Two (8.0%) patients underwent surgical resection of BM before radiotherapy. Among all patients, 8 patients received ≥2 courses of radiotherapy for BM. Eighteen patients (72.0%) were treated with SBRT (stereotactic body radiation therapy) or SRS (stereotactic radiosurgery). Six (24.0%) patients received both SBRT/SRS and WBRT (whole brain radiation therapy), and one (4.0%) patient only received WBRT. Fourteen (56%) patients received radiotherapy before anti-PD-1 therapy. Eleven (44%) patients had started anti-PD-1 therapy before radiotherapy. All

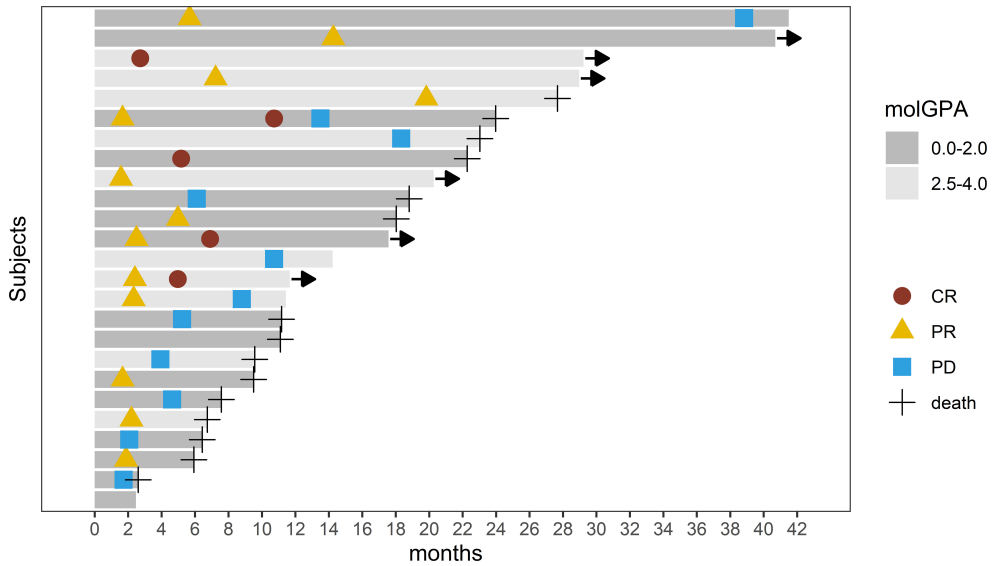


**FIGURE 2 |** Swimmer's plot showing intracranial response and survival after patients with brain metastasis received radiotherapy and anti-PD-1 therapy. CR, Complete Response; PR, Partial Response; PD, Progression Disease. Arrow implies ongoing response.

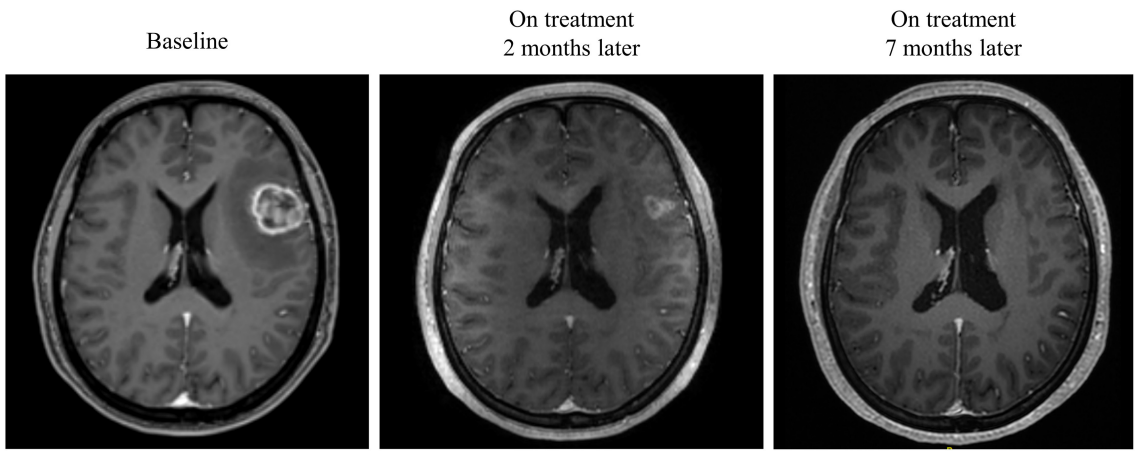


**FIGURE 3 |** Radiological examples of intracranial complete response. A 40-year-old female diagnosed with melanoma BM was given anti-PD-1 therapy and radiotherapy. MRI images show a complete response 7 months later.

patients started receiving anti-PD-1 therapy in very recent years. Eighteen (72%) patients started receiving ICI therapy in 2018 and 2019, 5 (20%) patients in 2017, 2 (8%) patients in 2015. The median treatment period of anti-PD-1 monoclonal antibody was 10.77 months (range, 0.7–27.97). In the current study, we also calculated molGPA score which has been shown to be significantly correlated with survival of melanoma patients (24). The majority of patients (60%) had a molGPA score of 0–2.

## Treatment Efficacy

Intracranial response after patients treated with radiotherapy and anti-PD-1 therapy was shown in **Figure 2**. The objective intracranial response was observed in 15 (60%) patients with 5 (20%) patients achieving CR (complete response). And 6 (24%) patients showed an ongoing intracranial response at the time of data analysis. The MRI images of a CR-obtained case are shown in **Figure 3**. One female patient receiving anti-PD-1 therapy combined with radiotherapy achieved intracranial CR 7 months after diagnosis. Another patient diagnosed with multiple BMs was treated with anti-PD-1 therapy following radiotherapy had a partial response 5 months later (**Figure 4**).

During the entire follow-up, 11 (44%) patients experienced intracranial PD (progressive disease), and 15 (60%) patients died. The median intracranial PFS was 10.73 months (range, 1.67–38.83 months), and the 1-year intracranial PFS was 61.9% (95% CI, 44.1–86.9%) (**Figure 5A**). The intracranial PFS was not significantly different between patients with melanoma-molGPA score of 0.0–2.0 and those with 2.5–4.0 ($p = 0.7$) (**Figure 5B**).

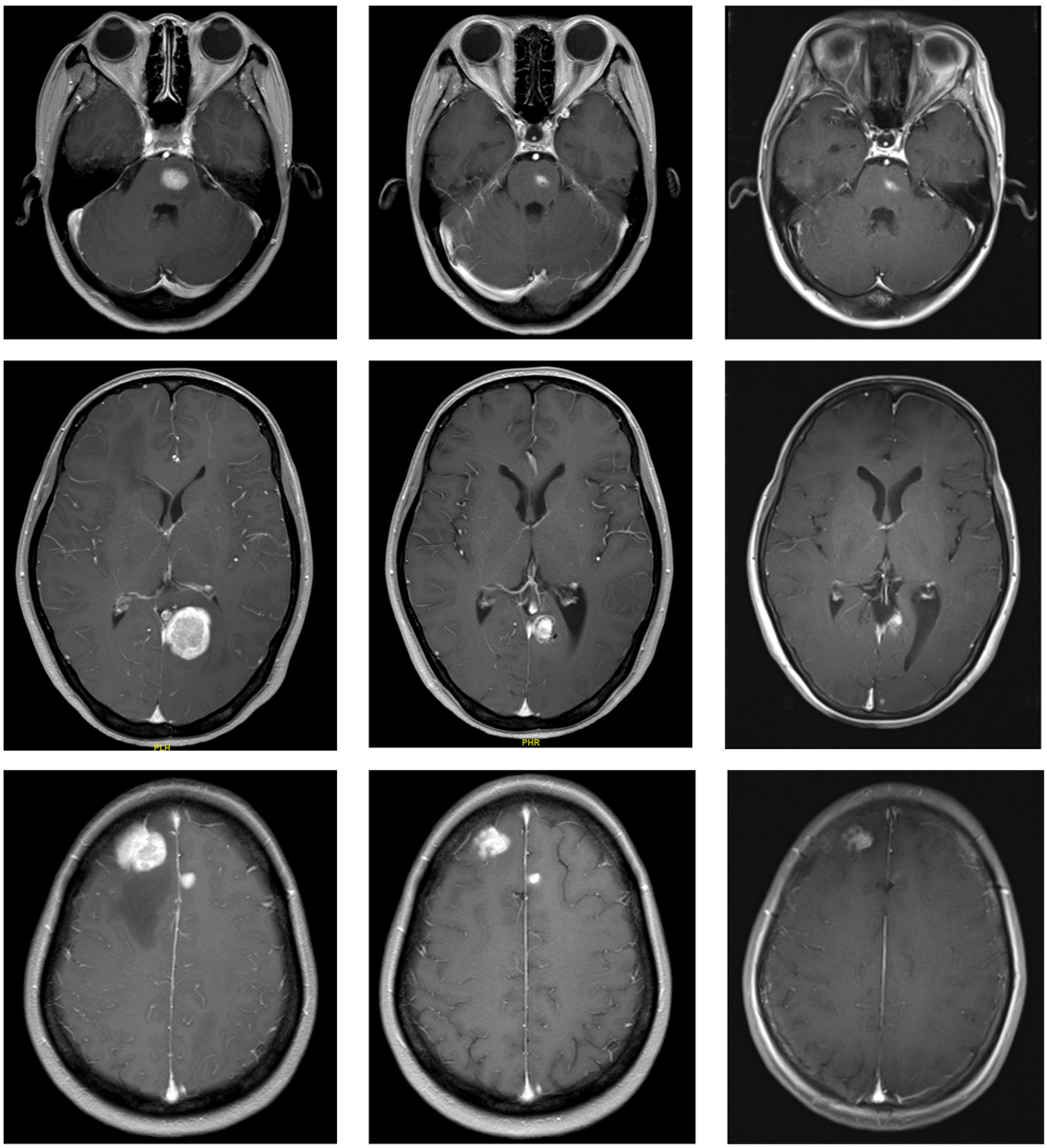


**FIGURE 4 |** Radiological examples of intracranial partial response. A 33-year-old female diagnosed with multiple melanoma BMs was treated with anti-PD-1 therapy following radiotherapy. MRI images show a partial response 5 months later.

The median OS was 15.87 months (range, 2.47–41.50 months), and the 1-year OS was 62.5% (95%CI, 45.8–85.2%) (**Figure 6A**). There was no statistically significant difference in OS between patients with a molGPA score of 0.0–2.0 and their counterparts of 2.5–4.0 ($p = 0.087$) (**Figure 6B**).

## Treatment-Related Adverse Events

The treatment-related AE (adverse events) were present in **Table 3**. There was no Grade 3 or higher treatment-related AE. The most frequently reported AE were rash ($n = 10$, 40.0%) and pruritus ($n = 9$, 36.0%). Other reported AE included skin hypopigmentation ($n = 3$, 12.0%), fatigue ($n = 4$, 16.0%), anorexia ($n = 4$, 16.0%), myalgia ($n = 3$, 12.0%), edema ($n = 1$, 4.0%), aminotransferase increased ($n = 1$, 4.0%), bilirubin increased ($n = 1$, 4.0%), tinnitus ($n = 1$, 4.0%), and hearing impaired ($n = 1$, 4.0%). Grade 2 immune-related psoriasis was reported by 1 patient (4.0%). Three patients (12.0%) had grade 1-2 hyperthyroidism or hypothyroidism. One patient

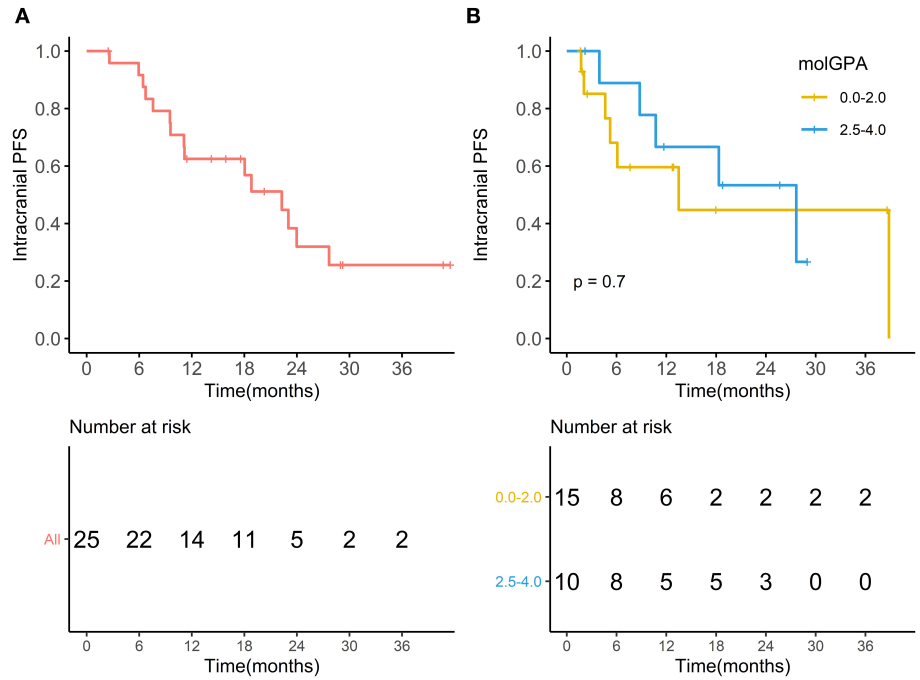


**FIGURE 5 |** Intracranial progression free survival. **(A)** Intracranial progression free survival of all patients with BM treated with radiotherapy and anti-PD-1 therapy. **(B)** Intracranial progression free survival of patients with molGPA 0.0-2.0 vs. those with molGPA 2.5-4.0.

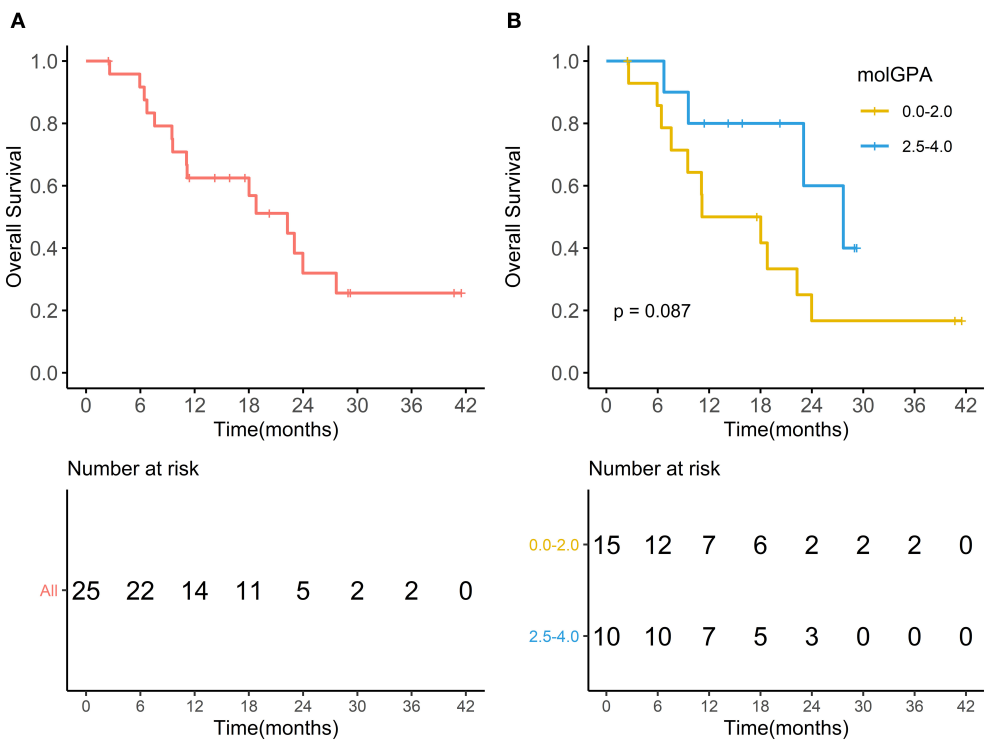


**FIGURE 6 |** Overall survival. **(A)** Overall survival of all patients with brain metastases treated with radiotherapy and anti-PD-1 therapy. **(B)** Overall survival of patients with molGPA 0.0-2.0 and those with molGPA 2.5-4.0.

**TABLE 3 |** Treatment-related adverse events.

| CTCAE category | Grade 1 | Grade 2 | Grade 3–5 | All (%) |
|---|---|---|---|---|
| Rash | 9 | 1 | 0 | 10 (40.0) |
| Pruritus | 7 | 2 | 0 | 9 (36.0) |
| Skin hypopigmentation | 3 | 0 | 0 | 3 (12.0) |
| Fatigue | 3 | 1 | 0 | 4 (16.0) |
| Anorexia | 4 | 0 | 0 | 4 (16.0) |
| Myalgia | 3 | 0 | 0 | 3 (12.0) |
| Edema | 1 | 0 | 0 | 1 (4.0) |
| Aminotransferase increased | 0 | 1 | 0 | 1 (4.0) |
| Blood bilirubin increased | 1 | 0 | 0 | 1 (4.0) |
| Tinnitus | 1 | 0 | 0 | 1 (4.0) |
| Hearing impaired | 1 | 0 | 0 | 1 (4.0) |
| Other skin disorder (psoriasis) | 0 | 1 | 0 | 1 (4.0) |
| Hyperthyroidism or Hypothyroidism | 2 | 1 | 0 | 3 (12.0) |
| Dysphasia | 1 | 0 | 0 | 1 (4.0) |

*CTCAE, Common Terminology Criteria for Adverse Events.*

developed grade 1 dysphasia after radiotherapy. Overall, no patient discontinued treatment due to AE.

## DISCUSSION

Chinese melanoma patients show distinct clinical and molecular characteristics. Most Chinese patients were diagnosed with acral or mucosal melanoma which was believed to be associated with a worse prognosis (4, 6, 7). Compared with the Caucasian patients, T-cell inflammation, TMB (tumor mutational burden), and antigen presentation machinery of the Chinese patients are lower (5). MAPK (mitogen-activated protein kinase) pathway and TERT (telomerase reverse transcriptase) promoter gene mutations are also differentially represented in the Chinese population (4). However, few studies have reported the effectiveness of radiotherapy combined with systemic therapy in Chinese melanoma patients with BM, let alone radiotherapy combined with immunotherapy. Therefore, we systematically evaluate the efficacy of Chinese melanoma patients with BM treated with radiotherapy and anti-PD-1 therapy in the present study, and favorable outcomes are reported. We demonstrated that intracranial objective response was achieved in 60% patients. The median intracranial PFS and OS were 10.73 months (range, 1.67–38.83 months) and 15.87 months (range, 2.47–41.50 months), respectively. And the 1-year intracranial PFS, and OS were 61.9% (95% CI, 44.1–86.9%), and 62.5% (95%CI, 45.8–85.2%), respectively.

Generally, the prognosis of melanoma patients with BM is extremely poor. The median OS is only weeks for patients with untreated BM, and only 1.2–2.1 months for patients treated with BSC (best supportive care) (10, 12–14). When melanoma patients with BM were treated with local therapy combined with or without systemic therapy, their OS was slightly longer. Data from Royal Prince Alfred Hospital showed that the median OS of patients with BM receiving surgery and radiotherapy, surgery, radiotherapy was 8.9, 8.7, 3.4 months, respectively (12). A study from M. D. Anderson Cancer Center reported that the median OS of patients with BM undergoing surgery, SRS, chemotherapy, WBRT was 9.8, 7.7, 4.6, and 3.9 months, respectively (10). As targeted therapy using BRAF/MEK inhibitors has greatly improved the prognosis of patients with metastatic melanoma, the efficacy of these inhibitors has also been explored in patients with BM. When patients were treated with dabrafenib for progressive BM after previous local treatments, the median OS was 7.33 months (16). The median OS for patients receiving vemurafenib with or without local treatments (radiotherapy or surgery) was 8.9–9.6 months (17).

More recently, immunotherapy has shown impressive and favorable responses in melanoma patients with BM. A study evaluated the efficacy of 18 melanoma patients treated with PD-1 inhibitor pembrolizumab, 4 (22%) patients achieved brain metastasis response (18). In another study, objective response was observed in 46–57% of patients who were treated with nivolumab and ipilimumab (19, 20). Median OS of 9.9 months was reported in patients receiving anti-PD-1 therapy with or without local therapy at five major melanoma centers in Australia (21). Preclinical and clinical data have demonstrated the synergistic anti-tumor effect of immunotherapy and radiotherapy (25–30). Data from Memorial Sloan-Kettering Cancer Center indicated that the median OS of patients receiving ipilimumab and SRS was 12.4 months (27). In another study, the median OS of patients who were treated with ipilimumab and radiotherapy in Brigham and Women's Hospital and Dana-Farber Cancer Institute was 14 months (28). In the current study, intracranial objective response was observed in 60% patients, and a median OS of 15.87 months was reported in patients treated with anti-PD-1 therapy and radiotherapy. Moreover, the patients in our study developed brain metastases earlier. The median time from initial diagnosis of melanoma to cerebral metastasis was 2.7 years in the U.S, and 3.1 years in Australia (11). In the present study, the median time from primary diagnosis of melanoma to BM was 19 months. This suggests that Chinese melanoma patients with BM may benefit more from the combination of anti-PD-1 therapy and radiotherapy.

Several studies have compared the outcome of patients with BM receiving different types of systemic therapies following radiotherapy. At Melanoma Institute Australia, the median OS of patients receiving anti-CTLA4, anti-PD-1, BRAFi ± MEKi, and no systemic drug therapy at the time of SRS was 7.5, 20.4, 17.8, and 10.8 months, respectively. However, the statistical analysis results were not reported (30). Another study from H. Lee Moffitt Cancer Center and Research Institute showed that 12-month OS rates of anti-PD-1 therapy, anti-CTLA-4 therapy, BRAF/MEKi, BRAFi, and chemotherapy following SRS were 48, 41, 65, 24, and 10%, respectively ($p = 0.01$) (29). It seems that patients treated with targeted therapy following SRS had a better OS. Prospective randomized clinical trials are required for further verification. And more data about the efficacy of systemic therapies and radiotherapy in Chinese melanoma patients with BM are demanded to guide individualized therapy.

There are some limitations to this study. First, this study is retrospective. And the data analyzed was from single center.

Although we recorded patient survival data as accurately as possible, the data were not collected in a standardized and prospective manner. Further prospective and multi-centers clinical trials are required to demonstrate the efficacy of the combination of anti-PD-1 therapy and radiotherapy in treating melanoma BM. Secondly, since anti-PD-1 therapy has been used to treat Chinese melanoma patients in very recent years, the sample size of the present study is relatively small. In this study, we analyzed the value of melanoma-molGPA score in predicting intracranial PFS and OS for patients with melanoma BM. Perhaps due to the small sample size, we cannot find the significant difference in intracranial PFS and OS between patients with high and low molGPA scores. Larger sample size is warranted to further verify the results.

In conclusion, the median OS of patients with melanoma BM who received both radiotherapy and anti-PD-1 therapy was 15.87 months in the present study. It provides evidence of combining anti-PD-1 therapy and radiotherapy in the management of melanoma BM. However, differences in patient-related factors may affect the outcome, and formal randomized clinical trials are required to determine whether anti-PD-1 therapy and radiotherapy have a synergistic anti-tumor effect in the setting of melanoma BM. Current ongoing clinical trials will provide further prospective evidence. A pilot study (NCT02716948) is investigating the side effects of SRS and nivolumab in treating patients with melanoma metastases in the brain or spine. Another pilot study (NCT02858869) is also exploring the adverse effects of pembrolizumab and SRS for Melanoma BM. The randomized ABC-X trial (NCT03340129) is evaluating the efficacy of ipilimumab and nivolumab with or without concurrent SRS in patients with asymptomatic, untreated melanoma BM. As the treatment paradigm for patients with melanoma BM evolves, choosing the appropriate systemic treatment or combination therapy and the optimal sequence of systemic and local therapies will be the next challenge for oncologists (21).

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of Sun Yat-Sen University Cancer Center. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

ZWP and LXL designed the study. LXL contributed to patients treatment and management. SW, CPY, and LLG collected and analyzed the data. SW wrote the manuscript. YC and LC provided suggestions for manuscript writing and data analysis. All authors reviewed and approved this manuscript.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
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# Application of New Radiosensitizer Based on Nano-Biotechnology in the Treatment of Glioma

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Glioma is the most common intracranial malignant tumor, and its specific pathogenesis has been unclear, which has always been an unresolved clinical problem due to the limited therapeutic window of glioma. As we all know, surgical resection, chemotherapy, and radiotherapy are the main treatment methods for glioma. With the development of clinical trials and traditional treatment techniques, radiotherapy for glioma has increasingly exposed defects in the treatment effect. In order to improve the bottleneck of radiotherapy for glioma, people have done a lot of work; among this, nano-radiosensitizers have offered a novel and potential treatment method. Compared with conventional radiotherapy, nanotechnology can overcome the blood–brain barrier and improve the sensitivity of glioma to radiotherapy. This paper focuses on the research progress of nano-radiosensitizers in radiotherapy for glioma.

**Keywords: nano-radiosensitizer, radiotherapy, radiation sensitization, nanoparticles, glioma**

## INTRODUCTION

Glioma is a tumor originating from glial cells, which is the most common primary malignant tumor in the brain (1). According to the grade of malignancy listed in the National Comprehensive Cancer Network (NCCN) Guidelines Version 1.2020 Central Nervous System Cancers (CNS), gliomas are classified into grades I to IV. Grade I lesions are benign, including pilocytic astrocytoma, multiform yellow astrocytoma, ganglion glioma, and subependymal giant cell astrocytoma. Grade II tumors include diffuse astrocytomas and oligodendrogliomas, which grow slowly, but can be highly differentiated. However, differing from pilocytic astrocytomas, these tumors infiltrate normal brain tissue and have a tendency to turn malignant. Grade III tumors include anaplastic astrocytoma and oligodendroglioma, which are characterized by high cell density and mitotic cells. The tumors of Grade IV are the most damaged and most common gliomas, including glioblastoma and gliosarcoma. Although we have made many efforts in the past few decades, glioma still has not been cured, and the median survival time of glioblastoma is still only 12 to 15 months (2, 3). The prognosis for patients with recurrent disease remains poor, with a median survival of only 25 and 40 weeks for recurrent glioblastoma (GBM) and recurrent anaplastic glioma, respectively (4).

Due to the active proliferation of glioma cells and the strong ability of invasive growth, the course of the disease progresses rapidly and is prone to recurrence and spread. As a routine treatment for glioma, radiotherapy has been used in clinical practice since 1970. The 2005 NCCN Glioma Treatment Guidelines recommend radiotherapy as one of glioma standard treatment methods (5–7).

Recently, radiotherapy has been developed rapidly, taking on an increasingly prominent role and position in the treatment of glioma, including conventional radiotherapy, three-dimensional conformal radiotherapy (3D-CRT), intensity-modulated radiation therapy (IMRT), and stereotactic radiotherapy. Conventional radiotherapy for gliomas mostly uses linear accelerators for whole-brain irradiation, which can easily cause damage to normal brain tissue and affect the radiotherapy dose in the tumor area. Radiotherapy technology has gradually shifted from whole-brain radiotherapy to local radiotherapy, together with improvements and research made when applying radiosensitizers, radiation doses, and radiation time intervals, in order to optimize the effect of radiotherapy, inhibit tumor progression, and improve radiation damage. However, radiotherapy for glioma still has some obvious shortcomings. For example, Roshan Karunamuni (8) found that radiotherapy for intracranial tumors can induce cognitive impairment, which is positively correlated with radiation dose. There was no significant difference in 5-year survival between patients with WHO grade II glioma (LGG) in the two groups who received 50.4Gy and 64.8Gy (9). NCCN recommends the use of preoperative and postoperative MRI imaging to determine the optimal tumor volume (GTV) and clinical target volume (CTV) before radiotherapy for gliomas. The clinical target volume (CTV) is an extension of the GTV (including Grade III gliomas, which increase the margin of 1 to 2 cm, and Grade IV gliomas, which increase the margin of 2 to 2.5 cm). Adult low-level glioma (WHO I or II) should receive 45–54Gy and 1.8v2.0Gy each time. For IDH wild-type low-grade glioma, increasing the RT dose to 59.4–60 Gy was considered. Anaplastic glioma and glioblastoma (WHO grade III or IV) recommend conformal RT (CRT) technology, including three-dimensional CRT (3D-CRT) and IMRT for focal brain irradiation, and the recommended radiation dose, with 60Gy and 2.0Gy each time or 59.4Gy and 1.8Gy each time. The initial radiotherapy plan was 46Gy and 2Gy each time.

The mechanism of radiotherapy is mainly divided into two types: direct damage and indirect damage. Direct damage is mainly caused by the direct action of radiation on organic molecules to produce free radicals to cause DNA molecules to break. Indirect damage is mainly caused by the ionization of water in human tissues by radiation (10). More and more studies have shown that the currently used low-liner energy transfer (Low-liner energy transfer LET) radiotherapy may promote the invasion and migration of gliomas (11). The radioresistance of gliomas is an important reason for the limitations of clinical radiotherapy. Rapid proliferation, high invasiveness, and radiation resistance are the main reasons behind unsatisfactory radiotherapy effects for gliomas. How to increase the radiosensitivity of glioma has become an important challenge (12–14).

The emergence of radiotherapy sensitizers provides new opportunities for radiotherapy for glioma. On the one hand, it can enhance the radiosensitivity of tumor cells; on the other hand, it can reduce the radiation dose and the adverse effects of normal brain tissue. When applied with radiotherapy, it can change the responsiveness of tumor cells to radiation, thereby improving the therapeutic efficiency. The killing effect of radiosensitizers on tumor cells is related to many factors, including tumor cell type, degree of cell differentiation, cell cycle, clinical stage, and anatomical classification (15). After treatment with radiation, DNA double-strand break (DSB) and DNA single-strand break (SSB) can be observed. Nevertheless, then some proteins related to DNA repair, such as DNA-dependent protein kinase (DNA-PK), and are activated to start the repair process. After that, the damaged cells return to normal cells eventually. In the process of radiation on cells, many factors determine the final results (16). Considering a single cell, it can enhance DNA damage and promote cell apoptosis or autophagy. Substances that inhibit DNA damage repair may enhance the killing effect of radiation on tumor cells to achieve the purpose of radiation sensitization. From the perspective of the tumor as a whole, the oxygen and state of the cells inside the tumor and the cell cycle distribution of the tumor cells have an impact on the killing effect of radiation. Most of the radiosensitizers used in the past refer to drugs with the abovementioned functions. With the continuous development of molecular biology, some small interfering RNA (siRNA) and monoclonal antibodies targeting radiation-sensitive genes have become new candidates for radiosensitizers (17, 18).

Adams and Fowler et al. divided traditional radiosensitizers into the following categories: DNA precursor base analogs (such as 5-BUdR), electrophilic radiosensitizers (including nitroimidazoles, nitroaromatic hydrocarbons, and nitro heterocyclic compounds), oxygen-like compounds, radiation damage repair inhibitors, mercapto inhibitors (such as 4-ethylmaleimide (NEM), neoarsphenamine, p-chloromer curibenzoate, iodoacetamide), cytotoxic compounds Sensitizer ($Cu^{2+}$), tumor vascular disrupting agent, and gene-related tumor radiosensitizer, etc. (19, 20). At present, the conventional radiotherapy sensitizers in clinic include 5-fluorouracil, platinum (such as cisplatin, carboplatin), gemcitabine, etc., which can enhance the radiotherapy sensitivity of tumor cells through different mechanisms of action (such as inhibiting DNA synthesis, promoting DNA double-strand breaks, regulating the cell cycle, etc.) (21, 22). However, these conventional radiotherapy sensitizers also have some drawbacks. With the combination of radiotherapy to treat tumors, 5-fluorouracil has a short half-life and requires long-term intravenous drip administration, which easily forms thrombus and causes nosocomial infections (23). Cisplatin is a widely used clinical radiotherapy (CRT) drug, which can kill many types of tumors (24, 25). Consequently, it can cause many adverse reactions, such as nausea, vomiting, neurotoxicity, ototoxicity. and

nephrotoxicity (26). 5-Iodine-2 deoxyuridine (IUdR) has been confirmed to have a significant radiosensitization effect on glioblastoma, but due to the short circulating half-life and the inability to pass the blood–brain barrier (BBB), its clinical application is limited (27). DNA double-strand repair inhibitors (DSBRIs) KU55933 were once considered as one of the most promising drugs to improve radiotherapy, but its clinical application remains due to its potential toxicity to normal tissues, inability to select-enter tumor cells, and poor solubilization (28). Misonidazole is a hypoxic cell sensitizer, which can enhance the antitumor effects of cyclophosphamide in preclinical studies (29). Formerly, it is expected to be an ideal radiotherapy sensitizer in terms of controlling radiation-resistant tumor cells and p53 mutant tumor cells (30). However, researchers in a randomized study found that Misonidazole did not improve the prognosis of cervical cancer radiotherapy compared with the placebo group (31), making people question the effectiveness of Misonidazole, with the toxicity of Misonidazol further studied. Trans sodium crocetinate (TSC) has been verified as a radiotherapy sensitizer. In a study of a C6 glioma model, the use of TSC improved the regression of GBM tumors after radiotherapy, increased survival, and achieved radiosensitization. The mechanism of action may temporarily increase tissue oxygenation of hypoxic glioma (32, 33). However, the effect on patients with glioma needs to be further explored. Carbon ion radiotherapy is an excellent way of radiotherapy, with great application prospects in glioma (34–36). However, its combination with nano-radiosensitizers remains to be studied.

Therefore, how to find a safe and effective radiotherapy sensitizer for glioma has become an urgent problem. With the rapid development of nano-science and technology, people are paying more and more attention to the role of nano radiation sensitizers in the treatment of glioma. Therefore, this paper will review the principle and types of radiosensitizers in radiotherapy for glioma and the research progress of radiosensitizers in radiotherapy for glioma

## ADVANTAGES OF NANO-RADIOSENSITIZERS IN RADIOTHERAPY FOR GLIOMA

Nanomaterials have been widely used to improve the efficacy of radiotherapy due to their good biocompatibility, inherent radiosensitivity, a high carrying capacity of multiple drugs, and enhanced penetration and retention in tumor tissues (37, 38). The research of nanomaterial-mediated sensitization of radiotherapy mainly focuses on the use of high atomic number nanoparticles (such as gold, silver, and bismuth) to enhance the radiation energy deposition in cells. With the development of polymer nanomaterials, the research on the treatment of glioma is increasing gradually. Small molecule drugs can be chemically bound and physically coated to target glioma tissues through the blood–brain barrier, thus improving the efficacy of radiotherapy for glioma.

### Nano-Radiotherapy Sensitizers Can Efficiently Cross the BBB and Target Gliomas

The blood–brain barrier (BBB) is the outer layer of blood vessels in the brain and spinal cord, which is highly selective for substance penetration. The barrier properties of a healthy blood–brain barrier are mainly due to the tight junctions between endothelial cells, which are stable by astrocytes and pericytes. Through complex design, the blood–brain barrier can prevent the passage of neurotoxins and microorganisms, and selectively allow oxygen and nutrients to enter the central nervous system, thereby maintaining homeostasis (39–44). BBB restricts the delivery of chemical drugs and becomes a difficult point in the chemotherapy of glioma. Therefore, the primary problem that nano-radiosensitizers used in radiotherapy for glioma need to solve is to cross the BBB and target the glioma tissue. Normally, nanoparticles cannot pass through the BBB, but when the tumor is present, BBB permeability increases, and nanoparticles can pass through. Compared with normal tissues, tumor tissues have an abundant blood supply, wide vascular space, and lack lymphatic drainage, making macromolecular substances or lipid particles have high permeability and high retention effects in tumor tissues, which can be called the high permeability and retention effect (EPR) of solid tumors. It can increase the drug concentration in tumor tissue through the EPR effect, which is passive transport. Our research group (45) used this effect to design an RT-sensitive liposome that is responsive to hypoxia as a novel DOX delivery system. The hypoxia radiosensitizer nitroimidazole combines with lipid molecules with hydrolysable ester bonds to form MDH, which is mixed with DSPE-PEG2000 and cholesterol to make MLP liposomes. Experimental results show that MLP liposomes can carry DOX and nitroimidazole across the BBB and can effectively stay in the tumor area. Hypoxia can induce the conversion of hydrophobic nitroimidazole into hydrophilic aminoimidazole through electron transfer, causing the instability of liposomes and releasing DOX. Meanwhile, MI enhanced the radiosensitivity of radiation-tolerant hypoxic cells due to electron affinity, and DNA damage caused by ionizing radiation was enhanced. The drug delivery system can effectively inhibit the growth of C6 glioma cells by combining radiotherapy and chemotherapy. Additionally, nano-radiotherapy sensitizers actively cross BBB by adding special ligands, antiboding and proteining to the surface engineering of nanoparticles to form multifunctional nanoparticles, with a strong BBB crossing efficiency and can selectively and specifically target CNS tumor tissues (46). It should be noticed that there is another Nano-radiotherapy sensitizer that was designed by our group called ALP-(MIs)n/DOX, and it also has an excellent ability to cross the BBB (47) (**Figure 1**). Zhang et al. (48) encapsulated the cyclin-dependent kinase inhibitor dinaciclib into lipid nanoparticles containing anti-PD-L1 antibodies, and RT induced the up-regulation of PD-L1 in glioma infiltrating TAMC (Tumor-associated myeloid cells). Lipid nanoparticles (LNP) targeting PD-L1 effectively target glioma tissues, inhibit PD-L1 or eliminate TAMCs, which are immunosuppressive

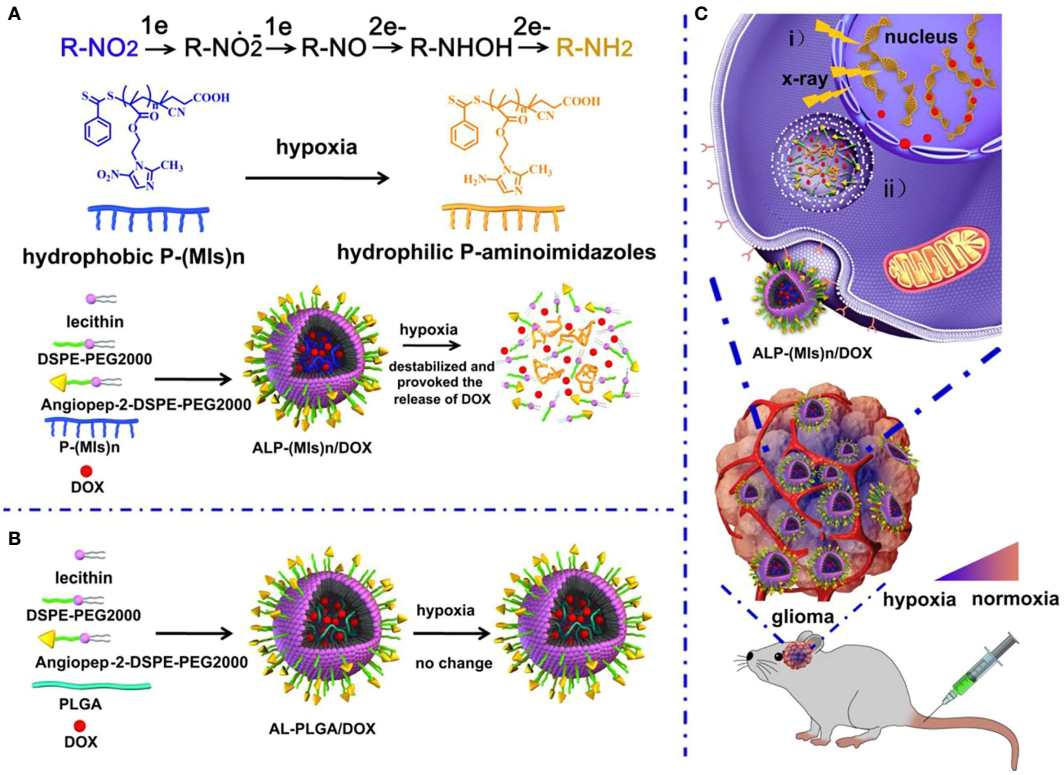


**FIGURE 1** | Schematic of the hypoxia-responsive and hypoxia RT sensitization ALP-(MIs)n drug-delivery system. **(A)** Mechanism of ALP-(MIs)n RT sensitization and DOX release under hypoxic condition and formation of ALP-(MIs)n/DOX. Six electrons are transferred in the complete reduction of nitro ($R\text{-}NO_2$) to amine ($R\text{-}NH_2$) under hypoxic conditions *via* a single-electron reduction catalyzed by a series of intracellular nitro reductases. **(B)** Formation of AL-PLGA/DOX as the control group. **(C)** Schematic illustrating ALP-(MIs)n applications: (i) Hypoxic cell radiosensitizer. ii. Hypoxia-responsive release of DOX into the cytoplasm, and then transports it to the nucleus to kill tumor cells (47).

cells, strengthen anti-tumor immunity, and extend the survival time of mice.

## Enhance the Efficacy of Radiotherapy for Glioma Through Radiation Energy Deposition

In terms of sensitization of radiotherapy, metal nanoparticles have been studied for many years as radiotherapy sensitizers. Metal nanoparticles with a high Z value have a high absorption capacity of radiation and can concentrate radiation energy on the tumor site (49). It is generally believed that these nanoparticles increase the cross-section of tissues or cells that react with radiation, facilitating the efficient deposition of high-energy radiant energy. From the formula of X-ray absorption coefficient μ and incident X-ray energy E and atomic coefficient Z: $\mu = \rho Z^4/(AE^3)$, the absorption coefficient μ is positively related to the fourth power of atomic coefficient Z, where ρ is the density and A the atomic mass (50, 51). Therefore, materials with high atomic coefficient elements have better X-ray energy absorption. The high Z-value nanoparticles after absorbing ray energy can produce a photoelectric effect, Compton effect, and Auger effect; this then generates a series of secondary electrons, such as the photoelectron, Compton electron, and Auger electron (52–54), which can directly interact with biomolecules locally or generate large amounts of ROS with water molecules. The principium above is shown in **Figure 2** (71). Tumor cells are then killed and the sensitization of radiotherapy is enhanced. The radiosensitization effect of AuNPs

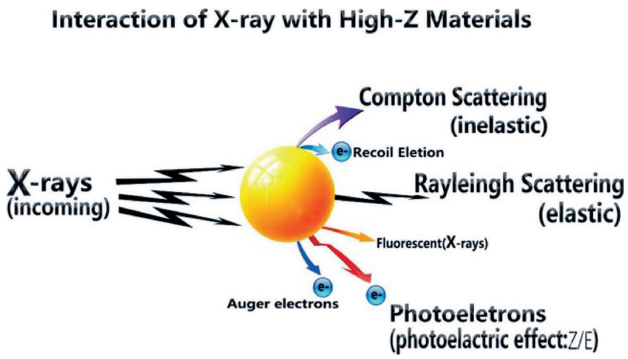


**FIGURE 2** | Radiant energy deposition to arouse secondary electrons (71).

depends on its size and the type of surface modification (55, 56). Silver, platinum, gadolinium, etc. have similar radiosensitization effects to gold nanomaterials. Liu et al. found that malignant glioma-bearing rats treated with silver nanoparticles (AgNPs) after radiotherapy effectively inhibited the proliferation of cancer cells and promoted the apoptosis of cancer cells (57).

## Enhance Radiotherapy for Glioma by Enhancing DNA Damage and Inhibiting DNA Repair

The radiotherapy resistance of tumors is mainly manifested in the double-strand breaks of tumor cells caused by radiation, and DNA itself has the ability to repair double-strand breaks (59). It is believed that the anti-radiation effect of tumors is due to hypoxia in tumor regions, which reduces DNA damage and enhances cellular defense mechanisms (60, 61). Therefore, DNA damage in glioma cells can be increased by increasing the oxygen content in the glioma region. In the meantime, the local oxygen of the tumor is more likely to produce ROS under the action of radiation, which increases the killing effect on the tumor. Many nano-radiotherapy sensitizers work by increasing the oxygen content of the tumor area (62, 63). Additionally, gliomas are usually resistant to RT due to their strong DNA repair activity (64, 65). The cytotoxicity of RT is mainly due to DNA damage, and double-strand breakage (DSB) caused by RT is the most serious type of DNA damage. If it is not repaired, it is deadly to the cells (66). Nanoparticles can inhibit DNA repair by inducing down-regulation of repair proteins, such as thymidylate synthase (67) (**Figure 3**), or inhibiting the DNA damage repair signaling pathway (68), thereby increasing the effect of radiotherapy. In terms of glioma, our research group designed a hypoxic radiosensitizer-prodrug liposome (MLP) as a carrier for the DNA repair inhibitor Dbait, which significantly inhibited the growth of glioma *in situ* in mice with the combination with radiotherapy (69).

## Can Effectively Transport Radionuclides to Achieve RIT

Radiotherapy is divided into two categories: external radiation therapy (EBRT) and internal radioisotope therapy (RIT). For EBRT, radiation beams such as high-energy X-rays, electron beams, or proton beams from outside the body are directly irradiated on the tumor, thereby inducing the death of cancer cells. For RIT, a minimally invasive method is used to introduce therapeutic radioisotopes into the tumor, such as direct infusion *via* a catheter (also called brachytherapy) (70, 71). Brachytherapy is not suitable for treating distant tumors due to the rapid elimination of radioisotopes *in vivo*. The combination of targeted nanoparticles with radioactive isotopes enables accurate isotope delivery, while nanoparticles for internal radiotherapy can also improve tumor vascular permeability, enhance retention effect (EPR), and increase uptake of the next wave of nanoparticles (38). In the treatment of glioma, nanoparticles were also widely used to deliver radionuclides (58, 72), which was proven to have good safety and feasibility (73). Allard introduced a lipid nanocapsule (LNC), which encapsulated $^{188}$Re($^{188}$Re(S$_3$CPh)$_2$(S$_2$CPh)[$^{188}$Re-SSS]) to form a lipophilic complex that can be used as a new type of

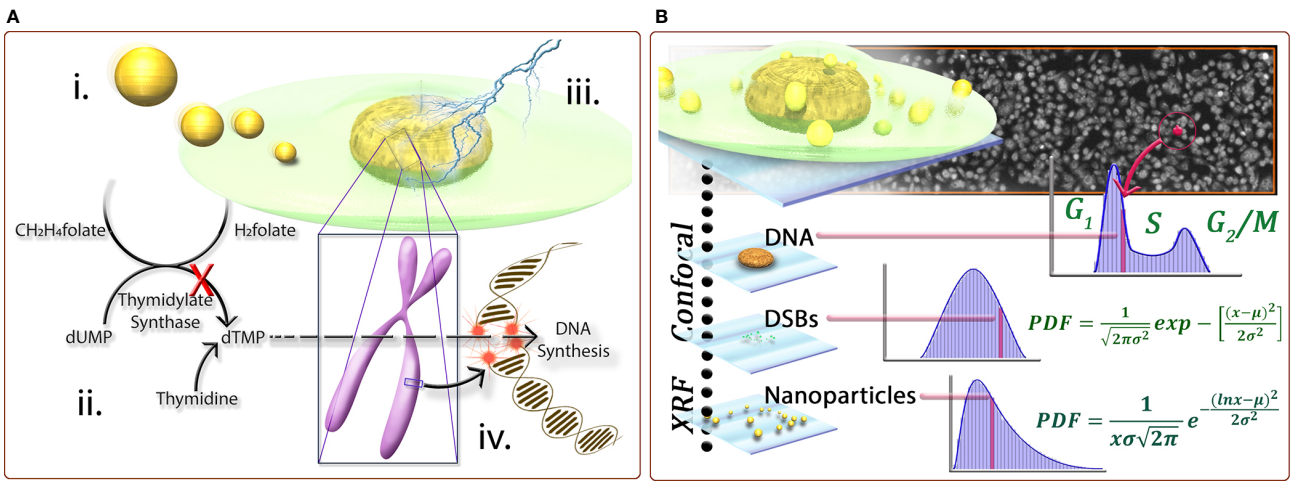


**FIGURE 3** | **(A)** Schematic representation of the following conceptst: (i) internalization of nanoparticles by cells can lead to the down-regulation of proteins, including thymidylate synthase (TS), important for DNA damage repair response; ii. due to the down-regulation of TS, the conversion of dUMP to dTMP is inhibited; iii. subsequently, when the DNA is subjected to insult by ionizing radiation causing doublestrand breaks; and iv. the normally effective homologous recombination pathway for repairing DSB's in S-phase cells is also inhibited, leading to a biological mechanism of radiosensitization. **(B)** A cross-correlative methodology developed provides a three-dimensional data set to compare cell populations and sub-populations with regard to nanoparticle dose–response at the single-cell level. Correlating biological markers imaged with laser scanning confocal microscopy with elemental content from synchrotron X-ray fluorescence microscopy for cell populations provides statistically significant, descriptive analysis of cell populations with regard to biological response for a quantified number of nanoparticles. For example, only cells with comparable numbers of nanoparticles are compared, or only cells in a certain phase are compared. The population behavior can be described by fitting functions and any individual cell from a population can be characterized by its biological markers coupled with its nanoparticle content (67).

radiopharmaceutical carrier. The results showed that the median survival of rats treated with 8Gy$^{188}$Re-SSSLNC was significantly improved. Compared with the control group, the median survival time increased by about 80%, with 33% of long-term surviving animals and when administered in LNC,$^{188}$Re tissue retention was greatly prolonged, with only 10% of the injected dose being eliminated at 72h (74). Interestingly, another study revealed that 188Re-activity gradient led to a bypass of immunosuppressive barriers, which can be used to treat glioblastoma (75).

## Nano-Radiotherapy Sensitizer Combined With Other Treatment Methods to Treat Glioma

Nano-radiotherapy sensitizers can not only be enriched at the tumor site by enhancing the penetration and retention effects and improving the targeting effect on tumor tissues, but they also can be combined with chemotherapy, immunotherapy, and other treatment methods. Meanwhile, the specific microenvironment of glioma is used to achieve effective drug delivery (76), improving the therapeutic effect of glioma.

Nano-radiotherapy sensitizer in combination with immunotherapy uses nano-delivery of inhibitory antibodies to block immune checkpoints. Due to the ability of nanomaterials to penetrate the BBB, immune-stimulating nanoradiation sensitizers can penetrate the BBB well and accumulate in glioma tissues. As mentioned above, lipid nanoparticles containing PD-L1 antibody not only have targeted functions but also inhibit PD-L1 and enhance T cell anti-tumor immunity and kill glioma cells in synergism with radiotherapy (48) (**Figure 4**). In addition, nanomaterials used as photosensitizers combined with photodynamic therapy (PDT) for radiotherapy have achieved significant effects on some other types of tumors (77, 78), which can also similarly kill glioma cells (79). In a study of high-grade glioma treatment, we found that photodynamic therapy (PDT) extended survival in patients, and in combination with intraoperative radiation therapy (IORT), improved survival even further (80). However, the application of nano-photosensitizer combined with PDT to the radiotherapy for glioma has not been reported in the literature.

Researchers found that enhanced autophagy of glioma promoter cells (GICs) contributes to the elimination of radiotherapy resistance (81). Liu et al. evaluated the radiosensitization effect of silver nanoparticles (AgNPs) on hypoxic glioma cells and found that the radiosensitization ability of AgNPs in hypoxic U251 cells and C6 cells was higher than that of normoxic U251 and C6 cells (82). The main reason for hypoxic radiation sensitization induced by siNPS is the promotion of cell apoptosis and the enhancement of destructive autophagy, suggesting that AgNPs can be used as excellent radiosensitizers in the treatment of hypoxic glioma. Paradoxically, earlier studies have found that gamma-ray-induced autophagy contributes to the radioresistance of these cells, and autophagy inhibitors may be employed to increase the sensitivity of GSCs to gamma-radiation (83).

Autophagy has a protective effect on inhibiting the radiosensitization of STAT3. Inhibition of autophagy and STAT3 may be a potential therapeutic strategy to improve the radiosensitization of glioma cells (84). Therefore, the effect of autophagy on radiosensitization of gliomas is still controversial (85, 86).

Emerging nano-radiosensitizers have developed rapidly currently. For example, near-infrared light combined with radiotherapy that converts light energy into heat energy (87), sonoporation sensitization radiotherapy (88), and nanoparticles of heterojunction structure can avoid the recombination of electrons and holes, improve photocurrent and photocatalytic activity, etc. (89).

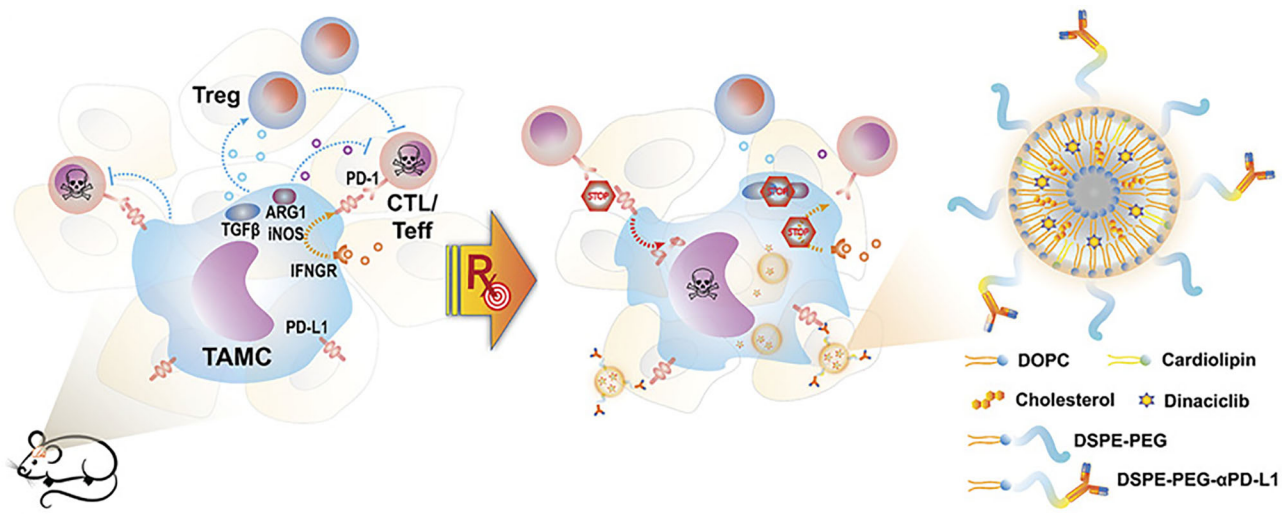


**FIGURE 4** | Schematic representation of nano-targeting of glioma-associated TAMCs. (CTL, cytotoxic T lymphocyte; Teff, effector T cell; PD-1, programmed cell death protein 1; IFNGR, IFN gamma receptor) (48).

## THE MAIN TYPES OF NANO-RADIOSENSITIZERS IN THE TREATMENT OF GLIOMAS

Nano-radiotherapy sensitizers can overcome a series of problems such as high toxicity, non-specificity, and obvious side effects of traditional sensitizers, making nano-radiosensitization treatments become a popular treatment for various malignant tumors including gliomas. According to the physicochemical properties of nano-sensitizers in existing research, the common nano-sensitizers (nanoparticles) in the treatment of glioma are divided into the following categories: 1. High-Z metal nano-radiotherapy sensitizers; 2. Common metal and its oxide nano-radiotherapy sensitizer; 3. Semiconductor nano-radiotherapy sensitizer; 4. Non-metallic nano-radiotherapy sensitizer material; and 5. Multifunctional nano-radiotherapy sensitizer. We draw a diagram (**Figure 5**) which summarizes the main species of nano-radiosensitizers and more details are shown in **Table 1**.

### High-Z Metal Nano-Radiosensitizer

A high-Z metal nano-radiotherapy sensitizer is the most in-depth research among various nano-material sensitizers because high-Z elements have a strong X-ray attenuation ability (50), which can increase the radiation dose of tumor cells in GBM tissues, thereby achieving the therapeutic effect of sensitization of radiotherapy (90). Gold, silver, platinum, and other high-Z precious metals have the advantages of low toxicity, easy preparation, controllable size and morphology, easy surface functionalization, high chemical stability, and good biocompatibility (91), which have natural advantages of of preparing bio-related nanomaterials. Recently, gold nanomaterials, the most studied among high Z metals, have been widely used in radiosensitization therapy of glioma (92). Yan Liu et al. used a one-pot green syn-thetic method to synthesize luminescent gold nanoclusters (AuNC) (93). Su-Yang Yang et al. used the strategy of cross-linked stable lipid nanocapsules (NCs) as a carrier to prepare a kind of inter-membrane cross-linked multilayer lipid vesicle (ICMV) containing amphiphilic gold nanoparticles (amph-NPs) to form Au-NCs. In vivo experiments on mice showed that the AU-NCS combined radiotherapy group had an obvious tumor-killing effect compared with the radiotherapy alone group (94). Yijin Liu et al. studied a mixed anisotropic nanostructure composed of gold (Au) and titanium dioxide ($TiO_2$). As a radiosensitizer, Au-$TiO_2$ nanoparticles (DAT) can significantly enhance the effect of radiotherapy (77, 93). In addition to nano-gold, nano-silver and nano-platinum materials have also been extensively studied (95). Haiqian Zhang et al. prepared a silver nanoparticle (AgNPs) for radiosensitization of hypoxic glioma cells, with the results showing that AgNPs can significantly improve the effect of radiotherapy in the radiotherapy of hypoxic glioma (82). Eva Pagá˘ cová et al. analyzed effects on radiation-induced γH2AX+53BP1 lesions of different nanoparticle materials (platinum (Pt) and gold (Au)), cancer cell types (HeLa, U87, and SKBr_3), and low-line energy transfer

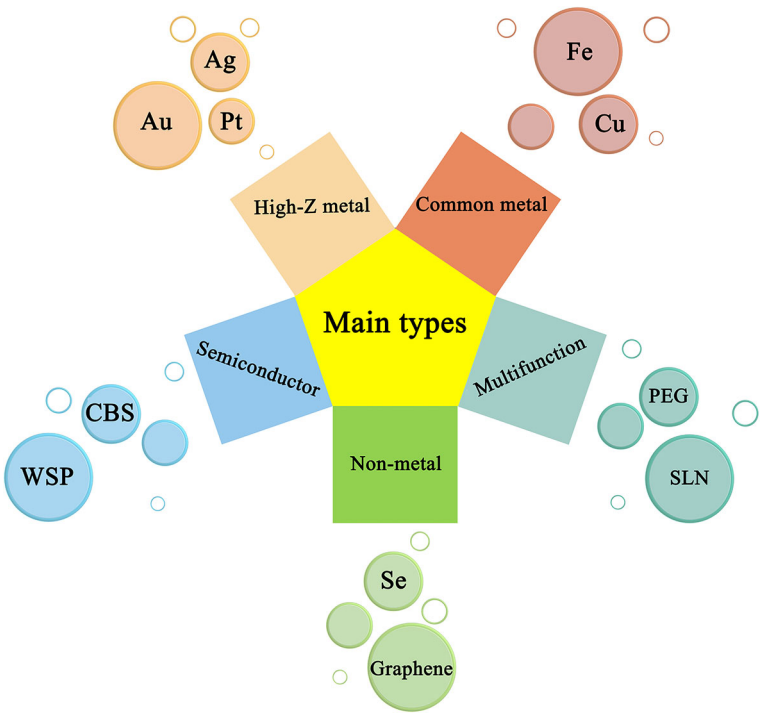


**FIGURE 5** | Representative nanomaterials and basic principles of action under types of nanoradiosensitizers.

**TABLE 1 |** Lists the types of glioma nano-radiotherapy sensitizers mentioned in the paper, including the type, name, and position of sensitizers.

| Main types | Based Nanomaterial | References |
|---|---|---|
| High-Z metal nano-radiosensitizers | Gold (Au) | (92–94, 96, 125) |
| | Silver (Ag) | (82) |
| | Platinum (Pt) | (96) |
| | gadolinium (Gd) | (98, 100, 126) |
| | Hafnium (Hf) | (99) |
| | Tantalum (Ta) | (97, 98, 101) |
| | Cerium (Ce) | (103) |
| | Terbium (Tb) | (104) |
| | Tungsten/Wolfram (W) | (105) |
| | Bismuth (Bi) | (106–110) |
| Common metal and its oxide nano-radiosensitizer | Iron (Fe) | (111, 112) |
| | Copper (Cu) | (113, 114) |
| | $Fe_3O_4$ | (64) |
| | $ZnFe_2O_4$ | (115) |
| Semiconductor nanomaterial sensitizer | $WO_{2.9}$-$WSe_2$-PEG (wsp) | (118) |
| | $Cu_3BiS_3$ (CBS) | (119) |
| Non-metallic nanomaterial sensitizer | Selenium (Se) | (107, 121) |
| | Graphene | (27, 122) |
| Multifunctional nano-radiotherapy sensitizer | SLN+EGFR+siRNA | (123) |
| | PEG+PEI+siRNA | (124) |

(LET) ionizing radiation (γ- and X-rays) dose (up to 4Gy) to evaluate its radiosensitization effect in gliomas (96). In addition to the above high-Z precious metals, other high-Z metal nanosensitizers also include gadolinium (Gd), hafnium (Hf), tantalum (Ta), cerium (Ce), terbium (Tb), tungsten (W), bismuth (Bi), and other metal elements with large atomic coefficients (97). Particularly, lanthanide metal-based nanoparticles are being developed and utilized due to their strong X-ray attenuation ability. Verry, C et al. designed a gadolinium (Gd)-based AGuIX nanoparticle for combined radiotherapy for patients with brain metastases, showing that the nanoparticle significantly improved the effect of radiotherapy (98, 99). Chen has developed a nano-sensitizer of titanium dioxide doped with gadolinium, which targets mitochondria for effective radiation therapy. With X-ray irradiation, nanosensitizers trigger the domino effect of ROS accumulation in mitochondria (99). Géraldine et al. used 9L glioma cell line (9LGS) tumor-bearing mice to inject a biodegradable gadolinium-based ultrafine nanoparticle (AGuIX nanoparticles) intravenously. They found that AGuIX particles do not leak out of normal blood vessels, allowing more particles to accumulate effectively in glioma tissue, increasing the sensitivity of radiation therapy (100, 101).

In addition to the metal gadolinium (Gd), the metal hafnium (Hf), as a high-Z metal, is often used in the X-ray manufacturing industry because it easily emits electrons. Pure hafnium has the advantages of plasticity, easy processing, high temperature resistance, corrosion resistance, and so on. It is an important material in the atomic energy industry, which has also been put into medical research and use. Min-Hua Chen proposed a nanoparticle that can enhance active oxygen: Hf-doped hydroxyapatite (HF: HAP). After exposing (HF: HAP) to gamma rays, the generation of ROS in the cell increases significantly (99). Jin J summarized the latest progress in radiation therapy (RT) and immunotherapy of nanoparticles (NPs) such as hafnium (Hf) and bismuth (Bi) and evaluated the feasibility of high-Z metals as nano-radiosensitizers (102).

Among high-Z metals, tantalum (Ta) has been widely used in the medical field because of its moderate hardness and excellent ductility. The excellent corrosion resistance is mainly due to the formation of s atable tantalum pentoxide ($Ta_2O_5$) protective film on the surface, which has also been used in the field of radiotherapy for glioma sensitization. Briggs discovered for the first time that tantalum ($Ta_2O_5$) nanoparticles showed a dose-enhancing effect on gliosarcoma cells with strong radiation resistance under 10MV irradiation. It is believed that the enhancement effect is due to the secondary electrons generated by the photoelectric effect, which increases the biological effect of radiation, indicating that tantalum $Ta_2O_5$ has a certain radiosensitization effect in the radiotherapy for glioma (101). Besides, cerium (Ce) is also a widely used high-Z metal in the medical field as the most abundant rare earth element in the earth's crust. Xiaoyan Zhong prepared Ce (Ce)-doped $NaCeF_4$: Gd and Tb fluorescent nanoparticles (SCNP or fluorescent scintillator). Due to the sensitization of Ce ions, Tb ions can trigger X-ray sensitive fluorescence (XEF) under X-ray irradiation to generate reactive oxygen species (ROS) in RDT, thereby increasing the sensitivity to radiotherapy (103). Runowski enriches the fluorescence effect of $CeF_3$ nanoparticles (NPs) by co-doping with $Tb^{3+}$ and $Gd^{3+}$ ($CeF_3$: $Gd^{3+}$, $Tb^{3+}$) for the treatment of deep tumors such as intracranial tumors (104).

As a new high-tech material, tungsten (W) is another high-Z metal that has been put into the medical field. According to Wang, J's research, tungsten sulfide ($WS_2$QDs) is a nanomaterial suitable for radiotherapy (RT) and photothermal therapy (PTT), proving that tungsten (W) can be used as a nano-radiosensitizer (105).

Bismuth (Bi) is a hot spot nano-radiotherapy material besides nano-gold materials. Hossain, M controlled the concentration of nanoparticles to 350 mg·g−1 under a radiation source of 50 kVp and found that the radiosensitization effect of nano-bismuth was 1.25 times and 1.29 times stronger than that of nano-gold and nano-platinum, respectively. Based on this, it is concluded that bismuth nanoparticles have a stronger sensitizing effect than gold and platinum nanoparticles with the same nanometer size, particle concentration, and action site (106). In the presence of bovine serum albumin (BSA), Fangxin Mao et al. synthesized ultra-small biocompatible $Bi_2Se_3$ nanoparticles by reacting hydroxyethylthioselenide and bismuth chloride in an aqueous solution BSA-$Bi_2Se_3$ shows a strong wide absorption rate, high light-to-heat conversion efficiency, and a strong radiation sensitization effect in the near-infrared (NIR) window (107). Huan Yu et al. synthesized bismuth sulfide nanoparticles (BiNP) and coupled them with immunoactive Ganoderma lucidum polysaccharide (GLP) and verified that GLP-BiNP has a dual role in tumor treatment through radiosensitization and immune activity (108). Guosheng Song used a partial cation exchange method, which took MnSe nanocrystals as a template to replace

manganese with bismuth in the outer layer to form a $Bi_2Se_3$ shell, to advance the blood supply of tumor tissue, increase oxygenation significantly, improve the effect of radiotherapy (RT), and kill tumor cells effectively (109). Fangmei Zhang et al. designed and prepared a multifunctional bismuth-based nano-olfactory, which was functionalized by S-nitrosothiol and named Bi-SNO (NPs). X-rays can break down the S-N bond and trigger the release of a large amount of NO (over 60μM). The prepared Bi-SNO (NPs) with a small volume (36 nm) has the ability to absorb and convert 808 nm near-infrared photons for photothermal treatment, as well as the ability to increase X-ray absorption and CT imaging sensitivity. Moreover, the synergistic effect of Bi-SNO radiation, photothermal, and gas therapy *in vivo* was further studied, to get a significant synergistic tumor inhibition effect (110).

## Common Metal and Its Oxide Nano-Radiosensitizer

Other common metal types with nanoradiosensitization effects include common non-high Z nanoradiosensitizers, such as nanoradiosensitizers, iron nanoradiosensitizers, and copper nanoradiosensitizers. Chengcheng Yang developed a polydopamine (PDA) coated Ge11 peptide conjugated iron oxide nanoparticles ($Ge_{11}$-PDA-Pt@USPIOs) with cisplatin as a carrier, based on ultra-small superparamagnetic iron oxide nanoparticles (PAA@USPIos) coated with polyacrylic acid, showing synergistic therapeutic effects of radiotherapy and chemotherapy under low temperature *in vitro* (111). Muhammed prepared SiO-MNP-coated iron oxide nanoparticles by co-precipitation and other methods to enhance the radiation sensitization effect by increasing the production of ROS (112).

For nano-copper sensitizers, Yu Fan et al. designed a therapeutic nano-platform based on the complexation of pyridine (Pyr) functionalized fifth-generation ($G_5$) polyamidoamine dendrimers with $Cu^{2+}$, which is used for radio-enhanced T1-weighted magnetic resonance (MR) imaging and coordinated radiotherapy and chemotherapy for tumors and tumor metastases (113). Chenyang Zhang designed a new smart radiosensitizer based on $Cu_2(OH)Po_4$ nanocrystals. Sensitizers can respond to both endogenous ($H_2O_2$) and exogenous (X-rays) stimuli simultaneously and can finally induce apoptosis and necrosis of cancer cells (114).

Some ferrite-based spinel structure nano-material sensitizers have also been reported. For example, Alireza Meidanchi synthesized superparamagnetic zinc ferrite spinel nanoparticles $ZnFe_2O_4$ by a hydrothermal method which is used as a radiosensitizer for cancer treatment. When exposed to gamma rays, the low-energy electrons produced in the nanoparticles further kill tumor cells. The use of biocompatible $ZnFe_2O_4$ nanoparticles (at a concentration of 100μg/ml) in radiotherapy can produce a synergistic response to radiotherapy. The killing efficiency of highly radiation-resistant cancer cells is 17 times that of traditional radiotherapy, so it is a reliable radiation sensitizer (115). Besides, the sensitizers of metal nanomaterials for glioma include some special new nanometal materials, such as metal-organic skeleton (Zr-MOF) nanoparticles (116) and room temperature liquid nanometals (LMs) (117). Moreover, some of the above nano metal materials not only directly affect the sensitization of radiotherapy but also act as multifunctional adjuvants in auxiliary imaging, such as X-ray diagnosis (116).

## Semiconductor Nanomaterial Sensitizer

In the field of semiconductor nanosensitizer materials, common semiconductor materials include silicon (Si), germanium (Ge), gallium arsenide (GaAs), and other compound semiconductors doped or made into other compound semiconductor materials. Among them, silicon is the most commonly used semiconductor material. Semiconductors have the following in common. The conductivity of a semiconductor is between a conductor and an insulator, which will change significantly when it is stimulated by external light and heat. Therefore, semiconductor materials have great potential in the application of sensitization of radio therapy. Dong Xinghua et al. discovered $WO_{2.9}$-$WSe_2$-PEG semiconductor heterojunction nanoparticles (WSP NPs), which can be combined with radiotherapy (RT), photothermal therapy (PTT), and immune checkpoint suppression therapy (CBT) to jointly enhance anti-tumor and anti-metastasis effects. Under X-ray irradiation, the nanosystem catalyzes the highly expressed $H_2O_2$ in TME, promotes the generation of non-oxygen-dependent reactive oxygen species, and enhances the effect of radiotherapy (118). Yiwei Kang et al. encapsulated small semiconductor copper bismuth sulfide ($Cu_3BiS_3$, CBS) nanoparticles and rare earth down-conversion (DC) nanoparticles in larger size zeolite imidazole skeleton-8 (ZIF8) nanoparticles and then loaded them with anticancer drugs Doxorubicin (DOX). Under X-ray irradiation, a moderate dose of CBS&DC-ZIF8@DOX composite material can achieve high (87.6%) tumor suppression efficiency and synergistic radiotherapy and chemotherapy (119).

## Non-Metallic Nanomaterial Sensitizer

The development of non-metallic nanomaterial sensitizers in the treatment of glioma has also been very rapid, such as selenium (Se) nanoparticles, graphene nanomaterials, etc. (120). Qian Huang et al. synthesized selenium nanoparticles by reducing tin dioxide with vitamin C. The selenium nanoparticles were used as sacrificial templates to react with copper ions to form copper selenide nanoparticles. The results showed that the dumbbell-like copper-gold selenide nanocrystals could be used as an effective radiosensitizer for enhanced radiotherapy (121).

In the treatment of gliomas, graphene nanomaterials have also made new progress in the field of sensitization and radiotherapy. Sakine Shirvalilou et al. used magnetic graphene oxide (NGO/SPIONs) nanoparticles (MNPs) coated with PLGA polymers as dynamic nanocarriers for IUDR to achieve 5-iodo-2 deoxyuridine (IUdR) entry into the blood–brain barrier (BBB). IUDR/MNPs were administered intravenously to tumor-bearing rats of the C6 glioma cell line under a magnetic field of 1.3T, and the synergistic effect of IUDR/MNPs and radiotherapy was found. Compared with radiation alone, increasing the ratio of Bax/Bcl-2 (2.13 times) can significantly inhibit tumor expansion (>100%) and prolong survival time (>100%). Inhibit the anti-apoptotic response of glioma rats, thereby enhancing the sensitizing effect of tumor radiotherapy (27). Lei Chen et al. developed $^{131}$I-labeled, polyethylene glycol (PEG) coated

reduced graphene oxide (RGO) nanoparticle. After intravenous injection, gamma imaging shows a significant accumulation of $^{131}$IRGO-PEG in tumor tissue. Reduced graphene oxide has a strong near-infrared absorbance, which can effectively heat tumors under near-infrared irradiation. The $^{131}$I emits high-energy X-rays due to ionization, which induces tumor killing and enhances the effect of radiotherapy on cancer cells (122).

## Multifunctional Nano-Radiosensitizer

A simple nanoradiotherapy sensitizer cannot meet the needs of clinical treatment for the characteristics of radiation resistance and immunosuppression of glioma. Functional nanomaterials can improve the radiotherapy sensitivity of gliomas in many ways. Erel-Akbaba G has developed a cyclic peptide iRGD (CCRGDKGPDC)-conjugated solid lipid nanoparticle (SLN) to deliver epidermal growth factor receptor (EGFR) and PD-L1 small interfering RNA (SiRNA), binding to targeted and immunotherapy for glioblastoma and enhancing the efficacy of radiation therapy by regulating the immune system (123). Forrest M. Kievit et al. prepared a nanoparticle (NP) composed of superparamagnetic iron oxide core, biodegradable chitosan, polyethylene glycol (PEG), and polyethyleneimine (PEI) coating. The NP can bind to siRNA and protect it from degradation and deliver siRNA to the area around the target nucleus to use an siRNA vector to inhibit the expression of APE1 and enhance the sensitivity of brain malignancies to RT (124). siRNA itself is a radiotherapy sensitizer. By carrying a certain radiotherapy sensitizer nanocarrier and combining immunotherapy, it can achieve double or even multiple sensitizers, which is also the research focus of future radiotherapy sensitizer nanocarrier.

# OUTLOOK

In summary, the combined application of nanoparticles and radiotherapy sensitizers can significantly improve the effect of radiotherapy. The special biological characteristics of glioma weaken the effect of traditional radiotherapy, and the excellent targeting and good biocompatibility of nano-radiosensitizers solve the difficulties of traditional radiotherapy for glioma. At present, nano-radiosensitizers have developed rapidly in the past few years, providing new research strategies for sensitization of radiotherapy and new ideas for radiotherapy for gliomas. As mentioned earlier, nanoparticles as radiosensitizers have shown great potential in tumor treatment. New drug delivery methods can also improve the sensitizing effect of radiosensitizers (127). Nano-radiosensitizers are characterized by low cytotoxicity, good targeting, good biocompatibility, and easy functionalization. They can pass the blood–brain barrier (BBB), and some of them have been used as radiosensitizers in clinical treatment (128). However, single-functional nanoparticles cannot fully meet clinical needs, and more and more researchers have focused on finding multifunctional nanoparticles that are more conducive to clinical transformation. Furthermore, improving the drug-carrying capacity of nanomaterials is a strategy to develop multifunctional platforms. Research on the radiation sensitization mechanism will provide targets for new radiation sensitizers, and interdisciplinary research will promote the further development of new radiation sensitizers (129).

# AUTHOR CONTRIBUTIONS

HL and YX was responsible for the overall idea of the article. YH was responsible for the abstract and the fourth part. XZ was responsible for the *Introduction* and *Outlook* and revised the format. HM was responsible for the writing of the second part. LL was responsible for the writing of the third part. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Radiation and CAR T-cell Therapy in Lymphoma: Future Frontiers and Potential Opportunities for Synergy

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CAR T-cell therapy has revolutionized the treatment approach to patients with relapsed/refractory hematologic malignancies; however, there continues to be opportunity for improvement in treatment toxicity as well as response durability. Radiation therapy can play an important role in combined modality treatments for some patients undergoing CAR T-cell therapy in various clinical settings. In this review, we discuss the current evidence for RT in the setting of CAR T-cell therapy for patients with hematologic malignancies and propose potential opportunities for future investigation of RT and CAR T-cell treatment synergy. Future research frontiers include investigation of hypotheses including radiation priming of CAR T-cell mediated death, pre-CAR T-cell tumor debulking with radiation therapy, and selection of high risk patients for early radiation salvage after CAR T cell therapy.

**Keywords: chimeric antigen receptor T cells, radiation therapy, large B cell lymphoma, immunotherapy, external beam irradiation**

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## INTRODUCTION

Chimeric antigen receptor (CAR) T-cell therapy has transformed our approach to patients with relapsed/refractory (R/R) aggressive lymphomas, with multiple therapies that have achieved high response rates and notable durable disease remissions in patients with otherwise dismal outcomes. Radiation therapy (RT) may be a valuable treatment modality that, when optimally combined with CAR T-cell therapy, could offer enhanced tumor control and reduced toxicity. In this review, we discuss the current evidence for RT in the setting of CAR T-cell therapy for patients with hematologic malignancies and propose potential opportunities for future investigation of RT and CAR T-cell treatment synergy.

## BACKGROUND

While most patients with diffuse large B-cell lymphoma (DLBCL) respond to frontline immunochemotherapy based regimens [typically rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP)], roughly 30–40% of patients are refractory to primary therapy or develop relapsed disease (1, 2). Until recently, the primary potentially curative salvage therapy approach included multi-agent platinum-based chemotherapy followed by high dose chemotherapy and autologous stem cell transplantation (3–5), with an associated

overall response rate (ORR) of roughly 60% and 3-year overall survival (OS) of roughly 50%. However, for patients that do not respond to second line therapy, median survival is exceptionally poor at roughly 4–6 months (6, 7). In a large, international multicohort retrospective study of patients with relapsed and refractory DLBCL, only 20% of patients were alive at 2 years (8).

CD-19 CAR T-cell therapy has ushered in a new era of therapeutic approaches for patients with R/R large B-cell lymphoma (9). Autologous T-cells are genetically engineered to express chimeric antigen reception molecules that target the CD-19 antigen on the surface of large B-cell lymphoma cells. Patients are administered lymphodepleting conditioning chemotherapy, most commonly fludarabine and cyclophosphamide, over 3 days prior to infusion of the autologous CAR T-cell product.

Autologous anti-CD19 CAR T-cell therapy with axicabtagene ciloleucel (axi-cel) induced ORR and complete response (CR) rates of 83 and 58%, respectively, among patients with R/R large B-cell lymphoma in the multicenter ZUMA-1 trial; responses were sustained among 39% of patients with a median follow up time of ∼27 months (10, 11). Based on these results, axi-cel was approved by the Federal Drug Administration (FDA) in October 2017 for R/R DLBCL, transformed follicular lymphoma, primary mediastinal large B-cell lymphoma, and high-grade B-cell lymphoma. Tisagenlecleucel (tisa-cel) was subsequently FDA approved for patients with large B-cell lymphoma based on the results from the JULIET trial demonstrating an ORR of 52% and CR rate of 40%; ongoing response at 6 months was observed in 33% of patients (12, 13). The TRANSCEND multicenter trial enrolled 344 patients with R/R large B-cell lymphomas who underwent apheresis for the production of lisocabtagene maraleucel (liso-cel). Among the patients included in the efficacy evaluation, the ORR was 73% and the CR rate was 53% (14). FDA approval for liso-cel is pending. The FDA most recently approved the first CAR T-cell product for adults with R/R mantle cell lymphoma, brexucabtagene autoleucel (bruxa-cel), based on the promising results of the ZUMA-2 trial which demonstrated responses in 93% of patients and CR in 67% (15). Roughly 57% of patients had sustained responses with a median follow up of 12.3 months (15). CAR T-cell therapy is undergoing active investigation in nearly all hematologic malignancies, with promising results emerging in Hodgkin lymphoma (16), multiple myeloma (17), and follicular lymphoma (18–20), among others.

While the high ORRs and notable proportion of patients achieving durable responses have been encouraging, there continues to be opportunity for improvement in treatment toxicity as well as response durability. Radiation therapy is a potential tool that, when coupled with CAR T-cell therapy, may offer the opportunity to improve outcomes.

Mechanistically, there is early evidence of potential synergy between radiation and CAR T-cell therapy, which provides additional impetus for investigation into their combined use. Potential complementary pathways that have been identified are mediated by effect of radiation on the tumor-microenvironment or in priming the local or systemic immune response. For example, preclinical studies show that low dose RT conditioning sensitizes antigen-negative tumor cells to CAR T-mediated apoptosis by making tumor cells susceptible to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated death (21). RT also enhances cytotoxic T-cell migration to irradiated areas, reverses T-cell exhaustion, and diversifies the T-cell receptor repertoire of tumor infiltrating lymphocytes (22). RT has complementary immunomodulatory activity through induction of increased major histocompatibility complex (MHC)-1 expression and liberation of antigens on irradiated cells, producing enhanced tumor-specific immunity via epitope spreading against irradiated and distant sites (23). In the following sections, we will discuss the potential role of radiation in CAR T-cell therapy with respect to the time at which radiation is administered relative to apheresis and CAR T-cell infusion. We propose that radiation therapy may have an important future role in tumor debulking, pre-infusion conditioning, and post-infusion rescue of residual or resistant disease.

## RADIATION AS BRIDGING THERAPY, BETWEEN APHERESIS AND CAR T INFUSION

During the period of CAR T-cell manufacturing, typically 3–4 weeks at minimum, patients may require bridging therapy to maintain control of disease and avoid the morbidity of symptomatic disease progression. Many of the initial clinical trials did not allow bridging therapy, however, in practice many patients require therapy for disease control prior to infusion of CAR T-cells. The optimal therapeutic regimen for bridging depends on the patient's treatment history and prior toxicities, however ideally enough time should be allowed between bridging and CAR T-cell infusion—a washout period—so as to allow recovery from adverse events, particularly if there is overlapping toxicity that may prompt treatment with steroids, which may blunt the CAR-T response.

Bridging therapy can include steroids for symptom control, radiation, chemotherapy or a combination. The results from several studies provide early evidence that RT as a bridging treatment can be safe and effective (**Table 1**). In an initial published report of RT as bridging prior to CD-19 CAR T-cell therapy from Moffitt Cancer Center by Sim et al. 12 patients were intended for RT bridging prior to axi-cel therapy (24). RT was initiated after apheresis in most patients (n = 10, 83%). Concurrent systemic therapy was administered to 7 patients (58%). Eleven patients went on to receive an axi-cel infusion. At a median follow up of 3.3 months, the ORR was 81.8% and CR was achieved in 45% (5 of 11 patients). Severe CAR T-cell toxicity defined as grade 3 or higher cytokine release syndrome (CRS) or immune effector cell-associated neurotoxicity syndrome [ICANS, previously termed CAR-T-cell-related encephalopathy syndrome (CRES)] occurred in 3 of 11 patients, consistent with the rates of this complication in the larger prospective studies. The authors also evaluated serum blood counts and observed neutropenia in 1/3 of patients after RT. White blood cell count and absolute lymphocyte counts also decreased slightly with RT. Anemia, thrombocytopenia, and neutropenia are also common after lymphodepleting conditioning therapy so the

**TABLE 1 |** Characteristics and results of initial reports of radiation therapy as bridging treatment prior to CD-19 CAR T-cell therapy among non-Hodgkin lymphoma patients.

| Study | Moffitt [Sim et al. (24)] | MDACC [Pinnix et al. (25)] | UPenn [Wright et al. (27)] |
|---|---|---|---|
| Patient population | R/R DLBCL, tFL | R/R DLBCL, tFL, PMBCL | R/R aggressive B-cell lymphoma |
| Product | Axi-cel | Axi-cel | Axi-cel ($n = 18$), tisa-cel ($n = 13$) |
| Total # Pts apheresed | 12 | 148 | 31 |
| Received CAR T, % | 92% ($n = 11$) | 84% ($n = 124$) | 100% ($n = 31$) |
| Received bridging therapy, % | 100% ($n = 12$) | 50% ($n = 62$) | NR |
| **Received RT bridging (%)** | | | |
| RT alone, % | 42% ($n = 5$) | 65% ($n = 11$) | 60% ($n = 3$) |
| CMT, % | 58% ($n = 7$) | 35% ($n = 6$) | 40% ($n = 2$) |
| Median RT dose, Gy (range) | 20 Gy (6–30) | 35 Gy (9–46) | 37.5 Gy (20–45) |
| RT fraction range | 2–4 Gy | 1.8–5 Gy | 2.2–4 Gy |
| **Timing of RT bridging** | | | |
| Before apheresis, % | 17% ($n = 2$) | 35% ($n = 6$) | NR |
| After apheresis, % | 83% ($n = 10$) | 65% ($n = 11$) | |
| **RT field size** | | | |
| Comprehensive, % | 42% ($n = 5$) | 53% ($n = 9$) | 60% ($n = 3$) |
| Focal, % | 58% ($n = 7$) | 47% ($n = 8$) | 40% ($n = 2$) |
| CRS grade ≥3, % | 8% ($n = 1$) | 6% ($n = 1$) | 0% ($n = 0$) |
| ICANS grade ≥3, % | 25% ($n = 3$) | 35% ($n = 6$) | 0% ($n = 0$) |
| Median follow up time (range) | 3.3 months (1.1–12) | 11.1 months (95% CI 9.9–12.3) | 12.3 months (9.8–19.9) |
| ORR | 81.8% | 100% for RT alone, 67% for CMT | 80% |
| CR rate | 45.5% | 82% for RT alone, 67% for CMT | 60% |
| 1-year PFS | NR | 44% RT alone, 25% CMT | 20% |
| 1-year OS | NR | 63% RT alone, 25% CMT | 80% |

*R/R, relapsed and refractory; DLBCL, diffuse large B-cell lymphoma; tFL, transformed follicular lymphoma, PMBCL, primary mediastinal B-cell lymphoma; axi-cel, axicabtagene ciloleucel; tisa-cel, tisagenlecleucel; Pts, patients, CAR T, chimeric antigen receptor T cell therapy; RT, radiation therapy; NR, not reported; CMT, combined modality therapy; Gy, gray; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; CI, confidence interval; ORR, overall response rate; CR, complete response; PFS, progression free survival; OS, overall survival.*

contribution of RT to these cytopenias is unclear. Ultimately this initial report demonstrated the safety and feasibility of an RT bridging approach.

In a retrospective series from MD Anderson Cancer Center (MDACC), the impact of bridging therapy was evaluated among 148 patients with R/R large B-cell lymphoma who underwent apheresis with the intention of delivering commercially available axi-cel therapy (25). In this study 16% of patients ($n = 24$) did not receive axi-cel therapy mainly due to progressive lymphoma. Among the 124 patients that received axi-cel therapy, 50% received bridging therapy including RT alone ($n = 11$), RT combined with systemic therapy ($n = 6$), or systemic therapy alone ($n = 45$). For all patients that received RT ($n = 17$), the median RT dose was 35 Gy. RT was administered after leukapheresis in 65% of patients ($n = 11$). In this study there was no difference in grade 3 or higher CRS or ICANS between any of the bridging or non-bridging cohorts. Interestingly, there was a trend toward decreased 1-year progression free survival (PFS) among patients who received any type of bridging therapy, at 29% compared to 44% in those who did not receive bridging treatment ($p = 0.06$). It is important to note, however, that patients who received bridging therapy (n=62) were more likely to have poor prognostic features at the time of apheresis such as Eastern Cooperative Oncology Group (ECOG) performance status of 2–3, international prognostic index (IPI) score of 3 or greater, bulky disease (defined as 10 cm or greater), and elevated lactate dehydrogenase (LDH). These characteristics have been shown to be associated with inferior survival outcomes in a large retrospective multicenter US Lymphoma CAR T Consortium study of R/R LBCL patients treated with standard of care axi-cel therapy (26). Therefore, it is unclear if bridging therapy itself is associated with shorter PFS or if confounding patient and disease factors are significantly contributing.

In the MDACC study, patients bridged with RT alone had a 1-year PFS of 44%, which was comparable to patients that did not receive bridging therapy (1-year PFS of 44%, $p = 0.52$). Both the cohort of patients bridged with RT combined with systemic therapy and the cohort bridged with systemic therapy alone had a 1-year PFS of 25%. The ORR and CR rates were higher for the patients that received single modality RT bridging at 100 and 82%, respectively, which were significantly higher than the ORR and CR rates for the systemic therapy alone cohort (67% ORR, $p = 0.03$ and 38% CR rate, $p = 0.01$), and compared favorably with the non-bridged cohort (82% ORR, $p = 0.13$ and 48% CR rate, $p = 0.04$). Taken together, this study demonstrated the efficacy of single modality RT as an effective bridging option for disease control prior to CAR T-cell therapy.

Similarly, Wright et al. conducted a retrospective study of 31 patients receiving tisa-cel or axi-cel for R/R aggressive B-cell lymphoma, of which 5 patients received bridging RT with a median RT dose of 37.5 Gy within 30 days of CAR T infusion (27). The study also included 26 patients that received non-bridging RT (delivered more than 30 days prior to CAR T infusion) or had no prior RT. No patients in the bridging RT group experienced grade 3 or higher CAR T related CRS or ICANS. Overall, CAR-T cell responses in the bridging RT and non-bridging RT groups were 80 and 64%, respectively. Lastly, Imber et al. presented their retrospective analysis of 11 patients with DLBCL or transformed follicular lymphoma who received bridging radiation prior to axi-cel ($n = 6$), JCAR017 ($n = 3$), tisa-cel ($n = 1$), or EGFRt/19-28z/4-1BBL CAR ($n = 1$) (28). The most common RT regimen was 20Gy in 5 fractions ($n = 6$). Local control was excellent but most ($n = 7$) had PD out of field prior to CAR infusion. Day 30 ORR was 100%, and of the 5 evaluable patients at day 90, 3 had continued complete metabolic response and 2 had PD (one with relapse in and out of RT treatment field

and one primarily out of field). RT did not seem to increase grade 3 or higher toxicities from CAR-T.

## RT Timing

Emerging data has suggested that oncologic therapies can impact the health and function of the autologous T-cells utilized for production of the CAR T-cell construct. T-cell fitness has been shown to be important for CAR T efficacy. CAR T composition and polyfunctionality was associated with both response and increased toxicity with a greater percentage of effector T-cells in responders vs. non-responders (29). Higher proportions of cycling CD4 T-cells and memory CD8 T-cells were associated with superior clinical response (30). Therapies that are likely to cause prolonged cytopenias, particularly in a patient who is older or less fit, could potentially have a greater negative effect on T-cell fitness. For instance, additional cycles of chemotherapy in patients with acute lymphoblastic leukemia, non-Hodgkin lymphoma (NHL), Hodgkin lymphoma, and acute myelogenous leukemia (AML) deplete naïve, effector memory T-cells and reduce T-cell proliferation capability (31). CAR T-cell fitness also varied by the number of prior lines of therapy received in the ZUMA-1 trial. Median CAR area under the curve (AUC) at Day 0–28 was substantially lower in patients who received 5 or more lines of prior therapy (11). Interestingly in an interim analysis of the ZUMA-12 trial that evaluated axi-cel therapy in the frontline setting for patients with high risk LBCL, the median peak CAR T cell levels and the median CAR T cell expansion levels were greater in the ZUMA-12 patient cohort as compared to the ZUMA-1 cohort (32). These observations suggest that exposure to multiple oncologic therapies can adversely impact the function of autologous cells used for CAR T-cell production. Bendamustine in particular may adversely affect T-cell numbers and function (33, 34).

The optimal timing of RT administration for patients that will undergo CAR T-cell therapy is currently unknown, however oncologists should ideally aim to deliver RT after apheresis. Caution should be exercised when RT is administered prior to T-cell collection. Even when limited RT fields that minimize bone marrow exposure are employed, RT has the potential to adversely impact circulating blood cells. Modeling studies have demonstrated that a single 2 Gy fraction of RT administered for a typical glioblastoma plan to the brain would deliver 0.5 Gy to 5% of the circulating blood cells and after 30 fractions, 99% of the circulating blood could receive at least 0.5 Gy (35). Lymphocytes are highly radiosensitive such that these low dose exposures could reduce lymphocyte counts and impair cell collection. Most importantly however, T-cell function could be impacted by even low dose RT, with subsequent effects on the autologous CAR T cell product that may impact treatment efficacy. However, while we generally recommend radiation after apheresis if possible to avoid impacting circulating T cells prior to apheresis, emerging evidence shows that local irradiation is not inherently immunosuppressive and large proportions of intratumoral T cells can survive clinically relevant doses of radiation (36). These tissue-resident memory T cells may be more radioresistant than circulating T cells and can mediate tumor control.

## RT Target and Dose

Overall, while the early data regarding RT bridging therapy is encouraging, the current data available include studies with limited patient numbers. Additional clinical validation and prospective studies are needed, particularly with regard to questions of optimal radiation dose and target. In the MDACC study, there was a trend toward improved PFS among patients treated with "comprehensive" RT that encompassed all known active sites of disease, compared to patients treated with "focal" RT with active lymphoma excluded from the RT field (25). Indeed, in that study, several patients treated with focal RT experienced relapse in sites that were active at the time of axi-cel infusion and not included in the bridging RT field. Comprehensive radiation may be most compelling for patients with disease in a contiguous or limited region(s) that can be safely encompassed within a radiation field without significant normal tissue toxicity (37). While the optimal dose of radiation is under investigation, early evidence indicates that hypofractionated radiation in clinical and pre-clinical settings can avoid lymphopenia and also result in recruitment of dendritic cells, priming of antitumoral CD8 T cells, and relatively low number of infiltrating regulatory T cells and thus may give us an early rationale for considering hypofractionation over conventional fractionation (38–40). To summarize the practical considerations when considering RT bridging, ideally RT should be delivered after apheresis if possible to minimize impact on T cell fitness, more comprehensive RT treatment may be helpful if it can be delivered safely with minimal toxicity, hypofractionated regimens can often be delivered safely and may result in a more favorable immune microenvironment, and minimizing toxicity that may require steroid treatment is advised.

## RT and Tumor Debulking

Decreased tumor burden prior to CAR T-cell infusion is associated with improved efficacy and decreased toxicity in R/R DLBCL (10). The overall response rate to axi-cel in ZUMA-1, as well as durability of response at 1 year, has been directly associated with lower tumor burden (10, 14, 41). Additionally, there was an association between decreased tumor burden and lower rates of treatment-related toxicity (Grade 3 or higher neurologic events and CRS). The relationship between tumor burden and efficacy was also observed in the US Lymphoma CAR T Consortium study of R/R LBCL patients treated with standard-of-care axi-cel, in which high LDH was the most significant predictive variable in multivariate analysis for shorter PFS and OS (26). High tumor burden was also associated with decreased event-free survival among adult ALL patients following CD19 CAR T-cell therapy (42). Finally, among 96 large B-cell lymphoma patients treated at Moffitt Cancer Center with commercially available axi-cel, elevated tumor burden as identified by high metabolic tumor volume (MTV) on PET-CT was associated with significantly shorter PFS and OS (43). These studies support the notion that optimal tumor debulking can improve CAR T outcomes. RT is a useful tool that can facilitate effective tumor debulking, particularly among patients with highly chemorefractory disease, however it is unknown if debulking with RT prior to CART infusion improves outcomes.

## RADIATION AS CONDITIONING THERAPY

There is early preclinical evidence that low dose radiation induces tumor cell susceptibility to CAR T mediated killing via TRAIL-mediated death (21). Even at ultra-low radiation doses of 1.8 to 2 Gy, ribonucleic acid (RNA) sequencing analysis of radiation-exposed tumors revealed the transcriptional signature of cells highly sensitive to TRAIL-mediated apoptotic death. If tumor cells are sensitized to CAR T-cell mediated killing through enhanced apoptosis, there is a rationale to investigate the potential role of low dose total nodal or total body irradiation or perhaps even targeted radionuclide approaches (44) as part of the CAR T conditioning regimen. This concept has not yet been clinically investigated, however is supported by reports of disease progression following CAR T therapy only in areas that harbored disease before CAR T infusion that were not included in the radiation field (25).

## RADIATION THERAPY AFTER CAR T-CELL RELAPSE

Radiation is an attractive early salvage option for patients after disease relapse or progression to CAR T-cell therapy, particularly if it potentiates CAR T-cell mediated death. In a case of a patient with R/R multiple myeloma who received steroids and palliative radiation to the spine for cord compression on days 6–20 after B-cell maturation antigen (BCMA) CAR T-cell infusion, there was a peak in T-cell receptor repertoire expansion, as well as interleukin-6 (IL6) and C-reactive protein (CRP), following RT at a time point later than would be expected with CAR T therapy alone (45). The patient had a complete systemic response, and, despite steroids, there was BCMA CAR T-cell persistence, raising the intriguing possibility that RT may influence both the local and distant treatment response. An early retrospective experience of radiation treatment in the salvage setting for non-Hodgkin lymphomas after CAR T-cell therapy shows that this approach may also be effective in aggressive B-cell lymphoma. In a review of 14 patients treated at Memorial Sloan Kettering Cancer Center with salvage radiation post-CAR T progression, Imber et al. reported median OS after RT of 10 months, with 3 patients bridged to allogeneic transplantation and all patients alive without evidence of disease at the time of analysis (46).

Perhaps an even more novel, personalized approach to selecting patients for radiation treatment after CAR T-cell therapy is warranted. A study of early molecular response (EMR) in R/R DLBCL patients treated with axi-cel revealed that patients who achieved an EMR, defined as a >5-fold reduction in measured plasma-derived cell free DNA (cfDNA) as early as day 7 after infusion, had increased durability of response (30). Patients with an EMR had a 75% CR rate at 3 months compared to 0% CR rate at 3 months for those without an EMR. For those patients who fail to achieve an EMR, there may be an opportunity for early radiation treatment in an effort to reduce disease progression in this higher risk patient population. Whether molecular response can be used to help select patients who may benefit from early salvage radiation treatment merits further investigation.

## CONCLUSIONS

CAR T-cell therapy has revolutionized the treatment approach to patients with relapsed/refractory hematologic malignancies, however, there remains opportunity for improving outcomes and toxicity. Radiation therapy can play an important role in combined modality treatment for patients undergoing CAR T therapy in various clinical settings. Future research frontiers include investigation of exciting hypotheses including radiation priming of CAR T-cell mediated death, radiation debulking to reduce tumor burden, and selection of patients at high risk of CAR T failure for early radiation salvage.

## AUTHOR CONTRIBUTIONS

PF and CP conceived and designed the manuscript. All authors contributed to the writing of the manuscript.

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# Optimizing the Treatment Schedule of Radiotherapy Combined With Anti-PD-1/PD-L1 Immunotherapy in Metastatic Cancers

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Immune checkpoint inhibitors (ICIs) targeting programmed cell death protein-1 (PD-1), and programmed cell death ligand-1 (PD-L1) have been approved for a variety of malignant tumors and are widely used to treat patients with metastatic disease. However, the efficacy of PD-1 inhibitors is limited due to tumor heterogeneity, high tumor burden, and "cold" tumor microenvironment. Radiotherapy can improve the anti-tumor effects of PD-1/PD-L1 inhibitors in various ways. As a new radiotherapy method, stereotactic body radiotherapy (SBRT) or hypofractionated radiotherapy (HFRT) provides higher doses per fraction to the target lesions, thus achieving immune activation effects and overcoming tumor resistance to anti-PD-1/PD-L1 treatment, which significantly improves the local and distant control of tumors. However, for different metastatic situations, radiotherapy plays different roles in the combination therapy. In oligometastatic status, radiotherapy can be used as a local radical treatment aiming to eliminate cancers in cooperation with systemic PD-1 inhibitors. In other circumstances, like bulky metastasis or multiple metastatic tumors, radiotherapy can be used as adjuvant to systemic immunotherapy. This review focuses on the underlying mechanisms and optimization strategies for the combination of radiotherapy and anti-PD-1/PD-L1 therapy in metastatic disease.

**Keywords: metastatic cancer, PD-1/PD-L1 inhibitor, radiotherapy, *in-situ* tumor vaccination, biological response modifiers**

## INTRODUCTION

Targeting programmed cell death protein-1(PD-1)/programmed cell death ligand-1 (PD-L1) is one of key achievements in cancer immunotherapy. PD-1/PD-L1 inhibitors have been approved for the treatment of many kinds of tumors, such as melanoma, renal cell carcinoma, lung cancer, esophageal cancer, head and neck cancer, bladder cancer, breast cancer and so on (1). However, the response rate of most tumors treated with PD-1/PD-L1 inhibitors as monotherapy is limited to 15–25% (2). The therapy is even ineffective in some tumors, such as microsatellite stable (MSS) colorectal cancer and pancreas ductal adenocarcinoma (2, 3).Therefore, considerable interest is being directed to use combinational treatments to amplify immunomodulatory effects and produce a synergistic effect to anti-PD-1/PD-L1 therapy (4).

Ionizing radiation can enhance the immune response by directly acting on tumor cell DNA, generating *in situ* tumor vaccine effects, and producing cytokines, which can crosstalk with immune cells, thus changing tumor microenvironment (5). Although "abscopal effect" has been identified more than 67 years, it is very rare to see this phenomenon caused by radiotherapy alone (6). For patients with multiple metastatic tumors, emerging data suggested that single site irradiation was not sufficient enough to boost synergistic effect (7). Over the years, many clinical trials have been launched aiming to examine the safety and efficacy of radiotherapy in combination with immunotherapy. In metastatic cancers, radiotherapy can be used not only as a local radical therapy in some oligometastatic conditions, but also as a sensitizer to PD-1/PD-L1 inhibitors in other circumstances like bulky disease or multiple metastases. However, the optimal radiation doses, fraction size, appropriate timing, irradiated sites, and numbers of irradiated targets have not yet been established. In this study, we mainly discuss the mechanisms and treatment strategies for radiation therapy in combination with PD-1/PD-L1 inhibitors.

## THE POTENTIAL MECHANISMS OF RADIATION ON IMMUNOMODULATION

### The Direct Killing Effect of Ionizing Radiation on Tumor Cells

The ionizing radiation affects the tumor cell DNA, causing DNA double-strand breaks and releasing into the cytoplasm (8). Cytoplasmic DNA can activate cyclic GMP-AMP synthase (cGAS) to synthesize cyclic GMP-AMP (cyclic GMP-AMP, cGAMP) and further activate stimulator of interferon genes (STING), which can promote type I interferon (IFN-I) synthesis, thus stimulating dendritic cells (DC) and T cell activation (9). However, the activation of the cGAS/STING pathway is closely related to the radiation dose. Preclinical experiments have shown that hypofractionated (8 Gy×3 fractions) but not ablative radiation (20 Gy single dose) can activate this pathway and induce an abscopal effect when combined with immune checkpoint inhibitors (ICI). When a single dose is 12–18 Gy, the expression of DNA exonuclease Trex1 is significantly increased, resulting in a decrease of cytoplasmic double-stranded DNA, which is not conducive for activating immune response (10).

### Ionizing Radiation Coverts Tumor Into an *in-situ* Vaccine

Radiotherapy is shown to cause tumor cell death associated with releasing tumor-associated antigens (TAAs), danger signals and cytokines which are highly immunogenic and related with initiation of an *in-situ* vaccine (11). The ionizing radiation can promote tumor cells releasing TAAs, especially tumor neoantigens (TNAs), into blood and induce immunogenic cell death (ICD) (12, 13). ICD is a form of regulated cell death that elicits an adaptive immune response and relies upon the antigenicity and adjuvanticity of dying tumor cells (12). TNAs have poor structure homology to self-epitopes and are recognized by self-reactive T cells (12). Accumulating evidence showed the favorable immunotherapy response in patients with high tumor mutation burden (TMB) was in consistent with more TNAs found in this type of cancers (14). Therefore, enhancing tumor antigenicity by inducing TAAs releasing could promote immunogenic response and efficacy of PD-1/PD-L1 treatment (14–16). Radiotherapy can increase the expression of TAAs and release TAAs by causing tumor cell damage, and further promote antigen cross-presentation by DCs and stimulate the activity of antigen-specific cytotoxic $CD8^+$T cells, thus eliciting long-term anti-tumor efficacy when combined with PD-1/PD-L1 inhibitors (17).

Ionizing radiation can also promote the tumor cells to increase the expression or release of danger-associated molecular patterns (DAMPs) and cytokines which are associated with initiation of adaptive immunity. Several ICD-associated DAMPs and cytokines are found to play important roles in ionizing radiation induced ICD. Calreticulin (CRT) is a ubiquitous calcium-binding protein in the endoplasmic reticulum which can provide DC with a phagocytic signal allowing DC to recognize dead cells and phagocytose (18). Human high mobility group box 1 (HMGB1) is another DAMP that can exert a powerful immunomodulatory effect by binding Toll-like Receptor (TLR)-4 and TLR-9. HMGB1 can further promoting DC maturation and migration to lymph nodes, cross-presenting antigens to naive T cells (19, 20). Adenosine triphosphate (ATP) binds to the purinergic receptor P2X7, which increases the expression of inflammatory cytokines and chemokines, and induces the phagocytosis and inflammasome activation of DC (9, 18). Subsequently activated DC can secrete interleukin (IL)-1β and promote the activation of interferon-gamma-producing $CD8^+$T cell (11). Cytokines like IFN-I, which is produced by activated STING/TBK/IRF3/ NF-κB signaling pathway, mediates the anti-tumor effect of DC (9, 18).Tumor cell nucleic acid derivatives and extracellular annexin A1 have important roles in initiating ICD and affect the strength and durability of adaptive anti-tumor immune response (21, 22). Other immunostimulants like heat shock proteins, chemokines also play important roles in priming adaptive immunity (23–25). Herein, ionizing radiation can induce ICD and convert tumors into an *in-situ* personalized vaccine, providing immunostimulatory effects (**Figure 1**).

### Ionizing Radiation Modulates the Tumor Immune Microenvironment

The presentation and recognition of tumor-associated antigens are very important for initiating adaptive immune response, however, a microenvironment with a high density of tumor-infiltrating lymphocytes (TILs) is also essential for eradicating tumor cells. Smyth et al. suggested the tumor immune microenvironment can be categorized into four types according to the infiltration of $CD8^+$T cells and the expression of PD-L1 (29), and in 2019 they reclassified in gene level based on a T cell inflammatory gene signature and TMB (30). Turan et al. suggest that three landscapes best define the cancer microenvironment: immune-active, immune-deserted, and immune-excluded landscape (31). Among them, the tumors

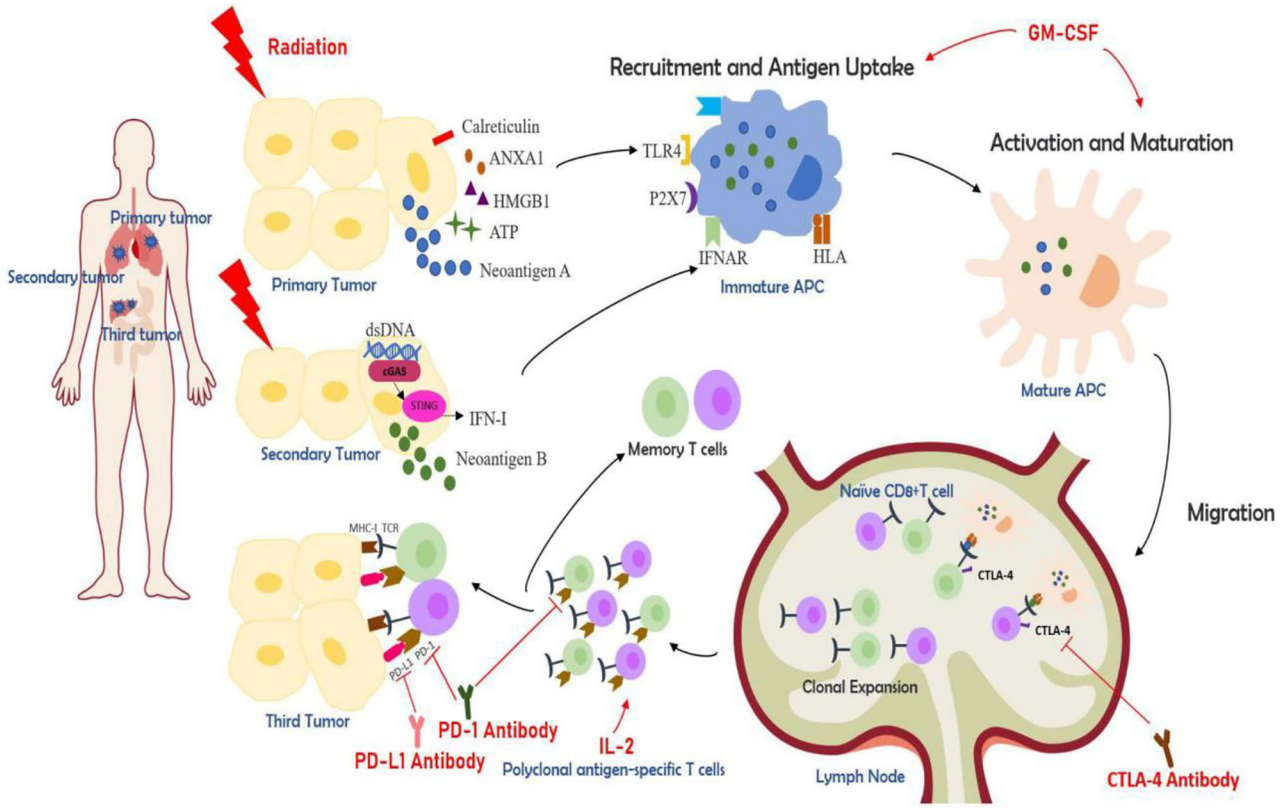


**FIGURE 1 |** The partial mechanisms of multisite radiotherapy combined with immune checkpoint inhibitors (ICIs) and biological response modifiers (GM-CSF or IL-2). Radiotherapy induces immunogenic cell death (ICD), which exposes and releases danger-associated molecular patterns (DAMPs) like calreticulin, HMGB1, ATP, ANAX1, and similar (12). cGAS-STING pathway is activated by the cytolytic double-strand DNA and results in the release of IFN-I (10). Radiation can also generate tumor neoantigens. Multisite radiotherapy can overcome the insufficient tumor-associated antigen (TAA) exposure caused by tumor heterogeneity (7). ICDs can recruit antigen-presenting cells (APC) like dendritic cells (DC). APCs can take up antigens and further be activated, which can be augmented by GM-CSF. DCs then migrate to lymph nodes, presenting antigens to T cells and prime a cytotoxic T lymphocyte (CTL)-mediated immune activation (26, 27). The activated CTLs initiate clonal proliferation and then travel to the irradiated lesions or distant tumor sites, exerting killing effects. The cytokine IL-2 is essential for the proliferation, differentiation, and survival of T cells (28). CTLA-4 antibody, PD-1 antibody, and PD-L1 antibody, known as ICIs, can increase CTL activation and boost the synergistic anti-tumor effects.

with immune desert microenvironment are also called "cold" tumors and generally resistant to ICIs (32). The "immune desert" microenvironment is characterized by the presence of a small amount of TIL and a large number of type II tumor-associated macrophages (TAM), myeloid suppressor cells (MDSC), regulatory T cells (Treg), and other suppressive immune cells (33). Both tumor cells and suppressive immune cells can produce molecules promoting tumor growth, such as vascular endothelial growth factor (VEGF), IL-10, transforming growth factor (TGF)-β, adenosine, and prostaglandin E2. These molecules can prevent DC activation and inhibit the activation of cytotoxic T cells (CTLs) and nature killer (NK) cells (34).

Ionizing radiation can modulate the tumor microenvironment and overcome the barriers of immune suppression. Chemokines like chemokine (C-X-C motif) ligand (CXCL)-9, CXCL-10, CXCL-16 are upregulated after irradiation. These chemokines have an important role in the recruitment of T cells into local tumor microenvironment and activation of T cells (35). Ionizing radiation can also convert TAM into TAM-1, which can secrete inducible nitric oxide synthase (iNOS), upregulate the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecules (VCAM) to facilitate lymphocytes infiltrating into tumor tissues (36, 37). Ionizing radiation can directly improve the killing ability of CTLs and NK cells. Tumors inhibit host immune response by downregulating major histocompatibility complex I (MHC-I), a key molecule of $CD8^{+}$T cell recognition, as well as secreting negative immune factors and recruiting immunosuppressive cells (17). However, radiotherapy can increase the expression of MHC-I and II molecules, Fas death receptors and stress ligands on tumor cells surface, which stimulates T cells and NK cells medicated cytotoxicity (38–40). Therefore, ionizing radiation can promote the infiltration of immune cells into the tumor microenvironment and directly improve the recognition and killing ability of T cells and NK cells, which potentially boosting the systemic efficacy of ICIs.

## EXPLORATION THE BEST MODE OF RADIOTHERAPY AND PD-1/PD-L1 INHIBITORS

Ionizing radiation is a double-edged sword. In addition to immune activation effects, it also has immunosuppressive effect (41). DNA double-strand breaks caused by ionizing radiation can activate ATM/ATR/Chk1 kinase signaling pathway, thereby up-regulating PD-L1 expression and inhibiting T cells activity (42, 43). Ionizing radiation can promote tumor cells to release transforming growth factor-β (TGF-β), IL-33, and other cytokines to increase the recruitment of Tregs (44). CD73 (ecto-5'-nucleotidase), which can be upregulated by ionizing radiation, can generate adenosine and increase Tregs in the tumor microenvironment (45). Tregs can induce effector T cells apoptosis, inactivation, dormancy, and inhibit the functions of B cells, NK cells, DC and macrophages (34). Therefore, it is not only necessary to consider how to exert the optimal immune activation effect of ionizing radiation but also how to avoid immunosuppressive effects when combining with anti-PD-1/PD-L1 therapy.

### Exploration of the Dose and Fraction Size of Radiotherapy

So far, the optimal dose and fraction schedule of radiotherapy to sensitize PD-1/PD-L1 inhibitors has not been determined. Many preclinical studies investigated the potential impacts on the immunity with different radiation doses. Kulzer et al. (46) found that hypofractionated treatment (5 Gy×3 fractions) could enhance tumor necrosis factor (TNF)-α, IL-6, and IL-8 levels comparing to conventional fractionated radiotherapy (2 Gy×5 fractions), suggesting that hypofractionated radiotherapy (HFRT) may promote the maturation and activation of antigen-presenting cells, especially DC. Lan et al. (47) found that HFRT could reduce MDSC infiltration into the tumor microenvironment in mice models. When combined with PD-L1 antibody, a higher tumor control effect was observed in HFRT treated mice comparing to those treated with conventional schemes (47). In fact, radiation doses exceeding 5 Gy per fraction can effectively and directly destroy tumor cells and render these cells' elements for *in-situ* vaccination (5, 20, 48). On the other hand, the conventional schedules are more likely to cause systemic lymphopenia which affects immunotherapy efficacy and associated with poor prognosis (49–51).

However, a higher single dose per fraction is not always associated with a higher immune activation effect. Evidence showed that 7.5–10 Gy×2–3 fractions could stimulate immune response with lower level of Tregs and achieve a better tumor control effect comparing to 15 Gy×1 fraction (52). Studies have also found that >12 Gy irradiation can inhibit the STING pathway and down-regulate IFN-I by up-regulating Trex1, which can decompose cytoplasmic double-stranded DNA. In contrast, the free double-stranded DNA is obviously elevated at a dose of 8–12 Gy, and the STING pathway is activated (10). Filatenkov et al. (53) found that hypofrationated irradiation (15 Gy×2–3 fractions) can reduce MDSCs when compared with a single dose fraction mode (30 Gy×1 fraction), thereby promoting higher activation of T cell function.

Some clinical trials have shown the clinical activity and safety of combination radiotherapy and PD-1/PD-L1 inhibitors in metastatic tumors. In the phase I trial conducted by Luke et al. (54), the 10–15 Gy×3 fractions scheme combined with pembrolizumab showed safe antitumor activity. The overall response rate (ORR) was 13.6% and <10% subjects experienced ≥ grade 3 adverse reactions. A phase II trial, PEMBRO-RT, examined the effect of 8 Gy×3 fractions radiotherapy combined with pembrolizumab in advanced metastatic non-small cell lung cancer (NSCLC). Comparing to the single pembrolizumab treatment without SBRT in control group, SBRT with pembrolizumab showed 36% ORR at 12 weeks (control 18%, $p = 0.07$), median progression free survival (PFS) of 6.6 month (control 1.9 month, $p = 0.19$) and median overall survival (OS) 15.9 month (control 7.6 month, $p = 0.16$) (55). In MDACC trial, where pembrolizumab was concurrently given with SBRT (50 Gy in four fractions) or HFRT (45 Gy in 15 daily fractions) as experimental group, no benefits in median PFS or OS were observed when compared with pembrolizumab without radiation therapy (56). But the pooled analysis of PEMBRO-RT and MDACC trials demonstrated that adding radiotherapy to pembrolizumab provided significant survival benefit (57). Moreover, subgroup analysis showed that 50 Gy in four fractions were significantly associated with better PFS (57), which needs further validation by a randomized phase III trial. The most common adverse events (AEs) in both trials were fatigue, respiratory related symptoms, rash, pruritus and weight loss. Generally, the AEs were mild and self-limiting in patients received pembrolizumab and radiotherapy, comparable with the safety profile in patients received pembrolizumab alone.

Radiotherapy schedules for patients with oligometastasis or multiple metastasis need tailored. The ESTRO/EORTC consensus on oligometastasis recommends combing local radical treatment with systematic treatment to eliminate the disease. Thorough local treatment can reduce the resistance to current systemic treatment and restore sensitivity to systemic therapy by eradicating metastasis (58). In oligometastatic tumors, the SABR-COMET study showed that radical or nearly radical SBRT (30–60 Gy in 3–8 fractions, 16–24 Gy in 1 fraction allowed for intracranial lesions) had significant OS benefits (the 5-year OS rate was 42.3 vs. 17.7%) compared to palliative treatment (8 Gy in 1 fraction or 30 Gy in 10 fractions) in the control group (59, 60). However, the number of patients with grade 2 or higher treatment-related toxicities was increased to 29% following the use of SABR compared with 9% in the control group. Therefore, for patients with multiple metastases, the accessibility and safety of radical treatment must be considered. Palliative radiotherapy may be more suitable for reducing tumor burden and enhancing the sensitivity of systemic therapy. Further research needs to investigate the combination of palliative HFRT and ICIs in patients with multiple metastases in order to determine the optimal dose and fraction size to enhance tumor response to immunotherapy without increasing treatment related toxicity. Meanwhile, radiation therapy schedule can be individualized based on different tumor pathological types, tumor sizes,

tumor locations, metastatic states, intrinsic radiosensitivity, and host characteristics (61).

In the trials of oligometastatic disease listed in **Tables 1**, **2**, radiotherapy was administered according to the lesion and clinical condition location, trying to achieve a radical dose [biologically effective dose (BED)>100 Gy] with 8–12 Gy per fraction in most of the trials. The palliative dose schedules of 6–15 Gy×3–5 fractions or a single dose of 20 Gy were given for multiple metastatic cancers. These trials helped us to determine the doses in different tumors and metastatic conditions in the future.

## Exploration of the Timing Schedule of Combination Therapy

Selecting an appropriate timing for combining radiotherapy and anti-PD-1/PD-L1 therapy is also crucial when designing the scheme. Preclinical data suggested that the PD-L1 expression significantly increased after irradiation. Higher level of PD-L1 expression was found at a single dose of 10 Gy comparing to 5 Gy, and at 48 h after radiation comparing to at 24 h (36). Dovedi et al. (65) found that highest expression of PD-L1 on tumor cells was at 3 days after radiotherapy, and PD-1 on T cells was upregulated 1–7 days after radiotherapy. *In vivo* preclinical data also suggested that concurrent anti-PD-1/PD-L1 antibodies administration with conventionally fractionated RT had longer survival time than those treated sequentially (65). However, there are other evidences suggested that different timing of radiation therapy and ICI therapy (concurrent or sequentially) can also produce synergistic effects (66–68). Herter-Sprie et al. showed that there was no significant difference among concurrently PD-1 antibody administrated with RT, and sequentially giving RT at 5 or 7 days after PD-1 antibody administration (69). Therefore, from the perspective of preclinical data, there are different results even some contradictions about the timing schedule, and there is still no conclusion of the optimal timing.

Although the optimal timing of combination of RT and ICI is not determined, this combinational therapy shows notable efficiency. In metastatic NSCLC, the experimental group given pembrolizumab within 1 week after SBRT showed better clinical effects compared with pembrolizumab administrated alone in the control group in PEMBRO-RT study (55). A phase I study for solid metastatic tumors showed that sequential administration of pembrolizumab after SBRT at multiple metastatic lesions achieved 13.2% in ORR and 13.5% abscopal effect in non-irradiated metastases (54). Regarding to investigation of best combinational timing, PACIFIC study showed stage III unresectable NSCLC patients who received durvalumab within 14 days after concurrent chemotherapy and radiotherapy (CCRT) had longer PFS than those received durvalumab over 14 days after completion of CCRT (70, 71). Similar result was reported that melanoma patients with brain metastasis who received PD-1 inhibitor and CTLA-4 inhibitor treatment within 4 weeks after stereotactic radiosurgery (SRS) had better results compared to those received PD-1 inhibitor and CTLA-4 inhibitor over 4 weeks after SRS (18). These trials implied that patient receiving PD-1/PD-L1 inhibitors immediately after radiotherapy might have better clinical outcome. However, there were several arguments. A phase I clinical study showed that ORR of simultaneous SBRT treatment after 3 cycles of PD-1 inhibitor was significantly better than that of SBRT followed by PD-1 inhibitor sequential treatment (72). The COSINR phase I trial evaluated concurrent or sequential ipilimumab, nivolumab, and SBRT in patients with stage IV NSCLC and found that the median PFS was 5.9 months in the sequential arm and 6.2 months in the concurrent arm, which showed no significant differences in two different timing schedule (73).

The safety and toxicity of radiotherapy and anti-PD-1/PD-L1 therapy are of great concern. Pembrolizumab given concurrently with SBRT or HFRT confirmed no clinical benefits in the MDACC trial but two patients had grade 4 adverse event which might be related to the concurrent scheme (56). Anti-PD-1/PD-L1 therapy may also lead to radiation recall pneumonitis (74). In the clinical studies listed above, it seemed the time intervals between radiotherapy and anti-PD-1/PD-L1 therapy were not associated with the rate of severe pneumonitis. Nonetheless, a study presented at the ESMO 2020 congress suggested that the application of anti-PD-1 drugs before or during thoracic radiotherapy increases the incidence of radiation pneumonitis compared to administration after radiotherapy (60 vs. 28%, $p = 0.01$) (75). Bang et al. showed higher overall toxicity when radiation was administered within 14 days of immunotherapy (39 vs. 23%, $p = 0.06$) but no significant differences in grade 3 AEs (76). These data seems that concurrent scheme has more adverse reactions and inferior effectiveness than sequential therapy, but it is still controversial due to the lack of randomized controlled trials. However, it is notable that the overall toxicity may also related with high BED, irradiated volumes and irradiated sites (77). Future studies are needed for better understanding of the efficacy and safety of different schedules and defining suitable patients for the options listed in **Tables 1**, **2**.

## Exploration of Appropriate Volume and Numbers of Irradiated Targets in Combination Therapy

In 2019, Chang et al. suggested using multisite radiotherapy for metastatic sites instead of single-site irradiation to boost the synergistic effect (7). Considering the heterogeneity among different metastatic sites, only one lesion irradiation in patients with multiple metastases might not be sufficient to expose new TAAs and promote immune cell infiltration to all metastatic sites. In addition, the increased tumor burden may lead to a decrease in the efficacy of PD-1 inhibitors (11, 78).Therefore, multisite irradiation can obviously decrease tumor burden, and consequently restore the tumors' sensitivity to anti-PD-1/PD-L1 therapy. However, multisite treatment undoubtedly increases the irradiated volume and adverse reactions. Treatment-related lymphopenia was associated with a less effective response to anti-PD-1/PD-L1 therapy and inferior survival (49, 50, 79). Therefore, it may be helpful to maintain the number and function of immune cells so that they can be recruited to initiate anti-tumor immune response. This might be

**TABLE 1 |** Trials testing radiotherapy in combination with PD-1/PD-L1 in advanced metastatic cancers that allowed only one irradiated lesion or did not mention the irradiated numbers.

| NCT number | Phase | Tumor type | RT regimen | PD-1/PD-L1 inhibitors | Treatment schedule timing | Trial design (arms) | Primary outcome | Status |
|---|---|---|---|---|---|---|---|---|
| NCT03988647 | II | Metastatic Merkel cell carcinoma | 9 Gy × 3f or 4–6 Gy × 5f | Pembrolizumab | RT will be given between the first and second cycles of immunotherapy | Single group | ORR | Recruiting |
| NCT03220854 | II | Advanced solid tumors | 6–12 Gy × 3–5f | Humanized anti-PD-1 monoclonal antibody | PD-1 inhibitor will be started after last SRT fraction (on same day) | Single group | Proportion of patients with improved disease control | Active, not recruiting |
| NCT03548428 | II | Oligometastase in Sarcoma | SBRT:3 to 5 fractions depending on tumor size | Atezolizumab | Not mentioned | Arm A: SBRT+Atezolizumab<br>Arm B:SBRT | PFS | Recruiting (62) |
| NCT02843165 | II | Advanced metastatic disease | 9.5 Gy × 3 allowed reduction (6 Gy × 3 Minimum Dose) | Anti-CTLA-4 and anti-PD-1/PD-L1 antibodies | SBRT will be delivered within 1–21 days of the start of Cycle 1 of the CBI | Arm A: CBI plus SBRT<br>Arm B: CBI | ORR | Recruiting |
| NCT04166734 | I/II | Advanced malignant pleural mesothelioma | 10 Gy × 3f | Pembrolizumab | Pembrolizumab will be given prior to SBRT | Sequential assignment Non-randomized | AE | Not yet recruiting |
| NCT03436056 | I/II | Metastatic NSCLC | 10 Gy × 3f<br>18 Gy × 3f dosed at the maximum tolerated dose | Pembrolizumab | Pembrolizumab will be given prior to SBRT | Sequential assignment Non-randomized | AE.To establish the recommended dose | Active, not recruiting |
| NCT02992912 | II | Metastatic tumors (colorectal cancer, NSCLC, RCC, sarcoma) | 15 Gy × 3f | Atezolizumab | Not mentioned | Single Group | PFS | Recruiting |
| NCT03115801 | II | Metastatic genitourinary cancers | 10 Gy × 3f | Nivolumab<br>Atezolizumab<br>Pembrolizumab | PD-1/PD-L1 inhibitor is administered on the day of radiation (Day 1) | Arm A:immunotherapy alone Arm B:Radiation and immunotherapy | ORR | Active, not recruiting |
| NCT02400814 | I | Stage IV NSCLC | Total of five fractions | MPDL3280A | Arm A:concurrent<br>Arm B:induction cohort<br>Arm C:sequential cohort | Arm A:SBRT Beginning on day 1 of course 1<br>Arm B:SBRT Beginning on day 1 of course 3<br>Arm C:SBRT prior to anti-PD-L1 | To determine best administration schedule of MPDL3280A and SBRT | Active, not recruiting |
| NCT04098432 | I/II | Locally advanced unresectable pancreatic adenocarcinoma | 8 Gy × 4f | Nivolumab | Nivolumab is given after SBRT | Single Group | AE | Recruiting |
| NCT03509584 | I | Pretreated advanced stage non-small cell lung cancer | 8 Gy × 3f | Nivolumab | Not mentioned | Arm I:HFRT+ Nivolumab<br>Arm II: HFRT+ Nivolumab + ipilimumab | AE | Recruiting |
| NCT04306926 | II | Advanced oligometastatic NSCLC | Give according to the location of the lesion and clinical condition. | TQB2450 | SBRT 3 days before TQB2450 | Single group | PFS | Not yet recruiting |

*(Continued)*

**TABLE 1** | Continued

| NCT number | Phase | Tumor type | RT regimen | PD-1/PD-L1 inhibitors | Treatment schedule timing | Trial design (arms) | Primary outcome | Status |
|---|---|---|---|---|---|---|---|---|
| NCT02599779 | II | TKI refractory metastatic kidney cancer (mRCC) patients | Dose and duration dependent on body site | Pembrolizumab | Arm-A: SBRT will be given at the time of progression on pembrolizumab and pembrolizumab will be continued. Arm B: SBRT will be given before the 2nd course of pembrolizumab and pembrolizumab will be continued. | Arm A: SBRT will be given at the time of progression on pembrolizumab and pembrolizumab will be continued. Arm B: SBRT will be given before the 2nd course of pembrolizumab and pembrolizumab will be continued. | PFS | Recruiting |
| NCT04547452 | II | Advanced metastatic HCC | 7–10 Gy × 5–8f | Sintilimab | The first course of sintilimab will be given within 4–6 weeks after completion of SBRT. | Arm A: Sintilimab and SBRT Arm B:Sintilimab | PFS | Recruiting |
| NCT03035890 | I/II | Metastatic NSCLC | 8–15 Gy × 3f 6–10 Gy × 5f | Nivolumab Pembrolizumab Atezolizumab | Concurrent | Single group | ORR | Active, not recruiting |
| NCT03122496 | I | Metastatic anaplastic thyroid cancer | 9 Gy × 3 f | Durvalumab | RT is given within 2 weeks after the completion of cycle 1 of durvalumab and tremelimumab | Single group | OS | Active, not recruiting |
| NCT03867175 | III | Metastatic lung cancer | 3–10 treatments of SBRT | Pembrolizumab | Not mentioned | Arm A:SBRT and Pembrolizumab Arm B:Pembrolizumab | PFS | Recruiting |
| NCT02826564 | I | Metastatic urothelial cancer | SBRT | Pembrolizumab | Arm A:Sequential Arm B:Concurrent | Arm A:SBRT prior to pembrolizumab Arm B:SBRT concurrent with pembrolizumab | AE DLT | Completed (63) |
| NCT03101475 | II | Colorectal cancer liver metastases | 10 Gy × 3 f | Durvalumab | SBRT is started 8–14 days after first dose of immunotherapy | Single group | ORR | Recruiting |
| NCT04167657 | II | Advanced NSCLC | 6 Gy × 5f | Sintilimab | Sintilimab is started no later than 3 weeks after radiation. | Single group | ORR | Recruiting |
| NCT04361162 | II | MSS pancreatic cancer | Not mentioned | Nivolumab | Concurrent | Single group | ORR | Recruiting |

*We searched "radiation and PD-1/PD-L1 inhibitors" in the clinicaltrials.gov database to identify studies with the following statuses: not yet recruiting, enrolling by invitation, recruiting, active, not recruiting, completed, and unknown status (Clinical Trial). A total of >60 trials were found. We identified the trials using radiotherapy with PD-1/PD-L1 inhibitors in advanced metastatic cancers (date of final query, 25 November 2020). Then we searched "radiation and immunotherapy" in the clinicaltrials.gov database as above. A total of >150 trials were found. We identified the studies of radiotherapy with PD-1/PD-L1 inhibitors in advanced metastatic cancers (date of final query, 25 November 2020). This list shows the trials that allowed only one irradiated lesion or did not mention the irradiated numbers. This list should not be considered comprehensive or exhaustive.*

*SABR, stereotactic ablative radiotherapy; PFS, progression free survival; CBI, checkpoint blockade immunotherapy; ORR, objective response rate; SBRT, stereotactic body radiotherapy; RCC, renal cell carcinoma; AE, adverse events; DLT, dose limiting toxicities; RT, raidotherapy; TKI, tyrosine kinase inhibitor; NSCLC, non-small-cell lung cancer; SCC, squamous cell carcinoma; HNSCC, head and neck squamous cell carcinoma; ICI, immune checkpoint inhibitor; HCC, hepatocellular carcinoma; OS, overall survival; ACC, adenoid cystic carcinoma; MSS, microsatellite stability.*

achieved through decreasing the exposure of circulating blood volume and avoiding irradiation at lymphoid tissue or medullary tissue, such as bone marrow, spleen, thymus, and lymphatic vessels (11).

For patients with oligometastatic disease, defined as number of metastases equal or <5 and restricted to no more than 2 organs, several studies have shown that active local treatment for all metastases can significantly prolong patients' OS with tolerable side effects (58–60). The phase II clinical study done by Bauml et al. (80) showed median PFS of 18.7 months (PEMBRO-RT: 6.6 months) and median OS of 41.6 months (PEMBRO-RT: 15.9 months) in patients with oligometastatic

**TABLE 2 |** Trials testing radiotherapy in combination with PD-1/PD-L1 in advanced metastatic cancers that allowed more than one irradiated lesions.

| NCT number | Phase | Tumor type | RT regimen | PD-1/PD-L1 inhibitors | Treatment schedule timing | Numbers of irradiated targets | Trial design (arms) | Primary outcome | Status |
|---|---|---|---|---|---|---|---|---|---|
| NCT03464942 | II | Advanced triple negative breast cancer | SABR 20 Gy × 1f or 8 Gy × 3f | Atezolizumab | PD-1 inhibitor will be started within 5 days of last SABR dose | 1–4 metastases with at least 1 untreated | Arm A:Single Dose Arm B:Fractionated Dose | PFS | Recruiting |
| NCT03283605 | I/II | Metastatic head and neck carcinomas | Not mentioned | Durvalumab Tremelimumab | SBRT will be administered between Cycle 2 and 3 of durvalumab and tremelimumab | 2–5 | Single group | AE PFS | Recruiting (64) |
| NCT03644823 | II | Advanced NSCLC | 6 Gy × 3f | Atezolizumab | Not mentioned | 1–2 | Single group | AE | Recruiting |
| NCT03812549 | I | Stage IV NSCLC | SBRT 10 Gy × 3f | Sintilimab | Sintilimab will be started within 7 days after radiation completed | At least 2 | Single group | AE and/or DLT | Recruiting |
| NCT04549428 | II | Advanced oligoprogressive NSCLC | 8 Gy × 1f | Atezolizumab | RT will be delivered concomitant to the 2nd dose of atezolizumab | All eligible metastatic and primary sites | Single group | ORR | Not yet recruiting |
| NCT04625894 | I | Oligometastatic gastrointestinal cancer | Multisite SABR (BED > 100 Gy) | Camrelizumab | SABR prior to PD-1 inhibitor | Multisite | Single group | DLT | Not yet recruiting |
| NCT02303366 | I | Oligometastatic breast neoplasia | 20 Gy × 1f | MK-3475 | SABT followed by MK-3475 | At least one metastases (to a maximum of five metastases) | Single group | Safety profile | Completed |
| NCT03223155 | I | Metastatic lung cancer | Three or five fractions of radiation | Nivolumab | Sequential Arm: nivolumab/ipilimumab between 1 and 7 days after completion of SBRT. Concurrent Arm: nivolumab/ipilimumab first and must complete planned SBRT to 2–4 sites within 2 weeks | 2–4 | Sequential Arm Concurrent Arm | AE | Recruiting |
| NCT03087019 | II | Recurrent or metastatic ACC | >5 fractions | Pembrolizumab | Concurrent | Up to 5 | Arm A: Pembrolizumab + Radiation Arm B: Pembrolizumab | ORR | Active, not recruiting |

*(Continued)*

**TABLE 2 |** Continued

| NCT number | Phase | Tumor type | RT regimen | PD-1/PD-L1 inhibitors | Treatment schedule timing | Numbers of irradiated targets | Trial design (arms) | Primary outcome | Status |
|---|---|---|---|---|---|---|---|---|---|
| NCT04535024 | II | MSS oligometastatic colorectal cancer | Target dose will be adjusted depending on site of the lesion and organs at risk (BED > 100 Gy). | Sintilimab | Starts within 1 week upon SABR completion | Sequence of irradiation for multiple metastases | Single group | ORR | Recruiting |
| NCT03825510 | II | Metastatic non-small cell lung cancer | 3–5 fractions of SBRT | Nivolumab or Pembrolizumab | PD-1 inhibitors start after SBRT | ≤3 sites | Single group | OS and acute toxicity | Recruiting |
| NCT02608385 | I | Advanced solid tumors | 3 or 5 doses of SBRT | Pembrolizumab | Pembrolizumab is given after SBRT | All sites in Oligometastatic tumors | Arm A: Dose Escalation Cohort. Patients will be enrolled to receive specific doses of SBRT to determine the best safe doses. Arm B: Large Volume Tumors Cohort. Tumors will be partially treated with SBRT. Arm C: Oligometastatic Cohort. All lesions will be treated with SBRT | Recommended SBRT dose in combination with pembrolizumab | Active, not recruiting |

*We searched "radiation and PD-1/PD-L1 inhibitors" in the clinicaltrials.gov database to identify studies with the following statuses: not yet recruiting, enrolling by invitation, recruiting, active, not recruiting, completed, and unknown status, with study type of interventional (Clinical Trial). A total of >60 trials were identified as trials using radiotherapy with PD-1/PD-L1 inhibitors in advanced metastatic cancers (date of final query, 25 November 2020). Then we searched "radiation and immunotherapy" in the clinicaltrials.gov database as above. A total of >150 trials were detected. We identified the studies of radiotherapy with PD-1/PD-L1 inhibitors in advanced metastatic cancers (date of final query, 25 November 2020). This list shows the trials that allowed more than one lesion irradiated. This list should not be considered comprehensive or exhaustive.*

*SABR, stereotactic ablative radiotherapy; PFS, progression free survival; CBI, checkpoint blockade immunotherapy; ORR, objective response rate; SBRT, stereotactic body radiotherapy; RCC, renal cell carcinoma; AE, adverse events; DLT, dose limiting toxicities; RT, raidotherapy; TKI, tyrosine kinase inhibitor; NSCLC, non-small-cell lung cancer; SCC, squamous cell carcinoma; HNSCC, head and neck squamous cell carcinoma; ICI, immune checkpoint inhibitor; HCC, hepatocellular carcinoma; OS, overall survival; ACC, adenoid cystic carcinoma; MSS, microsatellite stability.*

**TABLE 3 |** Trials testing radiotherapy in combination with PD-1/PD-L1 and cytokines (IL-2 or GM-CSF).

| NCT number | Phase | Tumor type | RT regimen | PD-1/PD-L1 inhibitors | Treatment schedule timing | Primary outcome | Status |
|---|---|---|---|---|---|---|---|
| NCT03474497 | I/II | Metastatic NSCLC, Melanoma, RCC, or HNSCC who have failed PD-1/ PD-L1 inhibitors | 8 Gy × 3f | Pembrolizumab | Radiotherapy will be delivered to the treatment lesion during the second cycle of therapy using an 8 Gy × 3 fractions palliative regimen.A total of four interleukin-2 treatments will be delivered into the treatment lesion by IT injection biweekly (at least 48 h apart) starting 24–96 h after the completion of radiotherapy and to be completed during the second on-trial cycle of Pembrolizumab. | Abscopal response rate | Recruiting |
| NCT03224871 | Early Phase I | Metastatic NSCLC | 8 Gy × 3f | Nivolumab | Nivolumab will be started on week 1 day 1, concurrent with radiotherapy | DLT | Completed |
| NCT03958383 | I/II | Melanoma | Palliative radiation therapy | Nivolumab | Phase IA: Participants receive hu14.18-IL2 fusion protein IT.<br>Phase IB: Participants undergo palliative RT and hu14.18-IL2 fusion protein IT as in phase IA.<br>Phase IC: Participants undergo palliative RT, receive nivolumab, and hu14.18-IL2 fusion protein IT as in phase IA.<br>Phase ID: Participants undergo palliative RT, receive nivolumab in combination with ipilimumab, and hu14.18-IL2 fusion protein IT as in phase IA. | AE<br>MTD<br>MAD | Recruiting |
| NCT04106180 | II | Advanced NSCLC | 8 Gy × 3f | Sintilimab | SBRT combined sintilimab and GM-CSF | ORR | Recruiting |
| ChiCTR1900026175 | I/II | Metastatic solid tumor | 8 Gy × 3f | PD-1/PD-L1 inhibitors | SBRT combined PD-1/PD-L1 inhibitors and GM-CSF | Safety<br>PFS<br>Incidence of abscopal effects | Recruiting |
| ChiCTR2000035817 | I/II | Advanced liver cancer | Not mentioned | Carrelizumab | SBRT combined PD-1/PD-L1 inhibitors and GM-CSF | PFS | Recruiting |

*We searched "radiation and IL-2" in the clinicaltrials.gov database to identify studies, and 18 trials were found. Data were obtained searching "SBRT and IL-2" in the clinicaltrials.gov database resulting in 4 trials, where 3 trials were on combining radiotherapy with PD-1/PD-L1 inhibitors and IL-2 (date of final query, 25 November 2020). Then we searched "IL-2" in www.chictr.org.cn database to identify studies; 17 trials were identified; no study met our requirements. Then we searched "radiation and GM-CSF" in the clinicaltrials.gov database. Thirty-seven trials were identified. Data were obtained searching "SBRT and IL-2" in the clinicaltrials.gov database to identify studies, and 5 trials were detected. We identified one study on radiotherapy with PD-1/PD-L1 inhibitors in advanced metastatic cancers (date of final query, 25 November 2020). Then we searched "GM-CSF" in www.chictr.org.cn database to identify studies; 10 trials were identified, where 2 studies were on combining radiotherapy with PD-1/PD-L1 inhibitors and GM-CSF. This list should not be considered comprehensive or exhaustive.*

*HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small-cell lung cancer; RCC, renal cell carcinoma; MTD, maximum tolerated dose; MAD, maximum administered dose; AE, adverse events; DLT, dose limiting toxicities; SBRT, stereotactic body radiotherapy; ORR, objective response rate; PFS, progression free survival; IT, intratumorally.*

(≤4) NSCLC treated with local treatment (surgery, radiotherapy, radiofrequency ablation) combined with a PD-1 inhibitor for all lesions. The results may suggest better survival benefit of radical radiotherapy done for all metastatic sites if applicable than done at only one site. However, benefits of maximizing irradiated sites with concurrent ICI therapy need to be examined in randomized controlled phase III clinical trials.

Multisite SBRT is relatively implementable in patients with oligometastatic disease and small tumor size. However, it is not practical to give all sites SBRT to patients with multiple metastases or bulky tumors. Partial tumor irradiation can be considered in certain conditions with controlled, tolerable toxicity. In the phase I trial mentioned above, patients with solid metastatic tumors administrated with multisite SBRT with pembrolizumab achieved 13.2% in ORR. Partial tumor irradiation was carried out if the target tumor volume was larger than 65 mL in these patients (54). Other partial irradiation strategies like novel SBRT targeted hypoxic segment, called bystander tumor volume (BTV), defined by PET and contrast-enhanced CT, showed very inspiring results suggesting a bulky tumor control rate of 95% (bystander effects) and non-irradiated metastases of 45% (abscopal effects) (81, 82). Other ways like spatially fractionated radiation therapy (SFRT, also known as GRID) can precisely treat target lesion with a non-uniform dose and minimize the toxicity to normal tissue. Preclinical evidence suggested that SFRT could further trigger immune responses

and abscopal effects, which might be a potential combination modality with PD-1 inhibitors, especially for bulky tumors (83–85).

The safety and efficacy of multiple cycles of HFRT with each cycle delivering to one lesion instead of one cycle simultaneous multisite radiotherapy combining with anti-PD-1/PD-L1 immunotherapy is tested in our clinical trial (ChiCTR1900026175), presented at the ASCO congress 2020. Participants who had solid tumors with multi-metastases failed to standard therapy were enrolled and treated with PD-1 inhibitors, radiotherapy and GM-CSF (PRaG regimen) sequentially. Three doses of 8 Gy or five doses of 5 Gy are delivered to tumor lesion based on its site and size. On the 2nd day after radiotherapy, PD-1 antibody is intravenously administered once, and GM-CSF 200 μg is subcutaneously injected daily for 2 weeks. At least 2 cycles of triple combination are required, and each cycle is repeated every 3 weeks with different lesions irradiated. After completion of PRaG regimen, maintenance therapy with PD-1 inhibitor is administered every 3 weeks until disease progression or unacceptable toxicity. Interim analysis showed a favorable short-term efficacy of 3-month ORR of 15.8% and PFS of 4.0 months with tolerable toxicity (86, 87). Currently the study is ongoing.

There are other ways to get more lesions irradiated to boost anti-PD-1 effects by combing SBRT with low-dose radiation therapy (LDRT). Welsh et al. proposed to promote immune response to cancer by utilizing high-dose and low-dose radiation synergistically. Clinical data provided a promising result, where 58% of the low dose target responded to a mean dose of 7.3 Gy (1.1–19.4 Gy), which was remarkably higher than no-dose lesions (18%, $p = 0.0001$) (88). The underlying rationale is high-dose radiation increases the release and presentation of antigens as well as activates immunity, while low-dose radiation promotes the infiltration of immune cells into the tumor microenvironment (88). On-going phase I study in metastatic NSCLC reported delivering SBRT in 30 Gy in 3 fractions to a small volume target and LDRT (2 Gy×1 fraction, 4 Gy×2 fractions, or 10 Gy×5 fractions) to a large lesion, with administering sintilimab within 1 week after radiotherapy completion, achieves an ORR of 78.6%. There are 80% of subjects experience grade 1–2 treatment-related adverse events (TRAE) and only 6.7% of subjects have ≥G3 TRAE (89).

It is not clear how many lesions irradiated are required to obtain the greatest immune sensitization effect and minimize side effects for patients with advanced multiple metastatic tumors. At present, there are no large randomized controlled studies. There are several clinical studies on SBRT irradiation of multiple metastases combined with PD-1 inhibitor therapy are underway (**Table 2**). In addition to investigate the optimal radiotherapy schedule to tumors, the metastatic sites and their biological behaviors should also be considered when selecting the irradiated targets (90). Clinical data showed that radiotherapy targeting to parenchymal sites, such as liver and lung, might cause a better systemic immune changes than targeting to non-parenchymal sites, such as brain and bone (91). In 2018, Pitroda et al. biologically identified three distinct molecular subtypes of colorectal liver metastases, which was related to clinical outcomes and was potential independently of established clinical risk factors (92). These finding suggested that the molecular subtypes of oligometastasis can predict a subset of patients who might benefit most from local treatment (90). Therefore, lesions selected for radiotherapy can not only be considered by numbers and volumes but also be determined according to the molecular characteristics of metastases.

## BIOLOGICAL RESPONSE MODIFIERS TO BOOST THE EFFECT OF COMBINATIONAL THERAPY

The addition of biological immunomodulators can further boost the effect of this combinational approach. Cytokines like IFN-α, IL-2, GM-CSF, TNF-α, IL-15, IL-12, have a synergistic action with radiotherapy (93). In this review, we are mainly focusing on IL-2 and GM-CSF (**Figure 1**).

The cytokine IL-2 is secreted by effector T cells and is essential for the proliferation, differentiation, and survival of T cells. Preclinical studies have shown that in mouse models of melanoma, colon and breast cancer, HFRT combined with IL-2 can produce significant synergistic therapeutic effects and enhance anti-tumor effects of $CD8^+$T cells and NK cells (94). Phase I clinical study showed that in metastatic malignant melanoma and renal cancer, SBRT combined with IL-2 was well-tolerated and provided an ORR of 66.6%. The possible mechanism is the activation of $CD4^+$ effector memory T cells by combinational treatment (28). To date, there is no available data in clinical trials for radiotherapy combined with IL-2 and PD-1/PD-L1 inhibitors. A small number of phase I/II clinical studies are currently underway (**Table 3**).

GM-CSF is also an immunomodulatory cytokine, which can promote the differentiation of monocyte/M1 type macrophages and DCs, enhance their activities and antigen presentation, and amplify the body's immune response (26, 95). Previous studies showed that the expression level of DC gene signature in renal cell carcinoma and NSCLC tissues was positively correlated with OS (27). Blocking PD-L1 on DC can reduce the isolation of PD-L1 from B7.1, thus enhancing the interaction between B7.1/CD28 and activating T cells (27). Animal experiments suggested that GM-CSF combined with ICIs can enhance the activity of innate immune cells by enhancing antigen presentation, indirectly recruiting T cells into the tumor microenvironment, and ultimately enhancing the efficacy of PD-1/PD-L1 inhibitor. Thus, GM-CSF may help to transform "cold" tumors into "hot" tumors (96).

Clinical studies have also demonstrated that GM-CSF can enhance the efficacy of immune checkpoint inhibitors. In a randomized controlled study of patients with unresectable stage III or IV melanoma, the median OS of the patients treated with GM-CSF and ipilimumab was significantly improved compared to the group treated without GM-CSF (97). Preliminary findings in patients with advanced cholangiocarcinoma showed pembrolizumab combined with GM-CSF improved 6 months PFS reached 35% with 7% of subjects having ≥ G3 adverse reactions, suggesting this combination is safe and obtained

good short-term effect (98). Evidence from combination PD-1/PD-L1 inhibitors with GM-CSF modified tumor vaccines also demonstrated synergistic anti-tumor effects (99–101). GM-CSF could also boost the immune effect of radiotherapy and induce abscopal effects. Prospective clinical study has shown that local radiotherapy combined with GM-CSF induces a 27% abscopal effect and improves patients' prognosis in patients with advanced solid tumors (102). To date, there is no report on triple combination therapy of radiotherapy, PD-1/PD-L1 blocker and GM-CSF. Our prospective study on HFRT combined with PD-1 blocker and GM-CSF in the treatment of advanced multiple metastatic solid tumors is ongoing (ChiCTR1900026175) (86, 87). Several phase II clinical studies of second-line SBRT combined with PD-1 inhibitors and GM-CSF triple therapy in solid tumors are ongoing (**Table 3**).

## SUMMARY

Combination treatment of radiotherapy and PD-1/PD-L1 inhibitors is a promising strategy for patients with metastatic cancers, where radiotherapy acts as a radical local treatment in oligometastasis and as an adjuvant therapy in multiple disease or bulky disease by directly damaging malignant cells, helping TAA releasing and antigen presentation, modulating tumor microenvironment. Addition of biological immunomodulators can further amplify the anti-tumor immune effects of this combinational treatment. Further research needs to optimize treatment schedule, maximize immune response and reduce adverse effects, through investigation of doses and fraction size of radiotherapy, the numbers and sites for irradiation, as well as the optimal timing of combination. It will provide solid evidence for this combinational treatment to support it widely accepted in clinical practice in the future.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## AUTHOR CONTRIBUTIONS

XZ, JP, and ZX helped to write and revise the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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# Radiotherapy in the Era of Immunotherapy With a Focus on Non-Small-Cell Lung Cancer: Time to Revisit Ancient Dogmas?

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Radiation-induced immune effects have been extensively deciphered over the last few years, leading to the concept of the dual immune effect of radiotherapy with both immunostimulatory and immunosuppressive effects. This explains why radiotherapy alone is not able to drive a strong anti-tumor immune response in most cases, hence underlining the rationale for combining both radiotherapy and immunotherapy. This association has generated considerable interest and hundreds of trials are currently ongoing to assess such an association in oncology. However, while some trials have provided unprecedented results or shown much promise, many hopes have been dashed. Questions remain, therefore, as to how to optimize the combination of these treatment modalities. This narrative review aims at revisiting the old, well-established concepts of radiotherapy relating to dose, fractionation, target volumes and organs at risk in the era of immunotherapy. We then propose potential innovative approaches to be further assessed when considering a radio-immunotherapy association, especially in the field of non-small-cell lung cancer (NSCLC). We finally propose a framework to optimize the association, with pragmatic approaches depending on the stage of the disease.

**Keywords: radiotherapy, immunotherapy, immune check point inhibitors (ICI), abscopal effect, lymphopenia, non-small-cell lung cancer (NSCLC), adscopal effect**

## INTRODUCTION

For more than a century, radiotherapy (RT) has been the cornerstone for the treatment of cancer. The classical radiobiological mechanisms underlying tumor cell death are well known, mainly involving deoxyribonucleic acid chain (DNA) damage, either directly or *via* water radiolysis and the production of free radicals and reactive oxygen species (ROS). The relative biologic effectiveness of radiation is influenced by several mechanisms known as the '5Rs': repair of sublethal damage,

repopulation, redistribution within the cell cycle, reoxygenation and intrinsic radiosensitivity, which mostly explains variations in radiosensitity/radioresistance for a given tissue/tumor (1).

Conventional RT consists in delivering once daily fractions of 1.8-2.2 Gy for 5-8 weeks, as this empirical approach turned out to achieve a differential effect between tumor cells and normal tissue. With the advances in dose delivery, patient immobilization and repositioning and tumor motion management, stereotactic RT has emerged, enabling the delivery of higher biological effective doses (BED) in fewer fractions and with a sharp dose fall-off.

Immunotherapy (IO) to restore and/or to boost anti-tumor immunity, especially with immune check-point inhibitors (ICI), has changed the standard of care in many fields of oncology for a decade. For example, the PACIFIC trial led to an unprecedented gain in progression-free survival (PFS) and overall survival (OS) for the management of non-resectable stage III non-small-cell lung cancer by adding durvalumab as maintenance therapy following chemoradiotherapy (2).

Along with the advances in anti-tumor immunity research, the deciphering of radiation-induced immune effects has led to the concept of a dual immune effect of RT with both immunostimulatory and immunosuppressive effects (3). Briefly, RT can release tumor-antigens (TA) along with the translocation of calreticulin to the tumor cell membranes, leading to tumor cell phagocytosis (4) and the activation of the cytosolic DNA sensing cGAS/STING pathway, with in turn induction of interferon β (IFN-β) (5), and the release of damage-associated molecular patterns (DAMPs) (such as heat shock proteins, high mobility group box 1 molecules (HMGB1) or adenosine triphosphate (ATP)). These DAMPs are recognized by toll-like receptors (TLRs) expressed at the surface of dendritic cells (DCs), and can promote processing and cross-presentation of TA by IFN-β-induced mature DCs (6). Following this immunogenic cell death, DCs then migrate to the tumor-draining lymph nodes and prime $CD8^+$ T cells (7), with in turn leukocyte extravasation and recruitment to the tumor site through chemokine secretion by tumor cells and other cell types in the tumor micro-environment (CXC motif chemokine ligand (CXCL)9, CXCL10, and CXCL16) (8, 9). Once T cells have infiltrated the tumor tissue, they encounter tumor cells with the radiation-induced expression of several surface molecules and receptors, such as MHC-I molecules (10), the TNF-R superfamily (11, 12) and ligands for the NKG2D receptor (13), leading to enhanced tumor cell killing by $CD8^+$ T cells and NK cells.

Together with this radiation-induced *in situ* "vaccination", RT can induce immunosuppressive effects *via* several mechanisms: upregulation of PD-L1 levels on tumor cells *via* IFN-γ released by $CD8^+$ T cells and of PD-1 levels on $CD8^+$ tumor infiltrating lymphocytes (TILs), contributing to T cell exhaustion (14, 15); direct depletion of circulating lymphocytes and lymphoid progenitors in primary and secondary lymphoid organs (16, 17); enhancement of immune suppressive pathways (mostly: HIF1α upregulation, increased colony-stimulating factor 1 (CSF1) levels, induction of TGF-β and generation of adenosine from ATP), which in turn lead to a suppressive tumor micro-environment (TME) with induction of $CD4^+CD25^+$ regulatory T cells (T-reg) proliferation, M2 polarization of tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSC) activation (18).

Overall, this dual effect can explain why RT alone is not able to drive a strong anti-tumor immune response with a so-called "abscopal" effect in most cases and underlies the rationale for combining RT with IO, not only to amplify the *in situ* vaccination effect but also to overrule immunosuppressive effects. This rationale has generated considerable interest in this field, and around 700 trials are currently ongoing assessing different regimens of such associations in oncology. However, while some trials have provided unprecedented results and shown much promise (2, 19–21), others have led to disappointment (22–24). These discrepancies leave many open questions regarding the optimal combinations of these treatment modalities.

This narrative review aims at revisiting the old, well-established concepts of RT relating to dose, fractionation, target volumes and organs at risk in the era of IO, in order to propose potential innovative approaches to be further assessed when considering an RT + IO association, especially in the field of non-small-cell lung cancer (NSCLC).

Searches for original and review articles in the PubMed and Google Scholar databases were conducted until September 2020. General search terms (including both Medical Subject Headings (MeSH) and free text words) included the following: "radiotherapy", "immunotherapy", "immune checkpoint inhibitor", "anti-PD(L)1", "abscopal effect", "lung cancer", "non-small-cell lung cancer", "lymphopenia". Individual bibliographies were reviewed for additional relevant references.

## WHICH RT + IO ASSOCIATION FOR WHICH OBJECTIVE?

To establish the best RT scheme in the context of an RT + IO association, one should first define the main objective of such an approach (25) (**Figure 1**).

Schematically, the first objective is to promote the *in situ* vaccination effect of RT, either by adding IO to a short course of ablative (i.e. tumoricidal) RT towards the whole tumor sites (in early-stage disease or oligometastatic disease; in this case, IO in itsef also addresses the micrometastatic disease), or by adding RT at one or several metastatic sites to IO (polymetastatic disease). Several IO agents potentially trigger such an effect: activation of DCs *via* TLR agonists (26) or CD40 agonists (27); enhancement of T-cell priming *via* CTLA-4 antagonists (28, 29), OX40 agonists (30) or PD-1/PD-L1 antagonists (as PD-1 acts by inhibiting signaling downstream of the CD28 costimulatory receptor following B7-ligation) (31); enhancement of killing by effector T cells, mostly *via* PD-1/PD-L1 antagonists (14, 15).

Another approach consists in counteracting immunosuppressive signals induced by conventional daily definitive (chemo-)radiotherapy schedules for locally advanced

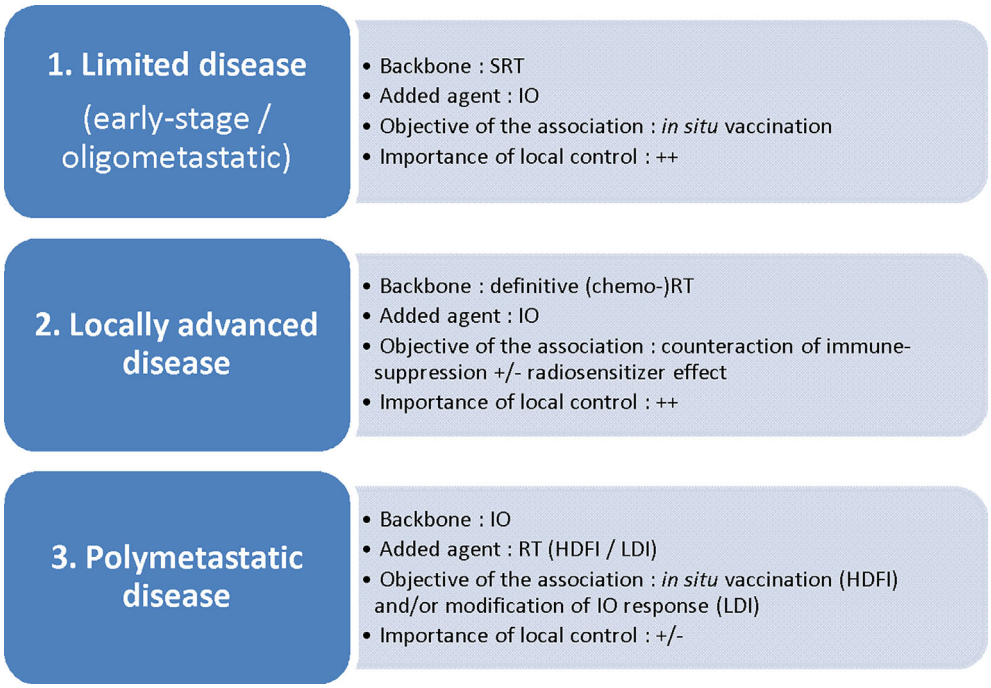


**FIGURE 1** | Disease setting and radiotherapy/immunotherapy combinations: which association for which objective? SRT, stereotactic radiotherapy; IO, immunotherapy; (chemo-)RT, (chemo-)radiotherapy; HDFI, high-dose per fraction irradiation; LDI, low-dose irradiation.

disease, due to the enhancement of immunosuppressive pathways as described above. In this case, IO and especially ICI can be used preferentially as a consolidative agent immediately following standard of care (chemo-)radiotherapy, then addressing the micrometastatic disease. Indeed, the restoration of the effective functions of TILs with ICI given concomitantly with tumor irradiation can be counterproductive owing to the profound suppression of TILs induced by daily RT. This is the rationale underpinning the PACIFIC trial, in which the addition of durvalumab following chemo-radiotherapy for stage III NSCLC led to the reduction of distant metastases and improved PFS and OS (2). The question as to whether IO can also act as a local radiosensitizer through a synergistic effect in this setting remains a matter of debate.

Finally, irradiation may serve as a strategy to modify the response to IO, in order to increase the immunogenicity of "cold tumors" through the homing of TILs or the reprogramming of the TME, inducing macrophage M1 polarization, for example (32).

## CONTROVERSIES ABOUT DOSE AND FRACTIONATION

Regarding the *in situ* vaccination effect, high dose per fraction irradiation (HDFI), usually through stereotactic radiotherapy (SRT), that delivers a few fractions with a high dose of radiation per fraction (generally above 6-8 Gy) is usually preferred, either as a tumoricidal schedule (e.g. 5 x 8 Gy) or as a non-tumoricidal schedule (e.g. 3 x 6 Gy). It has been shown that the release of intra-cellular peptides following single-fraction radiation took place in a dose-dependent manner *via* three main mechanisms with early and late effects: an increase in old protein degradation, upregulation of defined proteins through the response repair, and an increase in protein synthesis through the mTOR pathway activation. As peptides are the limiting factor, the increased intra-cellular peptide pool led to a dose-dependent increase in MHC class I presentation (10). Besides, Golden et al. showed that each component of immune-cell death following single-fraction radiation (calreticulin cell surface exposure, release of high mobility group box 1 (HMGB1) protein and release of ATP) was also induced in a dose-dependent manner from 2 to 20 Gy (33). Finally, Morisada et al. suggested a dose-dependent effect of radiation on both TA release and T-cell priming, with 8 Gy in a single fraction enhancing these pathways compared to 2 Gy in a single fraction, resulting in increased tumor cell susceptibility to T-cell-mediated killing (34). Importantly, Dewan et al. showed that a 3 x 8 Gy regimen was superior to 5 x 6 Gy in the induction of the abscopal effect and of tumor-specific T-cells, suggesting that this dose-dependent pro-immunogenic effect refers to the dose per fraction more than the total dose (35). However, the same group showed that HDFI (3 x 8 Gy 5 x 6 Gy), but not "ultra"-high single-dose RT (20 Gy x 1), was able to induce an abscopal effect when combined with anti-CTLA-4 (35). Vanpouille-Box et al. showed that the DNA exonuclease Trex1 is induced by radiation

doses above a threshold ranging from 12-18 Gy (36). During phagocytosis by myeloid cells, DNA fragments hidden in irradiated tumor cells are released from tumor-derived exosomes to the cytoplasm of myeloid cells (37), and cytosolic DNA stimulates the secretion of IFN-β through the activation of the DNA sensor cGAS and its downstream effector STING, in turn promoting the cross-priming of $CD8^+$ T cells (5). Above the threshold for Trex1 activation, DNA fragments are cleared from the cytosol, then precluding the secretion of IFN-β and T cell priming (36). Finally, while classical approaches tend to favor doses per fraction that are as high as possible in the context of SRT, these data suggest that the best SRT schedule for maximizing *in situ* vaccination in combination with IO is the delivery of 8-10 Gy fractions. In the context of early-stage NSCLC, several trials are currently assessing the benefit of IO in addition to standard of care SRT (NCT03110978; NCT03446547; NCT 03050554; NCT03383302). Some of them have implemented doses per fraction of around 10-12 Gy while another approach consists in a traditional fractionation of 3 x 18 Gy with addition of IO acting more as an adjuvant treatment than as a synergistic association to decrease the risk of regional and distant failures following SRT for high-risk stage I disease. In the context of NSCLC oligometastatic disease (generally fewer than five metastases), where SRT to all targets is now classically proposed as an ablative treatment (38, 39), the benefit of IO adjunction to SRT is being assessed in several trials with dose per fraction around 6-10 Gy (NCT03275597). In this perspective, when SRT to brain oligometastases is proposed in a context of IO, a hypofractionated schedule (e.g. 3 fractions of 8-10 Gy) could be better than a classical single fraction of 16-20 Gy. Such a schedule is being tested in patients with recurrent glioblastoma, in association with durvalumab (40). A provocative question is whether tumoricidal irradiation is absolutely required for localized disease when HDFI and IO are combined. This could pave the way for dose de-escalation with the definition of new therapeutic windows exploiting the synergy between RT and IO while decreasing radiation-induced toxicity. Finally, in the context of polymetastatic NSCLC disease, the benefit of the *in situ* vaccination effect of HDFI (tumoricidal or not) to one or several targets using doses per fraction of 6-10 Gy in addition to standard of care IO has been suggested (19–21) and is being assessed in the phase III NIRVANA-Lung trial NCT03774732.

When ICIs have been assessed as consolidation agents following standard of care definitive (chemo-)radiotherapy for locally advanced disease, RT has been delivered mostly in a conventional dose-fractionation schedule (1.8-2 Gy per fraction, one fraction per day, five days per week, to a total of 60-66 Gy for NSCLC) (2, 41, 42). The rationale behind this schedule is based on the linear quadratic model, whereby the optimal dose-fractionation regimen in order to kill cancer cells while sparing surrounding normal tissues may be established. However, the linear quadratic model accounts only for radiation cell killing and does not take the role of the immune system in antitumor responses into account (1). Therefore, the optimization of dose-fractionation chemoradiotherapy regimens for locally advanced disease in the context of IO combination requires careful consideration, as conventional fractionated regimens have been associated with lymphopenia and immune suppression in several types of cancers (16, 17). In this perspective, moderately hypofractionated (2.5-4 Gy per fraction) schedules could be of interest because the acceleration of treatment allowed by hypofractionated schedules could reduce the amount of blood passing through the beam and thus the duration and the severity of radiation-induced T-cell suppression and lymphopenia (43, 44). Indeed, in their study of 115 patients with unserectable stage III NSCLC treated by definitive RT, Zhao et al. found that overall treatment time within 4 weeks was significantly associated with a decreased risk of developing severe lymphopenia in multivariate analysis (44). Notably, in this setting of locally advanced disease, the question whether the addition of IO to (chemo-)radiotherapy can act as a radiosensitizer through a synergistic effect remains open for two main reasons. First, the majority of data regarding *in situ* vaccination have been obtained using a high radiation dose per fraction, corroborating the fact that the pro-immunogenic effects of radiation probably occur in a dose per fraction-dependent manner, provided that the 10-12 Gy threshold is not surpassed (10, 33, 34). Yet, the large fields required in the treatment of locally-advanced disease generally preclude the use of doses per fraction higher than 4 Gy. Second, data regarding the synergistic effects of moderately hypofractionated RT in association with IO are not consistent: while several preclinical studies suggest a benefit of hypofractionated over conventionally fractionated regimens, due to better $CD8^+$ T cell dependent primary and abscopal tumor control (45) and reduced recruitment of MDSCs into tumors through the downregulation of vascular endothelial growth factor (VEGF), a recent clinical series of 47 metastatic melanoma patients treated with ipilimumab and RT showed that fraction size ≤ 3 Gy vs > 3 Gy was associated with an improved rate of index lesion response outside the radiation field after adjusting for total radiation dose, site irradiated, timing of ipilimumab, and time from diagnosis to radiation treatment (46). Overall, hypothesizing a potential synergy of moderate hypofractionated RT in combination with IO, and considering the concern of early data of the toxicity of such an association (47) as well as the inconsistent data regarding the dose response effect following chemoradiotherapy in locally advanced NSCLC (48–50), dose de-escalated hypofractionated RT in combination with IO (and especially durvalumab consolidation) in stage III NSCLC is probably an approach to be investigated.

Finally, when considering RT as a response modifier of IO, low-dose irradiation (LDI) with one or a few fractions of 0.5 to 2 Gy has been shown to potentially increase the immunogenicity of "cold tumors" through several mechanisms: preferential induction of T-reg apoptosis compared with effector T cell cells (40); skewing macrophages from an M2 phenotype (promoting tumor growth) towards an inducible nitric oxide synthase-positive (iNOS+) M1 phenotype. These M1 macrophages in turn produce a range of chemokines which facilitate T-cell recruitment and normalize tumor vasculature, inducing T-cell tumor-infiltration (32). Furthermore, an original approach has been proposed combining both HDFI and LDI in

the context of the RT + IO association in order to generate *in situ* vaccination together with T-cell homing towards tumor sites (25). This hypothesis was corroborated in a preclinical study with bilateral mouse tumor models in which the authors suggested that HDFI of the primary tumor combined with LDI of the abscopal tumor and anti-PD-1 therapy achieved the best abscopal response, compared to HDFI + anti-PD-1, HDFI + LDI or LDI + anti-PD-1. The enhanced abscopal response was correlated with increased infiltration of $CD8^+$ effector T cells and upregulated expression of T-cell attracting chemokines (51). Clinical evidence of such LDI in association with HDFI has also been suggested in several reports. In a post-hoc analysis of three immunoradiation trials monitoring SRT with HDFI to a limited number of targets in association with IO, the out-of-field response of non-target lesions among 26 patients was statistically improved among low-dose irradiated lesions (mainly due to scatter dose related to anatomic proximity to another targeted lesion) compared to no-dose (<1 Gy) lesions (52, 53). Similarly, when LDI (4.9 Gy, range 2-8 Gy in 2 Gy fractions) was given intentionally to one large lesion together with ICI and HDFI, the low-dose treated lesion shrunk by 28.2% on average in 6 out of 9 patients with metastatic NSCLC (51). The RACIN trial is currently assessing the benefit of LDI to several lesions among advanced TIL-negative tumors in association with nivolumab and other agents (NCT03728179).

# CONTROVERSIES ABOUT IRRADIATED TARGET VOLUMES

## Which Target Volumes for Ablative Irradiation When IO Is Added?

### Tumor Irradiation

When tumoricidal irradiation of a limited disease burden (either non-metastatic or oligometastatic) is the main objective with the potential benefit of adding IO, several original approaches can then be considered to increase the therapeutic window in order to increase both the *in situ* vaccination effect and the local control while minimizing the toxicity.

One of the basic principles of RT is to ensure the full coverage of the tumor by the prescribed dose, using successive margins around the macroscopic target to account for microscopic disease (Clinical Target Volume – CTV – margins), target internal motions and patient set up (Planning Target Volume – PTV – margins). The aim is to avoid any lower dose regions which are classically associated with sites of recurrence. The correlate is the irradiation of a consequent amount of healthy tissues, with the risk of radiation-induced toxicity.

The reduction of the irradiated tissue volume would lead to the theoretical sparing of tumor-associated lymphocytes from the peri-tumoral TME, which can be rich in immune cells and can contain tertiary lymphoid structures (54). This sparing strategy could lead to a pro-immunogenic effect by sparing effector TILs which could otherwise be depleted following irradiation (54). It could also avoid the enhancement of proliferation and suppressive function of intra-tumoral T-reg, which has been shown following stereotactic irradiation (55). Such a reduction of the irradiated volume could be achieved through classical approaches of image-guided radiotherapy (IGRT) or gating/tracking strategies for mobile targets, aiming at reducing PTV margins (56). Another controversial approach would be to decrease or even to omit the Clinical Target Volume, based on the hypothesis that in the context of RT + IO, the benefit of sparing TIL would outweigh the benefit of eradicating microscopic disease. However, this hypothesis of a potential benefit from sparing peritumoral TME effector TILs arises from the notion that when large peritumoral volumes are irradiated, peritumoral TILs will mediate the local immune response as they are recruited *after* the irradiation, while those TILs present within or around the tumor at the time of irradiation, which are thought to be highly radiosensitive, are killed and cannot play an anti-tumor effector role. A recent preclinical study challenged this concept (57). The authors showed that many preexistent T cells not only survived following irradiation (yet with compromised proliferation), but also could mediate antitumor immunity *via* improvement of effector functions 9 days after irradiation as compared to T cells from unirradiated tumor (increased IFN-γ production and increased motility), without the contribution of newly infiltrating T cells. Furthermore, transcriptomic analyses suggested a T-cell reprogramming in the TME regulated by TGF-β with enriched signatures related to angiogenesis, adhesion or epithelial-mesenchymal transition, leading to a non-lymphoid tissue resident memory T-cell ($T_{RM}$)-like phenotype. These observations are fundamental, as not all T-cell subsets are equally sensitive, with $T_{RM}$ being more radioresistant than naïve or lymphoid tissue T cells (58, 59).

While an *in situ* vaccination effect has been shown to be crucial to achieve abscopal responses and to maximize systemic disease control, local control remains critical especially in the context of limited disease. In this perspective, partial tumor irradiation has also emerged as an innovative concept in order to widen the therapeutic window, especially for large tumors situated close to organs at risk where the classical approach of ablative RT to the whole target is challenging. While radiation oncologists usually make sure that the whole lesion receives the tumoricidal prescribed dose so that no area is underdosed, the partial irradiation approach consists in deliberately excluding a portion of the tumor from the radiation field. In two murine models, Markovsky et al. suggested that partial tumor volume irradiation (10 Gy, 15 Gy or 20 Gy delivered to 50% of the tumor using a 2 x 2 cm collimator) led to tumor responses similar to full tumor volume irradiation (10 Gy, 15 Gy or 20 Gy delivered to 100% of the tumor) *via* an immunostimulatory mechanism involving an increase in $CD8^+$ T-cell traffic throughout the non-irradiated portion mediated by an increase in ICAM (60). This led to the concept of ADscopal response (61), with an immune-mediated indirect therapeutic effect of RT "close to the irradiated target" ("bystander effect") rather than away from the target (ABscopal). Clinical data seem to corroborate this hypothesis, as large tumors (>65mL) partially irradiated exhibited local control similar to smaller fully irradiated

tumors in the NRG-BR001 phase I trial of SRT (3 x 15 Gy, 5 x 10 Gy or 3 x 10 Gy) in combination with anti-PD-L1. In the partial irradiation group, mean GTV size was 177 cc, and the mean volume of GTV excluded from the irradiated target was 113 cc (19, 61). This concept should be regarded with caution, however, since the "non-irradiated" portion receives non-tumoricidal but significant doses (scatter dose) that could be sufficient to elicit an immune response. Indeed, in the study by Markovsky et al., the non-irradiated tumor sub-volume received a dose of 5% (i.e. 0.5 Gy – 1Gy) or less of the primary in-field dose, and in the NRG-BR001 trial, the median isodose line covering the original GTV in the partially irradiated group was the 13% isodose line (i.e. 3.9 Gy – 6.5 Gy in 3 to 5 fractions). Therefore, we could hypothesize that the ADscopal effect is in effect a response to LDI. The phase II PembroX trial among patients with stage I-IIIA NSCLC is currently assessing the benefit of pre-operative SRT (1 fraction of 12 Gy) to half of the primary tumor following pembrolizumab (NCT03217071). The primary endpoint for this study is the change in number of TILs in the lung cancer tissue from before and after the neo-adjuvant treatment.

Finally, it has been recently suggested that the choice of the tumor portion to be irradiated in a partial irradiation approach could be successfully guided by metabolic imaging in order to focus on the hypoxic radioresistant portion. In this perspective, Tubin et al. proposed an innovative approach of Stereotactic Body RadioTherapy targeting Partial Tumor Hypoxic (SBRT-PATHY) clonogenic cells for the treatment of bulky locally advanced NSCLC not amenable to chemo-radiotherapy, with promising rates of ADscopal and ABscopal effects of 96% and 52%, respectively (62). The hypoxic area was defined with both $^{18}$FDG PET-CT and contrast-enhanced CT. No CTV or PTV margin was used to limit the surrounding irradiated tissue. In this context, the accurate identification of radioresistant areas within the tumor could be of particular interest to define relevant sub-volumes to be partially irradiated. Given that hypoxia is a classical contributor to radioresistance (63) and that tumor hypoxia was shown to correlate with poor outcome in NSCLC (64), hypoxia imaging, using PET-CT with specific tracers (FMISO (flouromisonidazole), Cu-ATSM (Cu(ll)-diacetyl-bis (A/4-methylthiosemicarbazone) or FAZA = fluoroazomycin) or oxygen-enhanced MRI would help in identifying such sub-volumes (65, 66).

### Tumor-Draining Lymph Node Irradiation

Once the estimated risk of micrometastatic spread is estimated to be high (generally over 10-15%), prophylactic irradiation of tumor-draining lymph nodes at a dose a 45-50 Gy for locally advanced disease (known as Elective Nodal Irradiation – ENI) is a classical approach in RT for several tumors such as head and neck cancers or cervical cancers. However, this practice is likely to disrupt a potential radiation-driven adaptive immune response, especially in the context of RT + IO.

In a preclinical model, Marciscano et al. showed that SRT + ENI in comparison with SRT alone restrained the adaptive immune response following SRT by modulating the chemoattractant and chemokine signature, leading to the reduction of tumor-specific effector T-cell intra-tumoral infiltration and an unfavorable balance between effector T cells and T-regs. Furthermore, ENI was shown to attenuate the combinatorial efficacy of RT and anti-CTLA-4 (67). Similar findings were recently obtained, together with the role of tumor-draining lymph nodes as a reservoir of "stem-like" anti-tumor CD8$^+$ T cells, which then differentiate into terminally differentiated effectors, and the detrimental effect of RT towards lymph nodes where such cell populations are expanding (68). Finally, a major study by Dammeijer et al. suggested that, while PD-1/PD-L1 blockade therapy is generally thought to reinvigorate progenitor-exhausted T cells and to relieve tumor T-cell-mediated suppression in the TME, tumor-draining lymph nodes are a major component of anti-PD-1/PD-L1-mediated tumor immunity. Indeed, PD-L1 is also expressed by non-tumor macrophages and DCs, and the authors showed that tumor-draining lymph nodes are enriched in PD-1$^+$ T cells. In addition, the selective targeting of PD-L1 only in tumor-draining lymph nodes demonstrated effective anti-tumor T-cell responses, and PD-1/PD-L1 interaction in tumor-draining lymph nodes, but not in the tumor, was correlated with prognosis in melanoma (69).

On the other hand, the omission of RT on pathologically involved lymph nodes when ENI is omitted could also be deleterious, not only because microscopic disease is not targeted but also because tumor cells confer tolerogenic features to tumor-draining lymph nodes (70).

Finally, innovative trials combining RT + IO for localized disease should be conducted to assess the benefit of omitting ENI for localized/locally advanced disease.

## Which Target Volumes for RT Added to IO?

### High Dose per Fraction Irradiation for *In Situ* Vaccination Effect

In the polymetastatic disease setting where tumoricidal irradiation of the whole tumor burden is not feasible, the optimization of RT to be added to the IO backbone is also critical to promote the pro-immunogenic effects of RT while ensuring acceptable toxicity.

Partial irradiation has already been discussed and can be proposed in this setting to induce both abscopal and adscopal effects. The NIRVANA-Lung trial has implemented such an approach (NCT03774732).

The choice of the best tumor sites to be irradiated is also of the utmost importance, since radiation-mediated immunogenicity differs according to the target due to inherent differences in organ-related TMEs. McGee et al. prospectively monitored the peripheral immune response following SRT to any organ. They found that SRT to parenchymal sites (liver or lung) but not to bone or brain induced changes in systemic immunophenotypes, including a decrease in total and cytotoxic NK cells, an increase in TIM3$^+$ NK cells and activated memory CD4$^+$ and CD8$^+$ T cells, and a decrease in circulating levels of chemoattractant chemokines (71). This differential pattern can thus be explained by differences in antigenic load and relative abundance of innate immune cells and lymphocytes between these organs. However, one cannot rule out the impact of different dose/fractionation

schedules between parenchymal lesions versus bone/brain lesions in that study. Moreover, in their phase I trial testing SRT + ipilimumab for metastatic tumors within lung or liver, a team from MD Anderson estimated that patients having received SRT to the liver as compared to the lung presented a transient increase in markers suggestive of enhanced peripheral T-cell activation, i.e. higher proportions of $CD8^+$ T cells expressing ICOS, GITR, and 4-1BB (72). However, this increased peripheral immune activation following SRT did not translate into clinical responses, as in the phase II trial assessing the same combination of SRT + ipilimumab, the same group found that the rates of clinical benefit of non-irradiated tumor volume were 31% for irradiated lung versus 14% for irradiated liver metastases (P=0.061) (53). This discordance could be due to the inherent adverse prognosis of liver metastases, but also to a lack of concordance between peripheral immune correlates and intra-tumoral immunologic patterns. More recently, Yu et al. observed that the presence of liver metastases negatively correlates with response to IO among patients with melanoma and NSCLC, independently of other established biomarkers of response, and that liver metastases, but not lung metastases, modulate immune function in animal models and in patients by reducing the number and the function of peripheral antigen-specific T cells. In-depth analysis revealed that hepatic $CD11b^+F4/80^+$ monocyte-derived macrophages can induce antigen-specific $CD8^+$ T cell apoptosis *via* the Fas-FasL pathway in the liver metastatic TME, suggesting that liver metastases siphon and eliminate antigen-specific $CD8^+$ T cells, creating a systemic immune desert in preclinical models. Interestingly, liver metastasis-directed RT in preclinical models was able to reshape the liver TME by eliminating immunosuppressive hepatic macrophages, thereby preventing antigen-specific T cell loss (73). These data provide a new synergistic explanation of how the association of RT and IO improves the efficacy of IO, and make liver metastases key tumor sites to be irradiated to promote systemic antitumor immunity.

Finally, the classical approach to induce the abscopal effect in the polymetastatic setting in association with IO is a single-site irradiation approach. It has not yielded strong evidence as two phase II trials in NSCLC and head and neck cancers failed to meet their objective of out-of-field overall response rate (22, 74). More recently, a multifactorial rationale has emerged to target as many lesions as possible in this context (75). First, the cytoreductive effect of multi-target irradiation potentiates the destruction of resistant subclonal populations. Second, due to differences in immunogenicity owing to distinct TME features between organs (71), a multitarget approach, preferentially in different organs, would potentiate the *in situ* vaccination effect. Furthermore, considering tumor heterogeneity, the release of a wide variety of distinct TAs would intuitively increase the chance of successful priming of anti-TA T cells and the constitution of a wide clonal T-cell repertoire, leading to an efficient $CD8^+$-mediated cytotoxic effect towards shared TA in distinct lesions. Formenti et al. thus suggested that the expansion of a large number of tumor-specific T-cell clones in peripheral blood correlates well with the achievement of abscopal responses in NSCLC patients treated with SRT + ipilimumab (20). Additionally, the irradiation of multiple sites could optimize the recruitment and the homing of immune cells through modification in microvasculature and the secretion of chemoattractant chemokines in those sites (8, 9). Finally, it has been shown that exhausted T cells arise from effector T cells, which gradually lose their effector functions and express multiple inhibitory receptors due to continuous T-cell receptor (TCR) stimulation from persistent antigen exposure, either in the context of chronic infections or of cancer (76). Therefore, a high tumor burden can be regarded as a source of persistent antigen exposure, so the maximal reduction in tumor burden through multi-target HDFI would lead to a decrease in T-cell exhaustion. This hypothesis has been suggested by Huang et al. who demonstrated that among patients treated with the anti-PD-1 agent pembrolizumab, the clinical benefit was strongly correlated with the magnitude of reinvigoration of exhausted $CD8^+$ T cells (as indicated by Ki67 expression and IFN-γ production), but above all with the amount of initial tumor burden, with greater tumor burden resulting in lower response rates. This led to the concept of a "reinvigoration-to-tumor burden" ratio as a positive predictive factor of response to checkpoint inhibitors when the ratio is high (77). These results are in line with several clinical reports revealing an increased benefit of ICI among patients with polymetastatic melanoma with lower tumor burden (78, 79), and major benefits of ICI in non-metastatic situations with a high risk of micrometastases (2, 80). Additionally, in a subgroup analysis from a randomized phase III trial comparing RT to 1-5 bone lesions (single dose of 8Gy) to the same RT + ipilimumab among patients with castration-resistant prostate cancer, the improvement in OS with the addition of ipilimumab favored those patients with fewer lesions (23).

Overall, several clinical trials have reported the results of multi-target SRT in association with IO in a polymetastatic setting (19, 81) and have yielded mixed results. Luke et al. reported the results of a phase I trial assessing SRT to 2-4 lesions (majority with 2 sites treated) followed by pembrolizumab in patients with heavily pretreated metastatic solid tumors. The overall response rate was 13%, and was similar to that from historical series of pembrolizumab alone (19). In the phase I/II from Welsh et al. among NSCLC patients, the best out-of-field response was similar between pembrolizumab alone and pembrolizumab + SRT to one to four lesions (81). However, the multi-target approach with irradiation of as many targets as possible should probably be preferred in the future. The phase III NIRVANA-Lung trial has implemented such an approach in its design.

In the context of oligometastatic disease, the added value of adjoining SRT to the whole tumor burden to IO is supported by a rationale which goes beyond the pure benefit of exclusive ablative RT suggested in several trials (38, 39, 82, 83). This rationale has been already partly discussed and includes the following: the optimization of the systemic response against subclinical disease through a multitarget strategy (and optimization of *in situ* vaccination) and of the reinvigoration-to-tumor burden ratio *via* a complete cytoreductive effect (77); the optimization of the

local antitumor immune response from the preexistent TILs (57); and the frequent failure in sites of initial disease under IO (84, 85). Accordingly, Bauml et al. performed a single-arm phase II trial in 45 patients with oligometastatic NSCLC (≤ 4 sites) who were treated by local ablative therapy followed by pembrolizumab (86). The median PFS was as high as 19.1 months (versus 6.6 months in historical series) and the 2-year OS was remarkably high (77.5%).

### Which Volume for Low-Dose Irradiation?

As previously discussed, LDI is able to reprogram the TME leading to T-cell homing towards tumor sites, and original approaches combining high-dose RT, low-dose irradiation and IO (triple therapy), are gaining evidence (51). Yet, the question of the number of lesions to be treated with LDI remains unanswered.

In a pragmatic approach, LDI could be performed for lesions not suitable for HDFI or with higher risk of toxicity, such as large lesions (more than 5 cm) or lesions near critical organs at risk, for example ultra-central lung lesions abutting the proximal bronchial tree (87, 88).

More provocatively, LDI could be delivered to large volumes such as whole-abdominal irradiation or even whole-body irradiation, aiming at targeting all tumor lesions. Several preclinical studies support this hypothesis (89, 90). Recently, Liu et al. used a combination of HDFI (8 Gy x 3) with low-dose total body irradiation (0.1 Gy) in syngeneic mouse models of breast and colon carcinoma and found an enhanced systemic anti-tumor response as compared to HDFI alone, by infiltration of $CD8^+$ T cells dependent on IFN-γ and alteration of the immunosuppressive TME of secondary tumors (89).

The concern of late toxicity from large volume irradiation remains, even with LDI, especially regarding myelosuppression or toxicity related to lung, liver or kidney injury (91). A promising alternative would be to irradiate the whole macroscopic lesions using intensity modulation radiotherapy (IMRT) techniques, while sparing bone marrow and any non-target organ.

## NEW CONCEPTS FOR DOSE TO ORGANS AT RISK: DOSE TO IMMUNE ORGANS AT RISK (iOAR)

### Impact of RT on Lymphocytes

Radiation-induced lymphopenia (RILP) has been known for decades and has been extensively described since then (92–95). It partly explains the immune suppressive effects following RT. Lymphocytes are the most radiosensitive cells within the body due to prominent apoptotic response pathways. Lethal doses to reduce the surviving fraction of circulating $CD4^+$ and $CD8^+$ T lymphocytes by 90% (DL90), 50% (DL50) and 10% (DL10) are only 3Gy, 2 Gy and 0.5Gy, respectively (96).

The mechanisms of RILP involve irradiation of circulating lymphocytes as well as lymphocyte-rich areas in lymphoid organs or, potentially, within the tumor (54). For example, patients who receive prophylactic lymph node irradiation experience more frequent and more profound RILP than those who do not receive it (97–99). The same observation has been made for patients with abdominal tumors who undergo irradiation of large splenic volumes (100). However, not all T-cell subsets are equally radiosensitive, with regulatory, activated and memory T cells having been shown to be more resistant than naive T cells, and with non-lymphoid $T_{RM}$ and intra-tumoral T-cells being more resistant than lymphoid tissue and circulating T cells (57–59, 101–103). Overall, RT mainly induces a decrease in naïve T cells, which drives a decrease in absolute lymphocyte count and an enrichment in T-regs, with no disruption of the functionality of T lymphocytes or the frequency of antigen-specific $CD8^+$ T cells (104, 105).

### Impact of Lymphopenia on Outcome

Classically, RILP affects more than half of patients receiving RT and is transient with a recovery mostly within 3-6 months after RT, but with prolonged depletion in some cases (16, 106). The adjunction of concurrent chemotherapy can increase the severity of RILP (107). The impact of RILP has been extensively explored in several tumor types (16). In the context of NSCLC, several studies have demonstrated the negative impact of RILP on OS and PFS (43, 108–110). Additionally, baseline lymphopenia has been negatively correlated with outcome following ICI for the treatment of solid tumors (111–113). Therefore, in the context of the RT-IO combination, attention should be paid to limiting the severity of RILP. A recent retrospective series suggested that among patients treated by ICI for metastatic tumors, RILP following palliative RT at onset of ICI therapy was associated with poorer outcome (114).

### Factors Predicting RILP

Apart from patient-related factors such as advanced age, smoking habits, comedications, baseline lymphopenia or even genetic factors (115), several factors related to the characteristics of RT have been associated with the incidence and severity of RILP. These factors are directly or indirectly correlated with the amount of circulating lymphocytes and lymphoid organs exposed to (even low) doses of radiation, and with the duration of exposure. Thus, considering blood flow, any factor leading to prolonged RT duration will increase the amount of blood passing through the beam, and could potentially increase the severity of RILP. For example, an increased number of fractions (through hyperfractionation with twice-daily fractions) has been shown to be a risk factor for RILP (43, 116, 117). Similarly, a low-dose rate should be avoided intuitively, although evidence is lacking.

Furthermore, irradiation of organs containing large blood volumes and/or with high blood flow velocity could be at risk of RILP. Recently, among 244 patients treated by chemo-radiotherapy for NSCLC, the heart volume receiving 20 Gy or more (V20Gy) and 40 Gy or more (V40Gy) was significantly correlated with the 1-month post-RT start neutrophil-to-lymphocyte ratio (NLR) (118). Similarly, Contreras et al. found that among patients with NSCLC treated with definitive RT (± chemotherapy), a heart V50Gy > 25% was significantly associated with a higher NLR 4 months post-RT (119).

Similarly, lung V5Gy was significantly and independently associated with post-RT lymphocyte nadir among 711 patients who received definitive RT for NSCLC (43). Abravan et al. found that mean heart dose and mean lung dose were correlated, and that a thoracic vertebrae V20Gy was correlated with grade ≥3 lymphopenia following thoracic RT (110). However, in a recent analysis, Joseph et al. did not find any correlation between heart or lung dosimetric parameters and severity of RILP in multivariate analysis, but rather demonstrated a negative correlation between integral body dose and post-treatment absolute lymphocyte count, suggesting the detrimental effect of a "low-dose bath" (108).

The amount of circulating lymphocytes exposed to radiation dose also seems to be correlated with the size of the gross tumor volume to be irradiated. For example, larger GTV were associated with lower lymphocyte nadir among patients treated for NSCLC (43) or glioblastoma (120). Similarly, the amount of spleen exposed to low/medium doses (V5Gy, V10Gy, V15Gy, V20Gy, mean dose) has been correlated with severe post-chemoradiotherapy lymphopenia in patients treated for locally advanced pancreatic cancer (100, 121).

Finally, several models have been proposed to estimate the dose delivered to circulating immune cells. Yovino et al. established an *in silico* model to estimate the radiation dose to circulating lymphocytes during a standard radiation treatment of 60 Gy in 30 fractions for glioblastoma. The model indicated that while a single fraction of 2 Gy delivered ≥ 0.5 Gy to 5% of the total blood pool, 99% of circulating cells had received ≥ 0.5 Gy after 30 fractions (120). Similarly, Jin et al. developed a three-step model to calculate the effective dose to the immune cells (EDIC) during thoracic RT, assuming the following: a) the dose to circulating immune cells including rapidly circulating ones in the heart, lung and blood vessels, and slowly circulating ones in the lymphatic system and blood reservoirs (a portion of veins/capillaries) is a surrogate for the EDIC; b) at each fraction, the radiation dose is uniformly delivered to all cells for rapidly circulating ones, and only to those in the irradiated volume for slowly circulating cells. In this model, the blood dose relating to the contribution of a given organ is approximated by its mean organ dose (MOD), the percentage of cardiac output, the percentage of blood volume it receives, the time for one blood circulation, the irradiation time and the number of fractions (120). Second, the EUD (Equivalent Uniform Dose) is determined from a blood dose/volume histogram (percentage of blood volume irradiated at a given dose). Third, the EDIC is the sum of the EUDs of each organ. In summary, the EDIC can be approximated as a function of the mean heart dose, the mean lung dose, the mean body dose and the number of fractions (122). Using this model, Ladbury et al. showed that among 117 patients with stage III NSCLC treated with definitive fractionated radiation, most of whom were receiving concurrent chemotherapy, a higher EDIC was correlated with a greater risk of grade ≥ 3 lymphopenia (123). Corroborating the impact of tumor volume on severity of RILP, they also found that the planning target volume (PTV) was strongly associated with the EDIC with a 1.7 Gy increase per liter ($p < 0.05$).

## Optimizing Dose to iOAR

To limit the impact of radiation dose to the host immune system, one can hypothesize that the limitation of radiation dose to circulating lymphocytes as well as to lymphocyte-rich areas in lymphoid organs could be beneficial. To do so, and especially for thoracic malignancies, RT planning should be performed in such a way that doses to relevant organs or structures are as low as possible. These organs include the following: heart (possible impact of V20Gy, V40Gy, V50Gy, mean dose) (110, 118, 119), lung (possible impact of V5Gy, mean dose) (43, 110), large vessels, non-involved draining lymph nodes, bone-marrow within spine (possible impact of V20Gy) (110) or pelvis mostly, spleen (possible impact of V5Gy, V10Gy, V15Gy, V20Gy and mean dose) (100, 121), gut and thymus in children.

Interestingly, by applying the global concept of EDIC to estimate the dose to the immune system as an OAR rather than focusing on separate OAR involved in the process of RILP, a secondary analysis of the RTOG 0617 trial found that EDIC was the strongest significant factor for OS, PFS and local PFS (LPFS) in multivariate analysis following chemo-radiotherapy for stage III NSCLC, with a high EDIC associated with worse outcome. While GTV, mean heart dose, mean lung dose and integral dose were significant factors in a multivariate model without EDIC, they were no longer significant when EDIC was added (122). These findings were validated externally by Ladbury et al. In their series of stage III NSCLC treated with radical RT, they found that EDIC was an independent factor for OS, LPFS and PFS. Furthermore, plotting OS and LPFS hazard ratios as a function of EDIC suggested that the most profound effect on OS and LPFS occurred when EDIC was above 6.3Gy (123). Similarly, EDIC was also an independent factor of OS among 92 patients with esophageal squamous cell carcinoma treated with neoadjuvant chemo-radiotherapy (124). However, since the EDIC model is a measure of radiation dose to circulating immune cells, this correlation could be confounded by other organs at risk or structures such as the spleen, bone marrow and lymph nodes. Furthermore, the model does not account for interplay between radiation and chemotherapy.

Finally, further investigation is needed to optimize the dose to immune-related OARs with defined thresholds, especially in the context of RT-IO combinations.

# INSIGHTS FROM DOSE DELIVERY

The modality of delivery of the radiation dose should be taken into account when investigating the immune effects of RT.

Intensity-modulated radiotherapy (IMRT) emerged in clinical practice around two decades ago as a technique for delivering a more accurate dose distribution than conventional 2-dimensional RT or 3-dimensional conformal RT (3D-CRT), with limited exposure of adjacent OARs, including structures located within a concave area of the PTV. To do so, an "inverse planning" is performed, where the treatment planner first determines the dose distribution for the target tumor and OARs, and then the optimization method determines the

intensity of the irradiation beam (125). In stage III NSCLC, IMRT as compared to 3D-CRT has been associated in dosimetric studies with improved PTV coverage, and with a decrease in the volume of whole lung receiving more than 20 Gy and in cardiac doses (126). This translated into a reduction in severe pulmonary toxicity and even improved OS in several large retrospective series (127–130). IMRT was also associated with decreased severe radiation pneumonitis as well as improved quality of life in secondary analyses of the RTOG0617 trial (131, 132). However, in comparison to 3D-CRT, IMRT increases the low-dose bath as a greater number of beams and monitor units are used, and several studies have shown an increase in lung V5Gy (126, 133). Concerns about V5Gy/V10Gy and fatal pneumonitis have been raised with IMRT in the context of post-operative RT for mesothelioma (134), however, no clear correlation has been established for IMRT in NSCLC, and it is commonly thought that the potential benefit of IMRT outweighs this risk in NSCLC. Nevertheless, as lung V5Gy was significantly associated with post-RT lymphocyte nadir among patients who received definitive RT for NSCLC (43), and given the negative impact of lymphopenia on outcome, attention should be paid to the low-dose radiation lung volume in the era of immunotherapy. This potential increased risk of lymphopenia with IMRT could be counterbalanced by a decrease in treatment time (beam on time) by using flattening filter free (FFF) radiation beams, which can provide high-dose rate beams (135).

Stereotactic RT is usually proposed for early-stage NSCLC in medically inoperable patients. Owing to the technical properties and characteristics of dose gradient, SRT is associated with low-dose bath to ensure a high conformal dose distribution around the target; however considering the small size of the lesions treated with SRT, this low-dose spread is usually limited. This could prompt to develop approaches of SRT-based sub-volume radiation boost following a conventionally fractionated course of RT for the treatment of locally advanced disease; this approach is currently being explored in stage III NSCLC (136).

Proton therapy is also gaining interest in locally advanced NSCLC owing to its dose distribution capabilities related to the release of proton energy, mostly at the end of the path, a phenomenon known as the Bragg peak phenomenon. Proton therapy has shown promise in reducing normal lung tissues receiving low-dose ranges, while maintaining dose constraints to other critical structures such as the heart, esophagus and spinal cord (137). This could explain the superiority of proton therapy over photon-based IMRT in terms of severe lymphopenia in patients treated with (chemo-)radiotherapy for glioblastoma (138), esophageal cancer (139, 140) or medulloblastoma (141). However, a phase II randomized trial comparing proton therapy and IMRT in the treatment of stage III NSCLC failed to show any advantage of proton therapy on toxicity or on local failures (142). In addition to this dosimetric advantage, proton therapy could have intrinsic immunomodulatory properties. A recent study suggests that proton therapy induces upregulation of surface molecules involved in immune recognition (HLA, ICAM-1 and tumor associated antigens), and translocation of calreticulin, in a manner similar to photon irradiation. The authors extended their

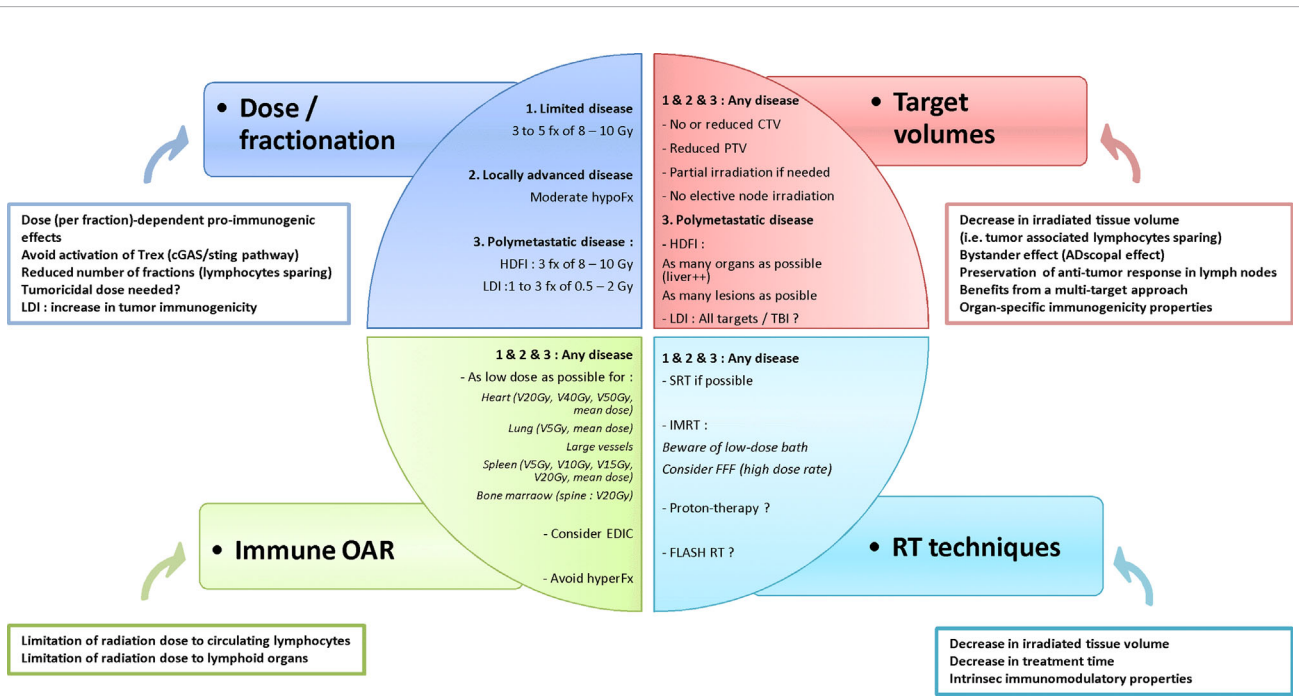


**FIGURE 2** | Hypothesis of framework to optimize radiotherapy-immunotherapy combination. fx, fraction; hypoFx/hyperFx, hypofractionation/hyperfractionation; CTV, clinical target volume; PTV, planning target volume; HDFI, high-dose per fraction irradiation; LDI, low-dose irradiation; OAR, organs at risk; VxGy, volume of organ receiving at least x Gy; EDIC, Effective Dose to Immune Cells; SRT, stereotactic radiotherapy; IMRT, intensity-modulated radiotherapy; FFF, Flattening Filter Free.

observations to cancer stem cells, which are classically resistant to radiation (143, 144). These results also support the association of proton therapy with T-cell mediated immunotherapy.

Finally, owing to their particular features, emerging unconventional approaches of RT may provide additional benefits when combined with IO (145). FLASH RT is one of these promising approaches. It is able to deliver radiation at ultra-high dose rate, which is thought to induce massive oxygen consumption; while tumors are generally already hypoxic, FLASH RT can induce transient protective hypoxia in normal tissues. Therefore, FLASH RT could enhance the differential effect between tumors and normal tissues as compared to conventional RT (146). The modulation of immune response with FLASH RT is not well established; however, together with a decrease in treatment time, some particular features associated with FLASH RT such as massive TA release or decrease in immunosuppressive TGF-β cascade activation may provide additional mechanisms of the synergistic effect of the RT – IO association (147, 148).

## DISCUSSION

The combination of RT and IO at any stage of cancer disease, i.e. from early stage to both oligo- and poly-metastatic disease, is offering new hope for the treatment of patients with malignancies. However, given the dual effect of RT upon the host immune system the RT schedule must be optimized whenever a synergistic effect of the combination of RT and IO is expected. To reach this objective, several traditional dogmas about RT might need to be revisited and challenged in this new therapeutic era, regarding dose, fractionation, target volumes, dose to organs at risk and dose delivery techniques. The main issues are summarized in **Figure 2**. Thus, both translational and clinical studies are necessary to better understand the mechanisms underlying the immune effects of RT and to provide a strong rationale for this combination. Along with the optimization of radiation dose delivery, biomarkers need to be validated to predict a synergistic effect of the RT – IO combination, based upon tissue analysis, circulating biomarkers, and quantitative imaging with radiomics (149, 150).

## AUTHOR CONTRIBUTIONS

JK designed the review, analyzed the data and drafted the manuscript. JM, CG-R, EC-J and MA analyzed the data and critically revised the manuscript. EC-J and MA supervised the writing. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Radiation Recall Pneumonitis After Treatment With Checkpoint Blockade Immunotherapy: A Case Series and Review of Literature

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**Background:** Radiation recall pneumonitis (RRP) is a poorly understood clinical syndrome in which patients develop radiation pneumonitis triggered by a systemic agent, often years after the completion of radiation therapy. Immune checkpoint blockade agents have only recently been posited as a trigger for RRP. Here, we present three cases of immunotherapy-induced RRP.

**Case Presentation:** Our first patient was diagnosed with primary lung adenocarcinoma, and 4.5 years after completing radiation therapy developed symptomatic RRP immediately following a second dose of nivolumab-containing immunotherapy regimen. Our second patient was diagnosed with primary bladder cancer metastatic to the mediastinum, which was treated twice with radiation therapy. He developed RRP in the days following his second course of ipilimumab-pembrolizumab which was months after his second course of radiation that he received. Our final patient was diagnosed with metastatic small cell lung cancer and received local consolidative radiation therapy in addition to whole-brain radiation. He developed RRP on the 11$^{th}$ day after concluding his 4$^{th}$ cycle of nivolumab-ipilimumab, approximately 7 months after having had completed chest radiation therapy.

**Conclusions:** Immunotherapy-induced RRP is a rare diagnosis which can present more focally than traditional immunotherapy pneumonitis and which must be clinically differentiated from other local processes such as pneumonia. Further research should explore the mechanisms underlying these radiation recall reactions as many patients receive radiation and immunotherapy during the course of their cancer treatment.

**Keywords: radiation, immunotherapy, pneumonitis, reaction, checkpoint, PD-1**

## INTRODUCTION

Radiation recall is a clinical phenomenon in which patients acutely develop signs and symptoms of inflammatory radiation toxicities or erythema within previously irradiated fields after initiation of a systemic therapy. Radiation recall typically arises after the timeframe during which any acute radiation toxicity would be expected (up to several years after completion of radiation therapy (RT)) and patients often tolerate re-challenge with the offending agent without recurrence of RRP (1). Numerous drugs have been linked to recall reactions, including cytotoxic antineoplastic medications, targeted cancer therapeutics, antibiotics, and even statins (2–4). While radiation recall has classically been described as a cutaneous reaction, more recently there has been increasing awareness of recall reactions occurring within other organ systems (2). One emerging diagnosis is radiation recall pneumonitis (RRP), a focal disease of lung parenchyma that clinically and radiographically resembles radiation pneumonitis, but which is temporally incongruent with radiation pneumonitis which typically occurs 1-3 months after conventional radiation (5). RRP has been described with chemotherapeutic agents (e.g. gemcitabine, doxorubicin, and docetaxel) (1, 6–8) and small-molecule kinase inhibitors (e.g. everolimus, sunitinib, erlotinib) (9–11). However, the literature is much sparser on RRP arising in the context of immunotherapy (12–14), with the largest case series containing only two patients (13). Here, we describe three cases of suspected immunotherapy-induced RRP treated at our institution.

## CASE DESCRIPTIONS

### Patient 1

Patient 1 64 y/o man with a 48 pack-year cigarette smoking history presented to an outside hospital with a chronic cough in 2012, which was refractory to antibiotic therapy. Imaging revealed an approximately 3.7x2.3 cm right-sided mass in the minor fissure with associated hypermetabolic hilar and mediastinal lymphadenopathy, as well as an ipsilateral pleural effusion which was pathologically negative. Biopsy *via* EUS diagnosed poorly differentiated adenocarcinoma, TTF-1 positive, CK 7, Napsin A positive, CK 20 negative, CK 5 negative, CD45 negative, EGFR wild-type, ALK wild-type, ROS1 wild-type. Staging imaging showed a right paratracheal lymph node measuring 3.2 cm, a subcarinal lymph node measuring 3.3 cm, and a right hilar lymph node 5.5 cm in maximal dimension, but no evidence of distant metastatic disease; he was thus diagnosed with a T2N2 stage IIIb non-small cell lung adenocarcinoma, per AJCC 7 criteria. He completed 4 cycles of carboplatin with pemetrexed, followed by concurrent cisplatin and radiation therapy (intensity-modulated RT (IMRT) 59.4 Gy in 33 fractions) completed 2013, with good radiographic response. Surveillance imaging detected multiple new parenchymal lung lesions, the largest of which was 1.1cm in the left upper lobe, as well as precarinal mediastinal adenopathy measuring 1.3x1.8cm. He was started on first line systemic therapy for advanced disease with pemetrexed, carboplatin, and bevacizumab. After progressing with bilateral pulmonary nodules on imaging he was enrolled on clinical trials, and in the ensuing years received: single-agent pembrolizumab (partial response, treated 11 months); single-agent nivolumab (progressed, treated 10 weeks); single-agent gemcitabine (partial response, treated 6 months); a second course of single-agent pembrolizumab (progressed, treated 1 month); single-agent docetaxel (partial response but discontinued due to toxicity, treated 5 months); and single-agent atezolizumab (progressed, treated 2 months). Throughout these courses of therapy, he developed metastatic disease to the left kidney, vertebral body, right sided ribs, and bilateral lung parenchyma.

In early 2018, he enrolled on a novel open-label immunotherapy trial, consisting of nivolumab combined with an experimental HDAC inhibitor. He received the first dose on 3/2018, and developed a cough approximately 2 weeks later, days following his second dose of the immunotherapy combination. After 2 weeks of symptomatic cough, on 5/2018 he started a course of empiric levofloxacin with no improvement. He completed his second cycle of therapy despite the cough becoming increasingly productive and having an increased home oxygen requirement. 2 weeks later, a CT of the chest with contrast revealed pneumonitis changes correlating to the previous IMRT fields (**Figure 1**), and was diagnosed with RRP. Cycle 3 was deferred and he started a tapered course of oral prednisone, 60 mg daily with excellent clinical response. After recovering, he tolerated a re-trial of monotherapy nivolumab (omitting the experimental agent) without a recurrent RRP. He has since received two courses of palliative radiation for chest wall lesions without complications.

### Patient 2

Patient 2 originally presented to an outside hospital in 2015 complaining of 2 years of gross hematuria that had progressed to blood clots in his urine. At that time, he was 63 years old with a 20 pack-years cigarette smoking history, and had no other known risk factors for urogenital malignancies. He was diagnosed with a T2G3 urothelial cancer of the bladder dome with extensive invasion in the muscularis propria *via* transurethral resection of bladder tumor (TURBT). In mid 2015, and underwent a restaging TURBT which re-demonstrated high-grade urothelial carcinoma invading the muscularis propria, and CT chest revealed an anterior mediastinal mass that was positive for metastatic urothelial cancer. He completed frontline therapy with cisplatin and gemcitabine in 1/2016 (cisplatin exchanged for carboplatin after acute kidney injury during cycle 3), after which he received palliative-intent RT with 30 Gy in 10 fractions to the mediastinal mass, completed 4/2016. He then was treated with an experimental immunotherapeutic agent on trial combined with durvalumab and has a durable response over 3.5 years. He was noted to progress in his mediastinum and this site was retreated

**Abbreviations:** RRP, radiation recall pneumonitis; RT, radiation therapy; IMRT, intensity-modulated radiation therapy; TURBT, transurethral resection of bladder tumor.

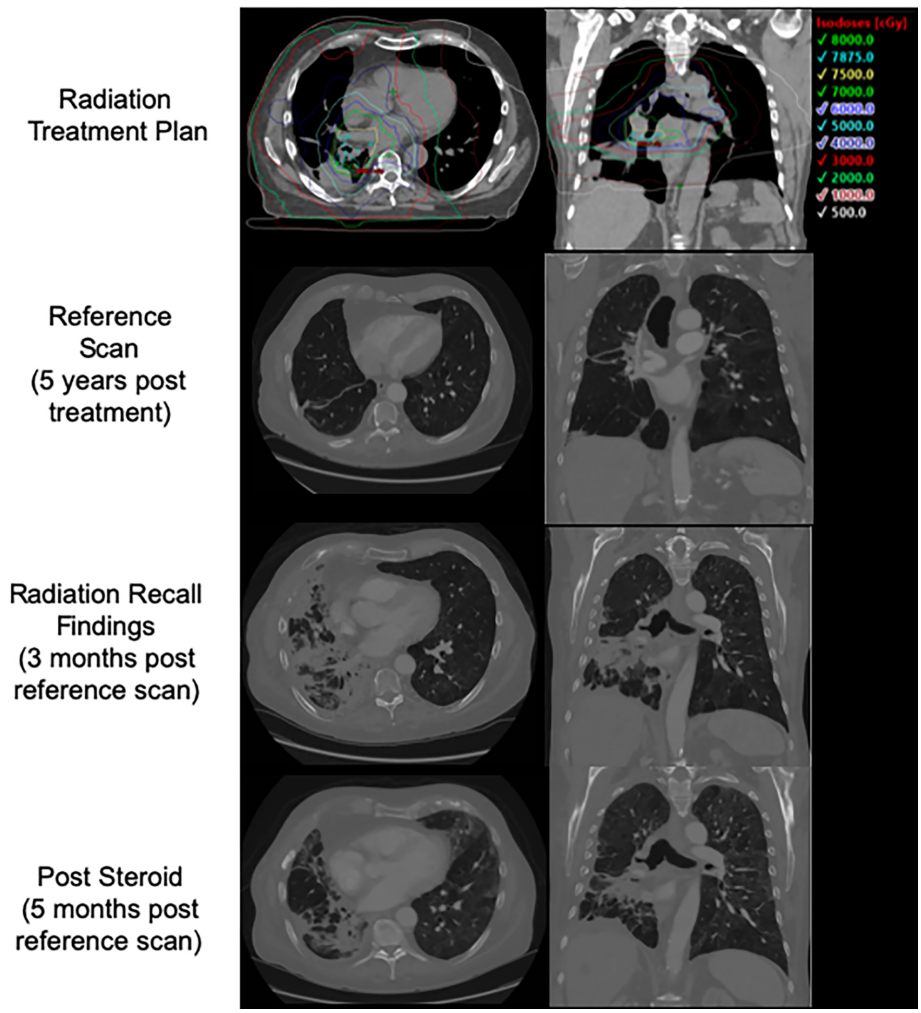


**FIGURE 1** | Patient 1's initial treatment plan, post treatment CT, CT at time of presentation with radiation recall, and CT following course of steroids with interval improvement.

using SBRT to 25Gy in 5 fractions, completed in mid 2019. This was followed by monotherapy pembrolizumab as a bridge to a clinical trial for which he was ultimately deemed ineligible, and in late 2019, ipilimumab was added to his pembrolizumab regimen. 3 days following his second cycle of pembrolizumab-ipilimumab, he presented to the emergency room complaining of abdominal and flank pain. He was found to have peritonitis from his necrotic primary tumor, as well as radiographic evidence of RRP of the left lung (**Figure 2**). However, the patient reported no symptoms associated with this, and was breathing normally on room air. Unfortunately, his clinical status rapidly declined, and he expired 12 days later in the hospital.

## Patient 3

Patient 3 presented in 2017 at age 52 with headaches, and was subsequently diagnosed with lung cancer metastatic to the brain. He was found to have small-cell lung cancer from a left upper lobe primary tumor and underwent surgical resection of a 4.1 cm. metastasis in the left cerebellum *via* posterior fossa craniectomy. He completed a cycle of cisplatin/etoposide while hospitalized, and then underwent whole-brain radiation therapy to 37.5 Gy in 15 fractions (completed mid 2017), followed by 4 additional cycles cisplatin and etoposide (completed 2 months later), which was followed by consolidative conformal RT to the primary tumor and mediastinum to 30 Gy in 10 fractions, completed in mid 2017. The patient subsequently developed 4 new brain metastases, which were treated with SRS in late 2017, followed by ipilimumab with nivolumab.

Seven months after completing thoracic RT, 11 days after receiving his 4$^{th}$ cycle infusion of nivolumab/ipilimumab, the patient developed acutely worsening left-sided chest pain, most severe upon inspiration and with no associated cough or fevers. He showed no infectious signs or symptoms, and was oxygenating well on room air. A CT scan of the thorax

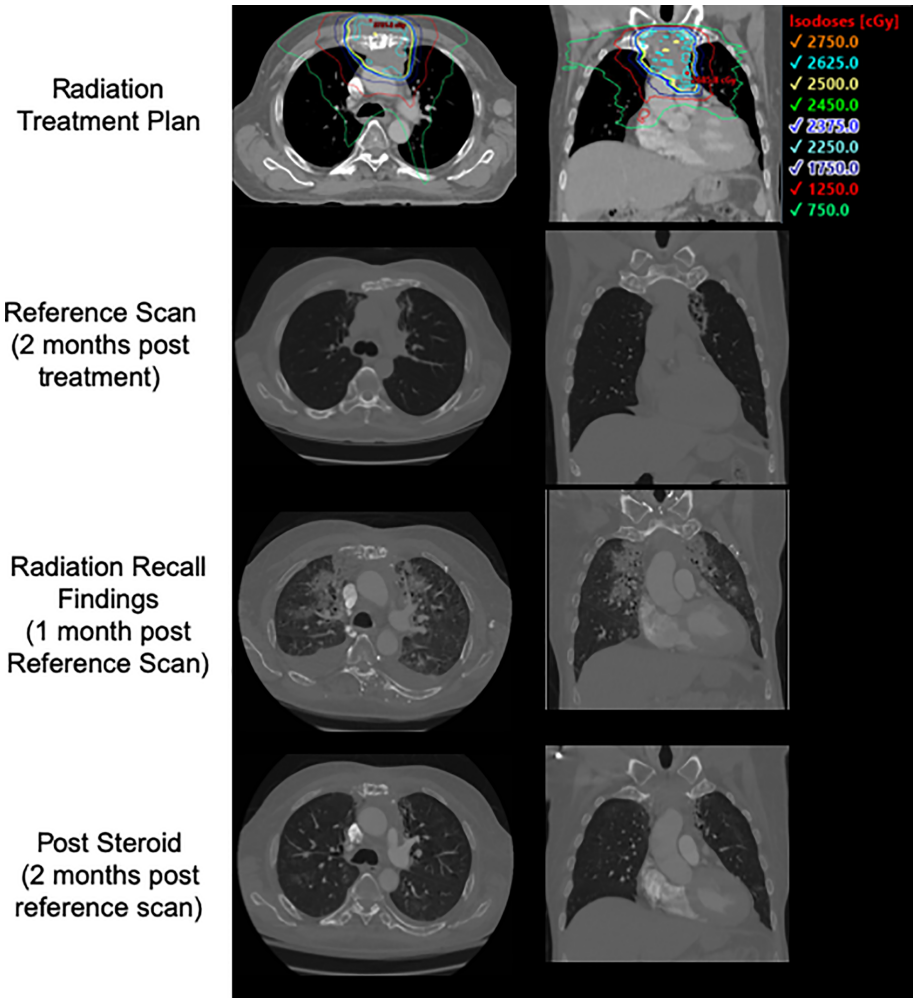


**FIGURE 2** | Patient 2's treatment plan, post treatment CT, CT at time of presentation with radiation recall, and CT following course of steroids with interval improvement.

revealed evolving left lung fibrosis consistent with a recall reaction (**Figure 3**). Infectious workup was unrevealing, and the patient responded rapidly to a tapered course of oral prednisone 50 mg daily. Upon resolution of his symptoms his medical oncologists elected to halt systemic therapies without a re-challenge. To date he has received ablative radiotherapy to the left adrenal gland and SRS twice to new brain metastases without complication.

## CONCLUSIONS

Immunotherapy-related pneumonitis is a well-documented (15, 16) adverse effect of immune checkpoint therapies. However, while prior RT may increase incidence of low grade pneumonitis in patients treated with these drugs (15, 17), radiologic manifestations of immunotherapy-related pneumonitis typically do not overlap with high-dose treatment fields (17), suggesting that RRP is indeed a distinct clinical entity. The pathophysiology of radiation recall remains an area of active investigation; authors have hypothesized radiation-induced: (1) sublethal stem cell damage/reprograming, in which surviving local stem cells lose future proliferative ability or develop aberrant inflammatory responses to systemic agents (2) hypersensitivity reaction in which radiation might lower the inflammatory response threshold, causing localized idiosyncratic drug reactions (3) changes to vascular permeability/proliferation causing local accumulation of systemic agents (i.e. pharmacokinetic effects), and (4) DNA damage and oxidative stress causing keratinocyte necrosis/depletion, amongst others (1, 6, 12, 18). Immunotherapy-mediated RRP implies that direct cytotoxic drugs may not be required to induce recall reactions. Additionally, the tolerance of some patients to re-challenge with checkpoint inhibitor therapies suggests that RRP is not simply

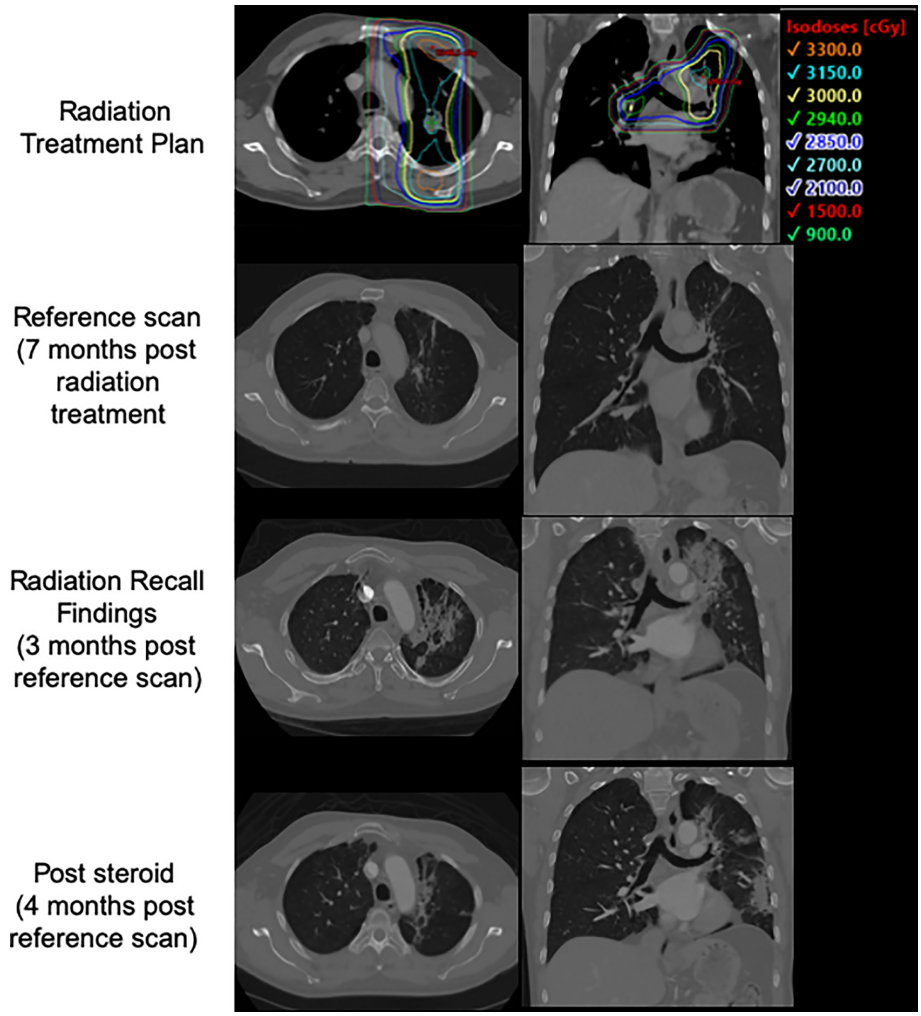


**FIGURE 3** | Patient 3's treatment plan, post treatment CT, CT at time of presentation with radiation recall, and CT following course of steroids with interval improvement.

the result of additive toxicities from these two therapies. These findings have led some to argue that recall reactions are non-immune inflammatory idiosyncratic drug hypersensitivities caused by RT-induced reprogramming of the inflammatory pathway in treated tissues (2). To date, however, there is no established mechanism for radiation recall.

Three recent publications have described a total of 4 cases of patients with primary lung cancers developing RRP following therapy with nivolumab (3 patients) or pembrolizumab (1 patient). In one case (14), a patient developed RRP both at the site of RT for her primary lung adenocarcinoma, and at the site of prior RT for breast cancer on the contralateral lung. Similarly to our patients, all of these patients were successfully treated with oral steroids, and one patient was reported to have tolerated a re-challenge with nivolumab without recurrence of RRP.

This is the largest single-institution immunotherapy-related RRP case series. Upon presentation with RRP, these patients had very focal/asymmetric findings (inconsistent with immunotherapy-related pneumonitis) corresponding closely with the distribution of prior radiation arising acutely post treatment with the offending agent(s), and radiographic (and symptomatic for patients 1 and 3) signs showed marked improvement following treatment with steroids. Dose in the regions demonstrating exudative changes during the recall pneumonitis ranged from 12.5 to 80 Gy (**Table 1**). Interestingly, all of our patients developed symptoms while on dual-agent immunotherapy (one of whom was receiving a novel experimental immunotherapeutic on a clinical trial); patients 1 and 2 had both tolerated immunotherapy(ies) (patient 1: nivolumab and pembrolizumab, patient 2: and an experimental agent with durvalumab, as well as pembrolizumab) prior to the treatment course that is believed to have triggered the RRP. Similarly to the literature on radiation recall reactions more generally, the only patient (patient 1) to trial a re-challenge of

**TABLE 1** | Dosimetric parameters including Max, Mean, V5, V10 radiation doses to the lung and heart in treated patients.

| | Bilateral Lung Max Dose (tGy) | Bilateral Lung Mean Dose (tGy) | Right Lung Mean Dose (tGy) | Left Lung Mean Dose (tGy) | Lung VS (%) | Lung V20 (%) | Heart Mean Dose (cGy) |
|---|---|---|---|---|---|---|---|
| Patient 1 | 8100 | 1803 | 2452 | 1303 | 86.4 | 34.4 | 2440 |
| Patient 2 | 5845 | 1134 | 1014 | 1280 | 69.5 | 10.7 | 819 |
| Patient 3 | 3327 | 795 | 447 | 1293 | 31.1 | 20.8 | 631 |

immunotherapy did so without a recurrence of RRP. If future studies find that patients on dual-agent immunotherapy have a higher propensity towards RRP, an interesting question remains of whether this is a synergistic toxic reaction or simply an additive effect with each agent contributing a minute increased probability of a recall reaction.

There are several limitations to this case series: our patients presented with a variety of primary cancers at different stages, and received distinct systemic therapies and sequencing of radiation therapy regimens. Patient 2, for example, received radiation therapy in two stages, first with palliative intent and subsequently for consolidation as his goals of care evolved. This limits this study to hypothesis generation, and increasing awareness of the potential of recall reactions in patients treated with immunotherapeutics. Additionally, with only 3 patients, it is difficult to identify if there is a temporal relationship between radiation dose and the grade of RRP. Furthermore, there remains the possibility that these reactions may represent a 'recall like-reaction' due to overlapping toxicity and increased risk of pneumonitis from immunotherapy and radiation therapy, or where immunotherapy could be impacting the timing or induction of a conventional radiation pneumonitis. Nevertheless, these cases serve to highlight the potential for radiation recall reactions in the setting of immunotherapy.

As immunotherapeutics have advanced to clinical and community practice, there has been continued monitoring of their toxicities and adverse effects (19), particularly with rare outcomes unlikely to manifest in smaller and controlled clinical trial populations. In each case presented here, patients developed acute radiographic changes consistent with pneumonitis within prior radiation fields months or years after having concluded treatment. In summary, these cases together with other cases from the literature suggest that radiation recall reactions and radiation recall pneumonitis can be associated with immunotherapies. Monitoring for recall pneumonitis and further investigation of the mechanisms underlying radiation recall reactions is warranted.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

Patients were enrolled on UCSD IRB approved studies and provided informed consent for use of data and publication.

## AUTHOR CONTRIBUTIONS

PR, WS, MC, SK, and ABS contributed to conception and design of the study. AS, JM, JH-G, AB, and JR managed and treated patients involved in this study. PR, WS, and MC performed analysis of patient data. PR wrote the first draft of the manuscript. PR, WS, and AS wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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# Current State of Personalized Genitourinary Cancer Radiotherapy in the Era of Precision Medicine

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Radiation therapy plays a crucial role for the management of genitourinary malignancies, with technological advancements that have led to improvements in outcomes and decrease in treatment toxicities. However, better risk-stratification and identification of patients for appropriate treatments is necessary. Recent advancements in imaging and novel genomic techniques can provide additional individualized tumor and patient information to further inform and guide treatment decisions for genitourinary cancer patients. In addition, the development and use of targeted molecular therapies based on tumor biology can result in individualized treatment recommendations. In this review, we discuss the advances in precision oncology techniques along with current applications for personalized genitourinary cancer management. We also highlight the opportunities and challenges when applying precision medicine principles to the field of radiation oncology. The identification, development and validation of biomarkers has the potential to personalize radiation therapy for genitourinary malignancies so that we may improve treatment outcomes, decrease radiation-specific toxicities, and lead to better long-term quality of life for GU cancer survivors.

**Keywords: genitourinary cancer (GU cancer), personalized radiation oncology, precision oncology, prostate cancer, testicular cancer, bladder cancer, renal cell carcinoma, precision medicine**

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## INTRODUCTION

Radiation therapy is instrumental in the management of many genitourinary malignancies. Technological advances in imaging, treatment planning, and treatment delivery have allowed physicians to deliver higher radiation dose to tumor or tumor bed while minimizing dose to surrounding normal tissue. Other advances in screening and other treatment options have translated to improvements in clinical outcomes for patients with genitourinary malignancies. Yet, the optimal management for malignancies on an individualized level is not well understood. There is an urgent need to incorporate more biomarkers to personalize radiation therapy in the era of precision oncology. Here, we review the progress in the identification, development, and validation of biomarkers for genitourinary malignancies to guide treatment recommendations, as well as highlight challenges and opportunities for further investigations in personalized radiation medicine.

# PROSTATE CANCER

Prostate cancer screening, risk stratification, and treatment have advanced dramatically in the past decades. Despite this, the optimal combination of treatments for an individual is not clear nor personalized at this time. The ideal management for an individual with prostate cancer is a complicated decision process, with more than one approach often available. It is therefore imperative to determine which patients are more likely to benefit from a treatment over another, both in terms of cancer control and quality of life, in keeping with precision medicine principles. In addition, it is critical to improve diagnosis and risk stratification with respect to detecting both clinically significant and biologically aggressive disease. Below, we briefly review the current state of various innovative predictive/prognostic tools from detection through risk stratification, as well as advances in radiation delivery to further target the prostate tumor biology.

## Detection and Screening

Prostate cancer detection has been aided by the use of magnetic resonance imaging (MRI). Early use in the 1990s allowed clinicians to evaluate for high-risk features such as extracapsular extension and seminal vesicle invasion (1, 2). Technology evolved and the inclusion of multiple parameters to evaluate the prostate, also known as the multiparametric MRI (mpMRI), has allowed for accurate localization of suspected prostate cancer lesions (3–6). At this time, tissue diagnosis with biopsy remains the gold standard, however mpMRI is a robust supplement in the diagnosis of prostate cancer. In addition, mpMRI has been increasingly incorporated into prostate biopsies by serving as a guide for "targeted" lesions that are not a part of the standard systematic biopsies. This can be accomplished using either a "cognitive" fusion biopsy or an MRI-transrectal ultrasound fusion biopsy. Both have demonstrated improved detection of clinically significant disease and overall disease burden in multiple studies (7–11). A recent phase 3 randomized trial determined that an MRI followed by selected targeted biopsy was noninferior to a standard 12-core transrectal ultrasound biopsy in detecting Gleason 3 + 4 (Grade Group 2) disease or higher (12). mpMRI will continue to play a large role in the detection of prostate cancer as well as in active surveillance.

There are multiple biomarkers that exist designed to be used to aid in the diagnosis of prostate cancer before a positive prostate biopsy (**Table 1**). Serum biomarkers notably include: 1) the Prostate Health Index (PHI) which combines total prostate-specific antigen (PSA), free non-protein bound PSA (fPSA), and an isoform of fPSA known as p2PSA (13); and 2) the 4Kscore, which incorporates serum biomarkers including total PSA, fPSA, intact PSA, and human kallikrein 2, as well as clinical variables to predict risk of high-grade PCa on the biopsy (14). Notable urinary biomarkers include prostate cancer antigen 3 (PCA3), which is a noncoding messenger RNA (mRNA) overexpressed in prostate cancer tissue and detectable in urine after a digital rectal examination (DRE) (15). The TMPRSS2-ERG genomic rearrangement can be detected in post-DRE urine samples with a specificity of 93% and a positive predictive value of 94% for prostate cancer diagnosis (16). TMPRSS2 is an androgen-regulated gene. ERG is a transcription factor that is overexpressed in ~50% of primary prostate tumors (17). TMPRSS2-ERG fusions are described in 30-50% of new prostate cancer diagnoses (18). There are multiple other biomarkers as per **Table 1** that can aid in the diagnosis of prostate cancer and have been shown to outperform PSA as a diagnostic tool, however their use in clinical practice is variable due to their limitations. There is a need to validate and compare these biomarkers against each other in a prospective manner before incorporating into routine clinical practice.

Molecular imaging, most notably, has exploded on the scene in the past few years with the development of several radiolabels specific for prostate cancer. Historically, the role of PET/CT was limited for prostate cancer diagnosis/staging. Multiple PET imaging tracers are being evaluated, with the top three most explored/promising summarized in **Table 2**. $^{11}$C-choline is a radiotracer that was previously explored for prostate cancer diagnosis, however its sensitivity and specificity values ranged from 72-87% and 62-84% respectively. In addition, choline-avid PET images could not reliably distinguish between benign and malignant lesions (19). Since that time, the PET compound $^{18}$F-fluciclovine demonstrated promise for prostate cancer detection, particularly in the setting of biochemically recurrent prostate

**TABLE 1** | Biomarkers for prostate cancer screening.

| Test | Biomarker | Positive |
|---|---|---|
| *Blood-based* | | |
| 4K | Total PSA, fPSA, intact PSA, human kallikrein 2 as well as clinical variables (age, DRE, and prior biopsy results) | ≥9% |
| Prostate Health Index | Total PSA, fPSA (free non-protein bound PSA), and p2PSA (isoform of fPSA)<br>Formula:<br>(2pPSA/fPSA)x$\sqrt{}$PSA | ≥25 |
| *Urine- post DRE* | | |
| PCA3 | Concentration of PCA3 mRNA relative to PSA mRNA | ≥35 |
| TMPRSS2-ERG | Detection of the fusion gene in post-DRE urine | ≥10 |
| MiPS (PCA3) + TMPRSS2-ERG | Combination of PCA and TMPRSS2-ERG | ≥35 + ≥10 |
| SelectMDX | RNA levels of *DLX1* and *HOXC6*<br>Also includes total PSA, PSA density, DRE, age, family history | ≥2.8RS |
| ExoDx | RNA content in extracellular vesicles, measuring RNA and calculating as sum of normalized RCA and RNA ERG | ≥15,6 |

*DRE, digital rectal examination.*

**TABLE 2** | Summary of top PET imaging tracers for prostate cancer.

| PET tracer | Production method | Half-life |
|---|---|---|
| Carbon 11 ($^{11}$C) choline | Cyclotron (onsite) | 20.3 min |
| Gallium 68 ($^{68}$Ga) PSMA | Generator | 67.7 min |
| Fluorine 18 ($^{18}$F) fluciclovine | Cyclotron (regional) | 109.8 min |

cancer (20). Generally, the sensitivity improves with a higher PSA relapse level. Due to its improved technique to detect prostate cancer lesions at lower PSAs in the recurrent setting compared to conventional imaging, this scan is FDA-approved for use in the post-treatment biochemically recurrent clinical setting. More recently, studies using targeted radiolabeling of the prostate-specific membrane antigen (PSMA) for PET/CT demonstrate promising data (21, 22). PSMA is a transmembrane protein that is overexpressed on prostate cancer tumor tissue; thus this can be targeted with a radioligand and lead to enhanced prostate cancer uptake and detection. Gallium 68 PSMA-11 has recently received FDA approval for use in the United States for PET imaging of PSMA positive lesions in patients with suspected prostate cancer metastasis as well as patients with suspected prostate cancer recurrence based on elevated PSA levels. Broad distribution of Gallium 68 PSMA-11 is being worked on. The proPSMA prospective trial demonstrated the superiority of PSMA PET-CT (n=150 men) compared to conventional imaging (n=152 men) for accuracy of identifying pelvic nodal or distant-metastatic disease, with a 27% absolute greater area under the curve (AUC) for accuracy over conventional imaging (92% [88-95] *vs* 65% [60-69] (23). PSMA PET imaging appears to be better than fluciclovine PET at lower PSA levels. A single institutional comparison between PSMA PET and fluciclovine PET in prostate cancer patients with biochemical recurrence after radical prostatectomy (PSA <2.0 ng/mL) found that, in 50 enrolled patients, detection rates were lower with fluciclovine PET (13 of 50, 26%) versus PSMA PET (28 of 50, 56%), with an odds ratio of 4.8 at the patient level (95% CI 1.6-19.2, p=0.0026) (24).

However, not all metastatic disease, particularly castrate-resistant metastatic disease, expresses PSMA. There are many ongoing trials further defining the role of Gallium 68 PSMA-11 PET in both the biochemically recurrent and diagnostic setting, particularly for advanced disease (25).

## Risk Stratification

It has become increasingly evident that using clinicopathologic characteristics alone to stratify patients into various risk categories to guide treatment decisions may be insufficient, as we learn more about heterogenous outcomes in risk groups. There are newer clinical staging/risk-stratification systems that demonstrate promise. These include the STAR-CAP (26) as well as the CAPRA score (27). However, there remains a need to identify and validate markers intrinsic to tumor biology to further stratify patients into risk groups. To that end, there are currently four commercially available gene panels that can be used for localized prostate cancer (**Table 3**). These can be used to further risk-stratify patients to guide personalized treatment decisions.

Prolaris (Myriad Genetics, Salt Lake City, UT) is a gene expression panel consisting of 46 genes (15 housekeeper genes, 31 cell cycle progression genes) which results in a cell cycle progression (CCP) score. It is designed for use on biopsy,

**TABLE 3** | Molecular tests for Prostate Cancer Risk Stratification.

| Genomic classifier | Test | Test independently predicts |
|---|---|---|
| PROLARIS | 46 genes (15 housekeeper, 31 cell cycle progression genes) to determine a cell cycle progression score | -Prostate cancer-specific mortality<br>-Biochemical recurrence<br>-Metastases<br>-Grade group ≥3 or pT3 at time of surgery |
| PROMARK | Expression of 8 genes | -Prostate cancer-specific mortality |
| ONCOTYPE | 17 genes associated with prostate cancer to create Genomic Prostate Score | -Metastases<br>-Prostate cancer-specific mortality<br>-Grade group ≥3 and/or pT3+ disease at time of surgery |
| DECIPHER | 22 RNA biomarkers | -Metastases<br>-Prostate cancer-specific mortality<br>-Postoperative radiation sensitivity<br>-Lymph node metastases<br>- Grade group ≥3 or pT3+ disease at time of surgery<br>-Biochemical failure<br>-Grade group ≥4 at time of surgery |

transurethral resection of the prostate (TURP) specimens, as well as radical prostatectomy specimens. There are a few studies that have evaluated the utility of this biomarker for clinical decision-making. Cuzick et al. used Prolaris on biopsy specimens and determined that the CCP score was the strongest independent predictor of death (28); separately the same group evaluated Prolaris in both prostatectomy and TURP specimens, demonstrating its strong performance as a prognostic factor for biochemical recurrence and time to death, respectively (29). Cooperberg et al. found that the CCP score was able to surpass the performance of a standard postoperative risk assessment score and had improved accuracy of risk stratification for outcomes for men with localized prostate cancer (30). Freedland et al. validated the Prolaris CCP score in the context of men receiving external beam radiotherapy, demonstrating its superior performance to predict recurrence and was associated with prostate cancer-specific mortality (31). Finally, a critical assessment of Prolaris by NICE determined that the use of Prolaris changed clinicians' treatment decisions in at least 47% of cases (32).

The Promark assay (Metamark Genetics Inc, Waltham, MA) uses the expression of eight different genes. This assay was validated using intact tissue biopsies and aids in classification for non-favorable pathology, providing independent prognostic data for stratifying patients (33).

A separate assay consisting of 17 genes was developed called Oncotype DX Genomic Prostate Score (Genomic Health, Redwood City, CA). The expression of these genes is incorporated into an algorithm to create a Genomic Prostate Score (GPS). The score was demonstrated to improve prediction of presence of adverse pathology (34, 35). Interestingly the first prospective study evaluating GPS after initial active surveillance found that there was no association of GPS with adverse pathology in those who underwent radical prostatectomy. There was also no association with upgrading in the surveillance biopsy (36).

The last assay to mention is the Decipher genome classifier (GenomeDx Biosciences, Vancouver, BC, Canada). This assay is based on the analysis of the expression of 22 genes. Decipher has been validated in multiple studies (37–41). A separate study found that in men who underwent radical prostatectomy followed by radiation, Decipher predicted both metastasis and biochemical recurrence (38). A meta-analysis published in 2017 confirmed the prognostic value of the Decipher score, independent from clinicopathologic variables. The meta-analysis included data from five studies in men who underwent radical prostatectomy. A low Decipher score was found to be associated with long-term disease control after surgery, while a higher score was found to be associated with a worse prognosis (41). There are multiple additional studies confirming the utility of Decipher in the post-operative setting, thus this is the assay with the strongest evidence to date. In addition, the Decipher test has been used in the intact setting. In a retrospective multicenter cohort study of 266 with very low, low, and favorable-intermediate risk men, it was found that the Decipher score was an independent predictor of adverse pathology, thus it is an aid to appropriately identify good candidates for active surveillance (42). In a cohort of men with intermediate-risk prostate cancer treated with radiation therapy alone, the Decipher score accurately predicted disease recurrence in these individuals at 5 years (area under the curve 0.78, 95% CI 0.59-0.91) (43). Decipher has been validated as part of a clinical-genomic risk group classification for localized prostate cancer to improve risk stratification, finding that 67% of patients would be reclassified from the standard NCCN risk-system by the new system (44). In an ancillary study of the NRG/RTOG 9601 trial, Decipher was validated and independently associated with distant metastases (hazard ratio [HR] 1.17, 95% CI 1.05-1.32, p=0.006), prostate cancer-specific mortality (HR 1.39, 95% CI 1.20-1.63, p<0.001), and overall survival (HR 1.17, 95%CI 1.06-1.29, p=0.002), after adjusting for age, race/ethnicity, Gleason score, T stage, margin status, entry PSA, and treatment arm (45). Based on this data, several trials are incorporating this risk classifier for stratification to either intensification/de-intensification treatment based on either high or low genomic risk, respectively (NRG-GU009, NCT0451371).

For advanced prostate cancer, studies have found that there are multiple mutations present in genes involved in the DNA repair pathways (DDR genes), particularly in patients with metastatic castration-resistant prostate cancer (mCRPC) (46–50). These mutations have been identified as a biomarker of response to poly ADP ribose polymerase (PARP) inhibitors and platinum chemotherapy. The PROfound trial was a randomized phase 3 trial evaluating PARP inhibitor olaparib in men with mCRPC with disease progression (51). Men had to have an alteration in prespecified genes with a direct or indirect role in homologous recombination repair and were divided into cohort A (at least one alteration in *BRCA1, BRCA2,* or *ATM*, n=245 patients) and cohort B (alterations in any of 12 other prespecified genes, n=142 patients). Imaging-based progression-free survival was longer in the olaparib group compared to control in cohort A (7.4 months versus 3.6 months, HR 0.35, 95% CI 0.25-0.47, p<0.001), yet this was less pronounced in cohort B. A significant benefit was found for olaparib in the overall population (cohorts A and B combined). Based on the promising early data (51–58), there are now multiple clinical trials ongoing to evaluate the utility of combining PARP inhibitors and platinum chemotherapy in prostate cancer patients with DDR mutations.

Separately, there have been multiple mutations associated with androgen receptor signaling in aggressive prostate cancer. A study of mCRPC patients with AR amplification who received first-line docetaxel resulted in a lower risk of death for patients with AR amplification, compared to those who received androgen receptor targeting agents (59). Similarly, in men with AR splice variant 7 (AR-S7), studies have found better outcomes with taxane treatment (60–63).

In men with mCRPC, loss of PTEN is common, leading to the overexpression of the PI3K/AKT pathway (47). This has been shown to lead to increased AR signaling and worse overall clinical outcomes (64), thus trials are underway to combine AR-targeted and PI3K/AKT inhibitors in these populations with PTEN loss.

A separate area of great promise for precision medicine and precision 'omics includes liquid biopsy techniques. This non-invasive technology can allow for biomarker discovery at multiple timepoints without need to rely on biopsies or other means to obtain tissue. Specifically, circulating tumor cells (CTCs), circulating tumor/cell-free DNA (ctDNA, cfDNA) provide snapshots of tumor cells and tumor-derived nucleic acids, respectively. These assays have demonstrated predictive and prognostic promise in metastatic prostate cancer (65) and early data in the localized setting is encouraging (66, 67).

## Advances in Radiotherapy Techniques, Treatment Delivery

Major advances in radiotherapy technique and delivery have led to the ability to target the prostate gland accurately, while largely avoiding normal tissues and sparing toxicity. This has allowed for dose escalation and improved treatment outcomes. Our improved understanding of the radiobiology behind prostate cancer has led to our current efforts and advances in techniques, while eventual integration with genomic tests/molecular understanding of a prostate tumor on an individual level can allow physicians to further personalize radiation therapy with these new techniques.

Innovations in imaging and other technologies have greatly contributed to our ability to "dose-escalate" prostate radiation treatment. For example, the use of a perirectal hydrogel spacer has been shown to be associated with lower dose to the rectum as well as decreased rectal toxicity (68, 69). A multicenter randomized controlled trial demonstrated a reduction in late (defined as 3-15 month) rectal toxicity severity in the spacer group, with 2.0% and 7.0% late rectal toxicity incidence in the spacer and control groups, respectively (p=0.04) (68). This is particularly beneficial for prostate cancer, as multiple hypotheses exist relating to the intrinsic radiobiology of prostate cancer. Emerging evidence suggests that such biology leads to greater sensitivity to increased fraction size (70). Other data suggest that prostate cancer harbors a lower $\alpha/\beta$ (a metric characterizing tissue/tumor sensitivity to radiation dose per treatment) compared to the surrounding normal tissues. This indicates that hypofractionated radiation (delivery of a higher dose to the prostate gland per treatment, for fewer total treatments) may improve cancer control. Thus, there has been great interest in moderate hypofractionation (generally accepted as 2.4-3.4 Gy per fraction (fx)) as well as ultrahypofractionation (generally accepted as >4-5 Gy/fx) (71, 72). Modern noninferiority trials have demonstrated excellent overall outcomes in comparison to standard fractionation (**Table 4**) (73–76). One superiority randomized trial comparing 75.6 Gy in 1.8 Gy/fx to 72 Gy in 2.4Gy/fx also demonstrated improved cancer control with moderate hypofractionation (77). This approach may be preferred for men with localized prostate cancer given the improved resource utilization and convenience. Ultrahypofractionated is an extreme form of hypofractionation, and there are several ongoing studies exploring its utility for localized prostate cancer. The HYPO-RT-PC trial demonstrated worse acute urinary toxicity with ultrahypofractionation (78). However, in the recently published PACE-B trial, ultrahypofractionation was not found to increase acute genitourinary or gastrointestinal toxicity (79). The ongoing RTOG 0938 trial is a phase II randomized trial evaluating 2 ultrahypofractionation regimens, 36.25 Gy in 5 nonconsecutive fractions or 51.6 Gy in 12 daily fractions; patient-reported outcome data did not demonstrate any significant difference between the two treatment schedules (80).

A separate method of "dose escalation" involves boosting visible tumor within the prostate that is visualized *via* multiparametric MRI with external beam radiation therapy. A recent phase III randomized controlled trial (FLAME) evaluated the utility of a focal lesion microboost in patients with intermediate- and high-risk prostate cancer (81). This demonstrated improved biochemical disease-free survival in the men who received the focal boost compared to the standard arm (HR 0.45, 95% CI 0.28-0.71, p<0.001), and there was no impact on toxicity or quality of life. With five years of follow-up, there was no difference in prostate cancer-specific survival nor overall survival for now, but this might become significant with longer follow-up.

Proton beam technology has the physical advantage of depositing energy in the tissue at the end-of-range, thus potentially sparing critical normal tissues such as the rectum and bladder in prostate cancer patients (82). Studies to date have not demonstrated an improvement in toxicity rates or clear benefit for protons. For example, a recent multi-institutional analysis of 1850 early-stage prostate cancer patients treated with either moderately hypofractionated photon or proton therapy on a registry demonstrated low rates of toxicity and no difference in late gastrointestinal or genitourinary toxicity (83). Yet, there are several ongoing trials evaluating protons versus photons for localized prostate cancer that will help to guide our understanding of the potential benefit for protons in this clinical space. A large ongoing randomized phase III trial of proton therapy versus intensity-modulated radiation therapy for low- to intermediate-risk prostate cancer called Prostate Advanced Radiation Technologies Investigating Quality of Life, or PARTIQoL (NCT01617161), as well as large prospective observational cohorts such as a Prospective Comparative Study of Outcomes with Proton and Photon Radiation in Prostate Cancer (COMPPARE, NCT03561220) and the Japanese multi-institutional prospective registry (UMIN000025453), will help to inform the debate between protons versus photons for localized prostate cancer.

Novel imaging techniques surrounding the identification and detection of prostate cancer as discussed above are changing how to treat this disease with radiation therapy, particularly in the post-operative setting. The role of PET/CT imaging was previously limited, however the introduction of novel imaging tracers including $^{18}$F-fluciclovine PET (**Figure 1**) and prostate-specific membrane antigen (PSMA) targeted agents has the potential to change clinical practice. Studies suggest that these novel radiotracers can modify radiation treatment intensification (84) as well as lead to an early improvement in failure rates (85). The LOCATE trial demonstrated increased detection of 1 or

**TABLE 4** | Moderate hypofractionation trials for prostate cancer.

| Trial | Type | Year | N | Trial arms | Median FU | Primary endpoint | Findings | Toxicities |
|---|---|---|---|---|---|---|---|---|
| PROFIT (73) | Noninferiority | 2017 | 1206 | 78 Gy/39 Fx *vs* 60 Gy/20 Fx | 6.0 y | Disease-free survival | HR (95% CI): 0.96 (0.74–1.25) | No significant difference in late toxicity |
| HYPRO (74) | Noninferiority | 2016 | 804 | 78 Gy/39 Fx *vs* 64.6 Gy/19 Fx | 5.0 y | Relapse-free survival | HR (95% CI): 0.86 (0.63-1.16) | Higher grade 2+ acute GI toxicity with hypoFx; Higher grade 2+ late GU toxicity with hypoFx |
| CHHiP (75) | Noninferiority | 2016 | 3163 | 74 Gy/37 Fx *vs* 60 Gy/20 Fx *vs* 57 Gy/19 Fx + 3-6 mo ADT | 5.2 y | Time to biochemical failure | HR (95% CI): 0.84 (0.68–1.03) 57 Gy/19 Fx inferior to 74 Gy/37 x | No significant differences but trend toward increased late grade 2+ GU toxicity |
| RTOG 0415 (76) | Noninferiority | 2016 | 1092 | 73.8Gy/41 Fx *vs* 70 Gy/28 Fx | 5.8 y | Disease-free survival | HR (95% CI): 0.85 (0.64-1.14) | Increased GI/GU late grade 2+ with hypofx |
| Hoffman et al. (77) | Superiority | 2018 | 206 | 75.6 Gy/42 Fx *vs* 72 Gy/30 Fx | 8.5 y | PSA failure | 8-y failure rate 10.7% (95% CI: 5.8%–19.1%) for 72 Gy *vs* 15.4% (95% CI: 9.1%–25.4%) for 75.6 Gy, P = 0.036 | Nonsignificant increase in late grade 2+ GI toxicity with hypoFx |

more recurrences using $^{18}$F-fluciclovine PET/CT in men with biochemical recurrence (122 of 213 patients, 57%), and 59% of patients had a change in management after the scan (86). Similarly, the FALCON trial demonstrated that the use of $^{18}$F-fluciclovine PET/CT in 104 men with biochemical recurrence resulted in 64% of patients with a change in treatment management (87). Multiple prospective trials are underway in various prostate cancer settings (diagnostic, localized, post-operative, recurrent, metastatic) to further standardize and validate its use in various clinical settings.

As precision oncology continues to evolve for the management of prostate cancer with improved biomarkers and improved detection of disease, the role of radiation therapy is also evolving, particularly with regards to the definitive management of oligometastatic disease. Oligometastatic disease refers to a stage where metastatic disease is still limited, and aggressive therapy directed at involved lesions may improve outcomes. The definition of oligometastatic prostate cancer varies, as the CHAARTED study defined oligometastatic disease as ≤3 metastases, and no visceral metastases (88), yet other studies

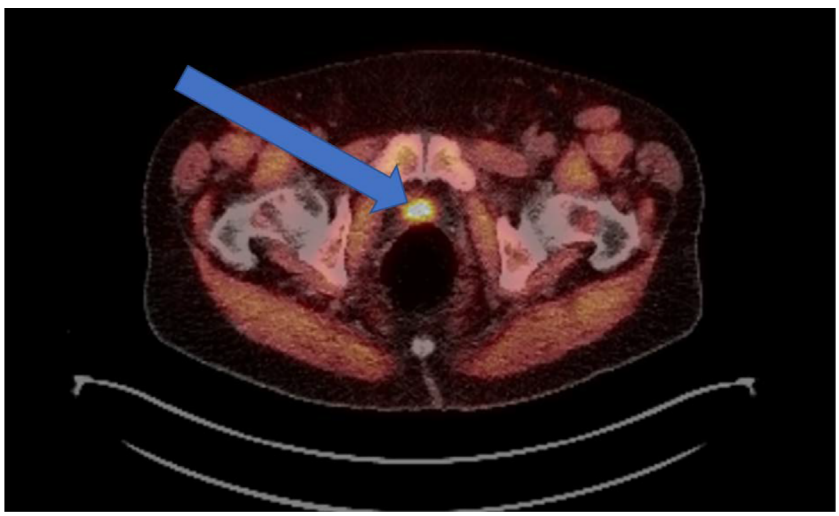

**FIGURE 1** | Example of an $^{18}$F-fluciclovine PET avid lesion in a biochemically recurrent prostate cancer patient. Demonstration of a 2.3-cm $^{18}$F-fluciclovine PET-avid lesion in the prostatectomy bed. Patient was post-radical prostatectomy and presented with a PSA of 0.84 ng/mL.

define oligometastatic disease as ≤5 metastases (89). In this space with evidence of overall limited disease, stereotactic ablative radiation therapy (SABR) may become a part of regular treatment management.

Both the HORRAD (90) and STAMPEDE (91) trials evaluated the role of definitive local treatment to the primary prostate in the setting of metastatic disease. In both trials, there was no difference in overall survival with the use of prostate primary-directed radiation therapy. However, on subgroup analysis in HORRAD, there was a trend toward benefit in overall survival with radiation therapy in men with low metastatic burden (defined as ≤5 metastases, HR 0.68, 95% CI 0.42-1.10). Based on this, analysis by metastatic burden was a prespecified subgroup analysis in STAMPEDE. The subgroup analysis met many of the subgroup analysis criteria put forth by Sun et al. (92). Aa survival benefit was found in favor of primary prostate radiotherapy in men with ≤3 metastases (HR 0.68, 95% CI 0.52-0.90, p=0.0098). A secondary analysis of this trial demonstrated a significant survival benefit in patients with lymph node only metastases (HR 0.60, 95% CI 0.33-1.09) as well as a failure-free survival benefit beyond 4 bone metastases up to 8 bone metastases (93). Overall, these are encouraging results but need to be further evaluated prospectively, particularly with new imaging modalities.

With respect to treatment of the metastatic lesions rather than the primary, the STOMP trial evaluated the benefit of metastasis-directed therapy (MDT, either surgery or radiation) in patients with biochemical recurrence after primary prostate treatment and ≤3 metastases, with a primary endpoint of ADT-free survival (94). After a median follow-up of 3 years, aggressive metastasis-directed therapy did increase ADT-free survival (median ADT-free survival was 13 months [80% CI 12-17 months] for surveillance versus 21 months [80% CI 14-29 months] in MDT group, p=0.11). Separately, the ORIOLE trial, a randomized phase 2 trial, evaluated observation versus SABR to metastatic disease in men with 1-3 metastases, found that, with a median follow-up of 18.8 months, SABR improved median progression-free survival (PFS, not reached versus 5.8 months, HR 0.30, 95% CI 0.11-0.81, p=0.0023) (95). This trial incorporated the use of PSMA-PET, thus this is a contemporary evaluation of SABR in the oligometastatic disease space. More work needs to be performed to further define oligometastatic disease (number of metastases, oligoprogressive versus oligorecurrent, etc), understand the benefit of treatment to the primary versus metastases (versus both), benefit in setting of standard and escalated therapies, and others, but there remain many exciting opportunities for exploration into these questions to define the role of radiation therapy in this space.

## BLADDER CANCER

There are two standard treatment options for muscle-invasive bladder cancer: 1) radical cystectomy, and 2) bladder preservation therapy (or trimodality therapy, TMT). Bladder preservation therapy is comprised of a combination of maximal transurethral resection of bladder tumor (TURBT), followed by chemoradiation. Molecular understanding of individual muscle-invasive bladder tumors may lead to predictive and prognostic biomarkers that can aid with treatment selection for individuals. Already, there are several promising candidates (96).

Bladder tumors frequently display mutations in DNA repair pathways, which likely drive bladder tumor development (97). MRE11 has demonstrated promise as a biomarker of radiation response. One study evaluated immunohistochemical staining of MRE11 in a cohort of patients treated with radiation alone (98). It was determined that patients with the lowest amounts of MRE11 staining had an associated worse 3-year cancer-specific survival. This was validated in a study evaluating MRE11 expression in tissues from 6 NRG/RTOG bladder-sparing radiation protocols. Low levels of MRE11 nuclear/cytoplasmic expression scores were associated with significantly higher disease-specific mortality (99). Other groups have demonstrated a similar association. Laurberg et al. demonstrated low MRE11 staining was associated with worse disease-specific survival in a cohort of 148 patients treated with bladder preservation (100). They also found no associated with MRE11 staining and outcomes among patients who were treated with cystectomy. In a study by Teo et al., a single-nucleotide polymorphism (SNP) in the *MRE11A* gene was associated with worse outcomes among patients treated with radiation therapy, but not among patients treated with cystectomy (101). Interestingly, this SNP was not associated with increased or decreased MRE11 measured by immunohistochemistry.

Further investigations into DNA repair pathway alterations have been performed more often in cohorts of those who received neoadjuvant chemotherapy followed by cystectomy or in those with metastatic disease. However, a small study of 48 patients treated with bladder preservation found deleterious mutations in DNA repair pathways, in particular, *ERCC2*, were associated with improved outcomes after chemoradiation (102). More work needs to be done in bladder preservation-specific cohorts.

Separately, alterations in signal transduction pathways have also been implicated in bladder preservation. In a study using patients enrolled in four prospective bladder preservation studies (RTOG 8802, 8903, 9506, 9706), EGFR expression assessed by immunohistochemistry was associated with improved outcomes in both univariate and multivariate analyses. Conversely, HER2 expression *via* immunohistochemistry was associated with poorer outcomes, specifically with reduction in complete response after chemoradiation (103). This latter finding was confirmed by Inoue et al. in a cohort of 119 patients treated with bladder preservation therapy (104). HER2 overexpression was associated with pathologic incomplete response and worse cancer-specific survival, suggesting resistance to chemoradiation. The RTOG 0524 phase I/II trial evaluated the use of trastuzumab in patients who were HER2/neu 2+ or 3+ along with concurrent paclitaxel and radiation, versus radiation and concurrent paclitaxel in patients who were HER2/neu-negative or 1+ by immunohistochemistry (105). It was found that both groups had similar complete response rates, thus suggesting that in patients

with HER2/neu 2+ or 3+ expressing tumors, the addition of trastuzumab mitigated the previously associated worse prognosis. This finding needs to be further evaluated in a randomized study but demonstrates the ability of biomarker-driven trials to improve outcomes in challenging diseases.

There has been work defining various molecular subtypes based on gene expression profiles (106–110). The subtypes, broadly characterized based on luminal and basal gene expression patterns, have been correlated with response to treatments including cystectomy and neoadjuvant chemotherapy. However, their association with response to chemoradiation is not clear. In one of the largest studies of molecular subtypes within a cohort of patients receiving bladder preservation therapy, specifically TMT, four subtypes were described, luminal, luminal-infiltrated, basal, and claudin-low (111). There was no association with complete response, disease-specific survival or overall survival within the cohort. Further investigation and validation with other TMT/bladder preservation cohorts is necessary.

In patients who are not eligible for concurrent chemotherapy, the use of carbogen and nicotinamide to modify hypoxia in tumors resulted in improved survival compared to radiation alone in the BCON trial (112). Upon further analysis by molecular subtype, patients with a basal subtype had greater benefit with hypoxia modification while those with a luminal subtype had no benefit (113). A 24-gene hypoxia signature was developed and validated in the BCON cohort and found that patients with "high-hypoxia" per the signature had improved outcomes compared to those with "low-hypoxia" with the use of hypoxia modification (114). Both the hypoxia signature as well as the molecular subtype have yet to be validated prospectively to guide use of hypoxia modification but serve as early tools to aid in development of future trials.

Finally, identifying biomarkers related to immune checkpoint inhibition (ICI) response in muscle-invasive bladder cancer is of critical importance, particularly given the potential for improved response when combining ICIs with radiation therapy. There are multiple ongoing clinical investigations into the potential synergy of combination ICI + radiation therapy in this patient population. The aforementioned work evaluating molecular subtypes within a cohort of patients receiving TMT also evaluated immune signatures based on gene expression, finding that signatures associated with T-cell activation and interferon-gamma signaling were associated with improved disease-specific survival in the TMT cohort (111). In a comparison cohort of patients with muscle-invasive bladder cancer treated with neoadjuvant chemotherapy and radical cystectomy, this association did not hold. These promising data demonstrate that immune-related biomarkers may have implications for TMT in muscle-invasive bladder cancer, and potentially the combination of TMT + ICIs. This will need to be further examined in the ongoing trials evaluating TMT + ICI, such as the INTACT: SWOG/NRG 1806 study, evaluating chemoradiotherapy +/- atezolizumab in muscle-invasive bladder cancer (NCT03775265); the KEYNOTE-992 study, evaluating chemoradiotherapy +/- pembrolizumab in muscle-invasive bladder cancer (NCT04241185); the CCTG BL13 trial, evaluating chemoradiotherapy followed by +/- adjuvant durvalumab; and the INSPIRE: ECOG-ACRIN/NRG EA8185 trial, evaluating chemoradiation +/- durvalumab in node-positive urothelial carcinoma (NCT04216290).

Liquid biopsy tools such as CTCs and ctDNA are similarly being investigated as prognostic biomarkers in bladder cancer as they are in prostate cancer (115, 116). CTCs and ctDNA will be collected and assessed in both the abovementioned INTACT and INSPIRE trials to determine their role as predictive biomarkers for overall outcomes after bladder preservation therapy. The presence of circulating biomarkers is also being explored in the surveillance setting post-treatment. One study in patients undergoing neoadjuvant chemotherapy followed by radical cystectomy found that the presence of ctDNA was prognostic for worse outcomes overall (115). This has not yet been evaluated in a TMT cohort but the data from INTACT and INSPIRE will help elucidate the role of liquid biomarkers in TMT-specific cohorts.

## Advances in Radiotherapy Techniques, Treatment Delivery

Advances in image-guidance for radiation therapy has facilitated both dose escalation, hypofractionation, and adaptive planning for bladder cancer patients. Older trials evaluated multiple options for radiation dose, fields, and frequency of radiation treatment, thus there is no standard at this time. The INTACT trial is very inclusive and allows a variety of radiation fields, per physician discretion. Regarding dose, a recent meta-analysis of two randomized, controlled, phase 3 trials in the UK demonstrated that a hypofractionated schedule of 55 Gy in 20 fractions is non-inferior to conventional fractionation (64 Gy in 32 fractions) (117). However, there appears to be a non-trivial increase in unacceptable gastrointestinal grade 3 toxicity when using hypofractionation in combination with immune checkpoint inhibitors, based on results from a phase I trial of atezolizumab and chemoradiation (50 Gy/20 fractions) for muscle-invasive bladder cancer (118) as well as a phase I trial of pembrolizumab and weekly radiation of 6 Gy per fraction to a dose of 36 Gy (119). Both trials had a small number of patients (n=8 and 5 respectively), and both were stopped early due to the dose-limiting toxicities observed. Thus, at this time, the INTACT trial uses conventional fractionation to avoid events that may contribute to dose-limiting toxicities.

To better delineate the primary bladder tumor, other imaging modalities are being explored that may aid in tumor-directed treatment. FDG-PET/CT may improve initial staging to better select patients for TMT, but physiologic uptake in the bladder limits its ability to better delineate the bladder tumor (120, 121). Multiparametric MRI is being explored to improve bladder tumor staging with advanced identification of muscle-invasion. MRI may also improve response assessment after bladder preservation therapy (122–125). At this time, further work is necessary to define the role of multiparametric MRI in the management of muscle-invasive bladder cancer.

Ongoing trials are evaluating the utility of adaptive planning for treatment of advanced bladder cancer. One such trial is the RAIDER study (NCT02447549), which is a randomized phase II trial of either standard planning and radiation delivery, adaptive image guided tumor-focused radiation, or adaptive image guided dose-escalated tumor boost radiation. The primary endpoint is the proportion of patients meeting radiation dose constraints to the bladder, bowel, and rectum in the dose-escalated group, as well as the proportion of patients experiencing severe late side effects following treatment.

In the field of precision oncology, there are many exciting opportunities for radiation in the treatment of muscle-invasive bladder cancer. As ongoing trials start to close and more study into potential biomarkers is completed, the resulting data will aid in our improved selection and treatment of candidates for bladder-preservation.

## TESTICULAR CANCER

In testicular seminoma, current treatment approaches have made this disease highly curable. Historically, radiation was the primary treatment for this disease, but the preferred treatment landscape has changed. For stage I seminoma, active surveillance is now the preferred treatment option (126). Emphasis on biomarkers of recurrence is necessary, as there are no current clinicopathologic variables that can be relied upon. Serum tumor markers are rarely elevated in a recurrence setting, and multiple studies suggest that they are unnecessary during surveillance follow-up (127, 128). Tumor size has been suggested as a risk factor for recurrence, however data is mixed on its prognostic ability (129, 130). miRNAs have demonstrated early promise as both diagnostic and prognostic markers (131, 132) but validation is required. The surveillance strategy consists of frequent computed tomography scans and follow-up. Yet, the seminoma population is very young, thus there is an emphasis to minimize irradiation. The Trial of Imaging and Surveillance in Seminoma Testis (TRISST, NCT00589537) evaluated the utility of decreased number of scans (from 7 to 3) as well as replacing CT scans with MRI (133). Results were recently presented at the 2021 GU Cancers Symposium and found that MRI is non-inferior to CT, and thus should be recommended, and a 3-scan schedule is non-inferior to 7 scans. The surveillance paradigm will likely shift given these recent findings, and this trial reaffirms that surveillance is both safe and effective in stage I seminoma.

For early stage II disease (specifically stage IIA), treatment options include either radiation therapy or chemotherapy (typically 3-4 cycles of etoposide/cisplatin/bleomycin). Radiation therapy is preferred over chemotherapy given the favorable tolerability and toxicity profile (126). However, greater precision is needed with selection of treatment. There are currently no tools to help inform the decision between radiation or chemotherapy in this clinical setting.

When radiation therapy is indicated, there have been efforts to further limit radiation dose to organs-at-risk in this young population. Originally, radiation was delivered using 30 Gy in 15 fractions in the adjuvant setting. Yet, the recognition of seminoma as highly radiosensitive led to the pivotal trial exploring 30 Gy/15 fractions versus 20 Gy in 10 fractions (134). After a median follow-up of 61 months, it was determined that 20 Gy was just as effective and non-inferior to 30 Gy. Further reduction in dose to organs-at-risk may be accomplished using proton beam therapy. Proton beam technology is a promising treatment modality for this patient population given its unique physical characteristics. A recent study comparing patients between proton beam therapy and photon-based treatment demonstrated excellent outcomes and no in-field secondary malignancies (135), although this data is limited with only 55 patients included and a median follow-up of 61 months. Separately, a dosimetric modeling study demonstrated superior sparing of organs-at-risk with protons as compared to photons. Proton beam therapy was estimated to avert 300 excess second cancers among 10,000 men treated at a median age of 39 and surviving to age 75 (136). Proton beam therapy should be strongly considered and further evaluated for men with testicular seminoma.

Decreased field size has been highlighted specifically to further limit dose to organs-at-risk. Emphasis on decreasing field size was evaluated in a trial for stage I testicular seminoma patients, randomizing patients to either a para-aortic strip or ipsilateral iliac lymph node irradiation (dog-leg field) (137). After a median follow-up of 4.5 years, the para-aortic strip was non-inferior to the dog-leg field and reduced toxicity; it is now accepted as standard-of-care for adjuvant radiation treatment for stage I seminoma. More recently, an analysis of metastatic lymph node positives respective to vascular anatomy was performed in seminoma patients and suggested modified treatment fields based on vascular anatomy to decrease normal tissue irradiation (138). This study demonstrated that the superior border of the treatment field can safely be decreased from the T10/T11 interspace to the T11/T12 interspace. In addition, this has led to a greater emphasis on tailored nodal treatment fields based on vascular, rather than bony, anatomy.

Overall, more work is needed in the field of biomarkers for testicular cancer, particularly as it relates to radiotherapy, in the surveillance setting, for treatment selection and for response to treatment.

## RENAL CELL CARCINOMA

The management of renal cell carcinoma (RCC) has been revolutionized by targeted kinase inhibitors (TKIs) as well as immunotherapy. Traditionally, RCC was deemed "radioresistant" and the role for radiation therapy was limited to mostly palliation. However, the rapid advancement of on-treatment image guidance, as well as highly conformal techniques to deliver a high-dose-per-fraction, has paved the way for stereotactic ablative radiation therapy (SABR) to play a role in definitive treatment of RCC (139–141). A 2019 meta-analysis of 26 studies targeting primary RCC with SABR demonstrated excellent local control and low grade 3-4 toxicity rates (142).

Regarding kidney function, a prior study of 21 patients with inoperable RCC demonstrated reasonable change in mean GFR at 2 weeks (+0.6 +/- 11.3 ml/min), 3 months (+3.2 +/- 14.5 ml/min), and 1 year (-8.7 +/-13.4 ml/min) (143). Ongoing trials are further evaluating the safety and efficacy of SABR to primary RCC (NCT02853162, NCT03108703, NCT01890590, NCT02613819, NCT03747133) and will help to establish the role of SABR for primary RCC.

Separately, there are numerous studies demonstrating a potential synergistic antitumor effect with SABR in combination with targeted therapies for metastatic RCC (mRCC). For example, SABR to an "oligoprogressive" lesion was found to extend the efficacy of sunitinib from 14 to 22 days (141). There is a lot of interest and ongoing trials evaluating the efficacy of combined immunotherapy with radiation, given case reports that have described an observed abscopal effect in the setting of both radiation and immune checkpoint inhibition (144). The phase II NIVES study (NCT03469713) is a single-arm study, evaluating the role of SABR to metastatic lesions in mRCC patients who receive nivolumab. Early data demonstrate a median PFS of 4 months, which is not much different from the nivolumab alone arm on CheckMate025, a trial randomizing mRCC patients to nivolumab versus everolimus (145). The RADVAX trial (NCT03065179) is a single-arm study evaluating the role of SABR to metastases in mRCC patients who receive both nivolumab and ipilumumab, with a median PFS of 8.2 months thus far. This is also not much different from the nivolumab + ipilumumab arm in CheckMate214, which randomized mRCC patients to either dual checkpoint inhibition or sunitinib (146). However, in the RAPPORT trial that was presented at the recent 2021 GU Cancers Symposium, patients with low burden of metastases received SABR (20 Gy x 1) and pembrolizumab (147). The treatment was well tolerated and the median PFS was 15.6 months, which is improved over the KEYNOTE-427 trial of pembrolizumab monotherapy (PFS of 7.1months). Further work is necessary to understand appropriate patient selection to a confer a benefit for SABR. The CYTOSHRINK trial is a phase II trial of nivolumab and ipilumumab +/- SABR to the primary RCC in mRCC patients (NCT04090710). Other trials are being opened in this space to evaluate the role of SABR to the primary or the primary + metastases in combination with immune checkpoint inhibitors to potentiate the effect of immunotherapy and improve outcomes in this disease space.

## SUMMARY

Advances in technology have led to a greater understanding of the molecular characterization of genitourinary cancers. Separately, developments in radiation therapy have led to improved tumor targeting as well as decreased dose to surrounding normal tissues. However, there is an urgent need to incorporate molecular information about various genitourinary malignancies to personalize radiation treatment. Just in the past few years, considerable progress has been made within the GU field with many promising biomarkers that have the potential to optimize radiation management that need to be validated. There remain many exciting opportunities for biomarker discovery as well as a need to validate the utility of biomarkers into initial management of genitourinary malignancies. We advocate for the incorporation of known tumor biomarkers into prospective clinical trials as well as for incorporation of translational studies for further biomarker discovery. Continued effort is necessary to one day fully integrate tumor biology to inform management decisions, with the ultimate goal of improving outcomes for our patients.

## AUTHOR CONTRIBUTIONS

Concept and design: SK and JE. Drafting of the manuscript: SK and JE. Critical revision of the manuscript for important intellectual content: SK and JE. All authors contributed to the article and approved the submitted version.

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# Immunotherapy Combined With Radiation Therapy for Genitourinary Malignancies

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Immunotherapy drugs have recently been approved by the Food and Drug Administration for the treatment of several genitourinary malignancies, including bladder cancer, renal cancer, and prostate cancer. Preclinical data and early clinical trial results suggest that immune checkpoint inhibitors can act synergistically with radiation therapy to enhance tumor cell killing at local irradiated sites and in some cases at distant sites through an abscopal effect. Because radiation therapy is commonly used in the treatment of genitourinary malignancies, there is great interest in testing the combination of immunotherapy with radiation therapy in these cancers to further improve treatment efficacy. In this review, we discuss the current evidence and biological rationale for combining immunotherapy with radiation therapy, as well as emerging data from ongoing and planned clinical trials testing the efficacy and tolerability of this combination in the treatment of genitourinary malignancies. We also outline outstanding questions regarding sequencing, dose fractionation, and biomarkers that remain to be addressed for the optimal delivery of this promising treatment approach.

**Keywords: immunotherapy, radiation therapy, renal cancer, bladder cancer, prostate cancer, genitourinary cancer (GU cancer), radiotherapy, immune checkpoint inhibitor**

## INTRODUCTION

Prostate, bladder, and kidney/renal pelvis cancers rank fourth, seventh, and eighth, respectively, in estimated cancer-related deaths in the United States in 2020 (1). Radiation therapy is a well-established treatment modality for genitourinary malignancies, with clinical utility in the definitive, adjuvant, and palliative settings. In localized prostate cancer, for example, radiation therapy is a curative treatment option with survival outcomes that have been shown to be equivalent to those of radical prostatectomy (2). In bladder cancer, radiation is a critical part of bladder-preserving trimodality therapy, which has comparable outcomes to radical cystectomy in well-selected patients (3). Renal cell carcinoma has traditionally been considered relatively radioresistant, but recent advances in radiation delivery and image guidance technologies have led to the development of

stereotactic body radiotherapy (SBRT), which enables the focal and conformal delivery of ablative radiation doses sufficient for the definitive treatment of primary renal cancer (4). In patients with metastatic cancer, palliative radiotherapy is frequently used to alleviate pain from bone metastases. In addition, emerging data suggests that in oligometastatic cancers with five or fewer metastatic lesions, the aggressive use of SBRT to ablate all sites of metastatic disease can lead to improved clinical outcomes (5, 6).

The last several years have also seen the rapid availability of immunotherapy drugs that increase overall survival in patients with a variety of cancers, including genitourinary malignancies (7). Immunotherapy utilizes the patient's immune system to induce tumor cell killing and can be either active or passive in nature. Active immunotherapy directly targets tumor cells and includes antibody therapy and chimeric antigen receptor T-cell therapy. In contrast, passive immunotherapy enhances the ability of the immune system to eradicate tumor cells and includes checkpoint inhibitors and cytokines. Among these approaches, immune checkpoint inhibitors have shown some of the most promising clinical activity to date. Currently available checkpoint inhibitors target two immune checkpoints: PD-1/PD-L1, which modulates T-cell activity resulting in immune response inhibition (8), and CTLA-4, an immunoglobulin expressed by activated T cells that downregulates immune response (9). FDA-approved therapies that target these immune checkpoints include atezolizumab, durvalumab, pembrolizumab, nivolumab (PD-1/PD-L1), and ipilimumab (CTLA-4), among others (10, 11).

Recent data suggest that radiotherapy and immunotherapy may act synergistically, and there has been mounting excitement about the possibility of combining these modalities to further improve outcomes in patients with genitourinary cancers. In this review, we discuss the pre-clinical mechanistic rationale for combining radiotherapy with immunotherapy, as well as emerging data from ongoing and planned clinical trials testing the efficacy and tolerability of this combination in genitourinary malignancies.

# BIOLOGICAL RATIONALE FOR COMBINING RADIOTHERAPY AND IMMUNOTHERAPY

## Radiotherapy Can Augment Immunotherapy

Several lines of evidence suggest that radiation can stimulate the tumor immune microenvironment, a concept that underlies a key rationale for combining radiotherapy with immunotherapy (12). In many cancers, the immune microenvironment becomes altered from a state of immune recognition/antagonism towards a state of immune escape, where the immune system becomes incapable of combatting the tumor (13). Biological changes commonly associated with immune escape include reduced MHC-class 1 expression, upregulated inhibitory ligands and cytokines, and increased numbers of myeloid-derived suppressor cells (14). Although the primary mechanism by which radiation causes local cell death is through the induction of DNA double-strand breaks (15), radiation has been shown to be immunogenic through the direct and indirect activation of innate and adaptive immune response **(Figure 1)** (16). Local cell death caused by radiation instigates the direct release of tumor antigens and promotes the priming and activation of cytotoxic T cells. In addition, radiation can promote the ability of antigen-presenting cells to present tumor antigens to naive T cells through the stimulation of calreticulin, a calcium-binding protein that promotes phagocytosis (17, 18). Conversely, radiation has also been found to downregulate the presence of CD47, a protein that signals down-regulation of phagocytosis (19). High radiation doses have been shown to increase MHC-1 expression, increasing the likelihood of tumor-specific peptide presentation by antigen-presenting cells to naïve T cells (20). This phenotype, in conjunction with increased expression of death receptors such as Fas, facilitates the immune system's ability to kill tumor cells by enhancing the visibility of the tumor

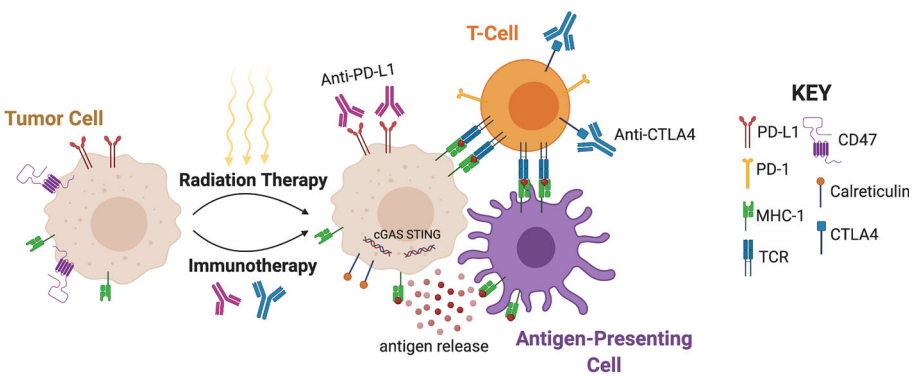


**FIGURE 1** | Mechanisms underlying synergy of radiotherapy and immunotherapy. Radiation promotes the ability of antigen-presenting cells to present tumor antigens to naive T cells through antigen release, stimulation of calreticulin, and downregulation of CD47. MHC-1 expression and the subsequent antigen presentation leads to interaction with T-Cell Receptors (TCR). Moderate doses of radiation also activate a type I interferon response through the sensing of cytoplasmic DNA *via* cGAS-STING. Radiation can upregulate PD-L1 and CTLA-4, and therefore immunotherapy can augment radiation efficacy by targeting these pathways. (Created with BioRender.com).

to cytotoxic T cells (21, 22). Moderate doses of radiation have also been shown to activate a type I interferon response in tumor cells through the sensing of cytoplasmic DNA derived from tumor micronuclei *via* the cGAS-STING pathway (23–26). Through these different processes, radiation therapy ultimately creates a proinflammatory microenvironment that instigates immune activation in a manner that may be synergistic with immunotherapy.

### Immunotherapy May Augment Radiotherapy

Not all tumors will respond to radiation, despite administration of definitive doses. Although the reason for radioresistance remains unclear, one hypothesis is that immune-mediated mechanisms may be involved (27). It is important to note that although radiation can be immunogenic, it can also be immune-suppressive. Radiation can directly kill immune cells in or near the tumor through DNA double strand breaks and apoptotic cell death, which in turn may negatively impact T cells in peripheral circulation (28). For example, a retrospective study of prostate cancer patients treated with (N=36) or without (N=95) pelvic nodal irradiation demonstrated a higher risk of radiation-related lymphopenia with pelvic nodal irradiation (29). Indirectly, while activation of type 1 interferon through cGAS-STING induces recruitment of effector T cells and antigen presenting cells (30), it can also upregulate transforming growth factor β (TGF-β), which triggers an immune-suppressive environment (31–33). Radiation can also drive the recruitment of myeloid-derived suppressor cells (MDSCs) (34), which serve as critical mediators of immunosuppression and inhibit effector T cells as well as induce Tregs (35). Increased infiltration of Tregs into the tumor microenvironment through radiation can downregulate the immune response (36). As a result, radiation's impact on MDSCs and T cells may promote tumor growth, local invasion, and subsequent metastases (37). Thus, therapies that counteract this effect by augmenting T-cell function may lead to improved control of the tumor (38). Radiation can also alter the balance of key immune checkpoint pathways including PD-L1 and CTLA-4. Radiation temporarily upregulates PD-L1 in mice with bladder cancer (39). The binding of the PD-L1 protein to the inhibitory checkpoint molecule PD-1 reduces the proliferation of antigen-specific T cells in lymph nodes (40). Similarly, radiation can upregulate the CTLA-4 receptor in T cells, leading to a downregulated immune response (41, 42). Thus, an important rationale for incorporating immunotherapy into radiotherapy regimens is to augment the efficacy of radiation by selectively targeting these immune suppressive effects.

### Radiotherapy and Immunotherapy Are Synergistic

Compared to other cancer treatments, tumor response to immunotherapy is often slower and may result in transient increases in tumor burden, even in patients who have an effective immune response (43). Radiotherapy could potentially greatly reduce the growth of such tumors, thus enabling patients to respond to the immunotherapy for longer periods of time (44). In a similar vein, radiation can be used to prime the tumor for immunotherapy by increasing the susceptibility of tumor cells to immune-mediated treatment (45). Moreover, combining immune modulating agents and radiation may induce protective immunologic memory, which could prevent disease recurrence. Finally, reports in the literature suggest that combining immune checkpoint inhibitors and radiotherapy may result in increased frequency of the "abscopal effect," the immunogenic cell killing of untreated distant tumors (46). Although the potential mechanism for the abscopal effect may include radiation-induced stimulation of systemic recognition of tumor-related antigens, the overall rarity of clinical cases necessitates further investigation (46, 47).

## CLINICAL EVIDENCE FOR COMBINING RADIOTHERAPY AND IMMUNOTHERAPY

### Non-Genitourinary Cancers

Several clinical studies have demonstrated a benefit for the combination of radiotherapy and immunotherapy in non-genitourinary cancers, as reviewed comprehensively elsewhere (44). For example, in lung cancer, the PACIFIC trial enrolled 709 non-small cell lung cancer (NSCLC) patients previously treated with platinum-based chemoradiation and randomized them in a 2:1 ratio to receive either adjuvant durvalumab or placebo. Treatment with durvalumab resulted in an increase in median progression-free survival and 2-year overall survival (66.3% *vs* 55.6%, P=0.005) (48, 49). In a secondary analysis of the KEYNOTE-001 phase 1 trial of pembrolizumab in NSCLC, patients who had previously received radiation therapy prior to receiving pembrolizumab experienced an increased median progression-free survival and overall survival compared to patients without previous radiotherapy (50). In the PEMBRO-RT Phase 2 randomized trial, 76 NSCLC patients received either pembrolizumab and SRBT (3 x 8 Gy within 7 days prior to the first cycle) or pembrolizumab alone. The study found that pembrolizumab preceded by SBRT resulted in a doubling of the overall response rate at 12 weeks (36% *vs* 18%, P=0.07) without any significant increase in toxicity, although this did not meet the prespecified endpoint for meaningful clinical benefit (51). Interestingly, subgroup analyses showed the largest benefit from the addition of radiation in patients with PD-L1 negative tumors.

### Prostate Cancer

Although numerous clinical trials are investigating the combination of radiotherapy and immunotherapy in genitourinary cancers (**Table 1**), only a few randomized trials have been published to date with mature results. In prostate cancer, a multicenter phase 3 trial investigated the use of ipilimumab *vs.* placebo after bone-directed radiotherapy (8 Gy x 1 fraction) in 799 men with metastatic castration-resistant prostate cancer that progressed after docetaxel (63). Ipilimumab therapy was associated with a trend towards increased overall survival that was not statistically significant (P=0.053). However,

**TABLE 1** | Active Phase II and III clinical trials combining immunotherapy with radiation therapy in genitourinary cancers.

| Cancer | Study | Eligibility | Design | Intervention | Planned Enrollment | Ref |
|---|---|---|---|---|---|---|
| Prostate | NCT01436968 | Localized PC | Phase III | RT + valacyclovir ± AdV-tK ± Aglatimagene besadenovec (CAN-2409) | 711 | – |
| Prostate | NCT02107430 | Localized High-Risk PC | Phase II | RT ± Dendritic Cells (DCVAC/PCa) | 62* | – |
| Prostate | NCT01807065 | mCRPC | Phase II | Sipuleucel-T ± RT | 51* | (52) |
| Prostate | NCT01818986 | mCRPC | Phase II | SBRT + Sipuleucel-T | 20* | – |
| Prostate | NCT03007732 | Newly Diagnosed Hormone-Naive Oligometastatic PC | Phase II | SBRT + ADT + Pembrolizumab ± TLR9 agonist (SD-101) | 42 | – |
| Prostate | NCT03795207 | Oligometastatic Recurrent Hormone Sensitive PC | Phase II | SBRT ± Durvalumab | 96 | (53) |
| Urothelial | NCT02662062 | MIBC | Phase II | RT + cisplatin + Pembrolizumab | 30 | (54) |
| Urothelial | NCT03171025 | Localized MIBC | Phase II | Chemoradiation with Adjuvant Nivolumab | 28 | (55) |
| Urothelial | NCT02621151 | MIBC | Phase II | RT + Gemcitabine + Pembrolizumab | 54* | – |
| Urothelial | NCT03421652 | Locally Advanced UC Ineligible for Chemotherapy | Phase II | RT + Nivolumab | 34 | – |
| Urothelial | NCT03775265 | Localized MIBC | Phase III | Chemoradiation ± Atezolizumab | 475 | (56) |
| Urothelial | NCT03950362 | BCG Unresponsive NMIBC | Phase II | RT + Avelumab | 67 | – |
| Urothelial | NCT04543110 | MIBC | Phase II | RT + Durvalumab | 25 | – |
| Urothelial | NCT03747419 | MIBC | Phase II | RT + Avelumab | 24 | – |
| Urothelial | NCT03702179 | MIBC | Phase II | RT + Durvalumab + Tremelimumab | 32 | (57) |
| Urothelial | NCT04216290 | Node-positive Bladder Cancer | Phase II | Chemotherapy + RT ± Durvalumab | 114 | – |
| Urothelial | NCT03915678 | anti-PD-1/L1 refractory Bladder Cancer ‡ | Phase II | RT + Atezolizumab + BDB001 | 247 | – |
| Urothelial | NCT03529890 | Locally Advanced UC | Phase II | Neoadjuvant RT + Nivolumab | 33 | – |
| Urothelial | NCT03115801 | Metastatic UC | Phase II | Atezolizumab or Pembrolizumab ± RT | 112 | – |
| Urothelial | NCT03511391 | UC ‡ | Phase II | (Pembrolizumab or Nivolumab or Atezolizumab) ± SBRT | 99* | – |
| Renal | NCT01896271 | Metastatic ccRCC | Phase II | SBRT + HD IL-2 | 26 | (58) |
| Renal | NCT03065179 | Metastatic ccRCC | Phase II | SBRT + Nivolumab + Ipilimumab | 29* | (59) |
| Renal | NCT02306954 | Metastatic RCC | Phase II | HD IL-2 ± SBRT | 84 | – |
| Renal | NCT02781506 | Metastatic ccRCC | Phase II | SBRT + Nivolumab | 7* | – |
| Renal | NCT01884961 | Metastatic ccRCC ‡ | Phase II | SBRT + HD IL-2 | 35 | (60) |
| Renal | NCT03050060 | Metastatic ccRCC ‡ | Phase II | hypofractionated RT + Nelfinavir + (Pembrolizumab or Nivolumab or Atezolizumab) | 120 | – |
| Renal | NCT02599779 | Metastatic RCC | Phase II | SBRT + Pembrolizumab | 35 | – |
| Renal | NCT03115801 | Metastatic RCC | Phase II | Nivolumab ± RT | 112 | – |
| Renal | NCT03469713 | Metastatic RCC | Phase II | SBRT + Nivolumab | 69* | (61) |
| Renal | NCT03511391 | RCC ‡ | Phase II | Nivolumab ± SBRT | 99* | – |
| Renal | NCT02992912 | Metastatic RCC ‡ | Phase II | SBRT + Atezolizumab | 187 | – |
| Renal | NCT04090710 | Metastatic RCC | Phase II | Ipilimumab/Nivolumab± SBRT | 78 | (62) |

*BCG, Bacillus Calmette-Guerin; ccRCC, clear cell renal cell carcinoma; HD IL-2, high dose IL-2; mCRPC, metastatic castration-resistant prostate cancer; MIBC, muscle-invasive bladder cancer; PC, prostate cancer; RT, radiation therapy; RCC, renal cell carcinoma; SBRT, stereotactic body radiation therapy; UC, urothelial carcinoma.*

**Actual completed enrollment.*

*‡For trials enrolling multiple cancer types, details are provided only for the GU cancer arms.*

subgroup analyses suggested that patients with favorable prognostic features such as the absence of visceral metastasis or anemia and normal alkaline phosphatase did have a significant improvement in survival with the addition of ipilimumab (63). In a phase 2 trial, 49 patients with oligometastatic hormone-sensitive prostate cancer were randomized to receive either the autologous cellular immunotherapy sipuleucel-T preceded by radiotherapy (30 Gy to a single metastatic site) or sipuleucel-T alone (52). Median progression-free survival was higher with the addition of radiotherapy (3.65 *vs.* 2.46 months, P=0.06), but this was not statistically significant. Overall, radiotherapy did not significantly enhance the humoral and cellular responses associated with sipuleucel-T.

Although these clinical trials have not demonstrated a definite benefit for the addition of radiotherapy to immunotherapy, results from additional ongoing clinical trials in prostate cancer are pending, including those testing PD-1/PD-L1 checkpoint inhibitors in combination with radiotherapy (**Table 1**). For example, in an ongoing phase 2 study (NCT03795207), 96 oligometastatic prostate cancer patients are randomized to either SBRT with durvalumab or SBRT alone in a 2:1 manner. Durvalumab (1500 mg/cycle) is administered one month prior to SBRT (3 x 9 Gy or 3 x 11 Gy) and continued until progression with a maximum of 12 months. The primary endpoint of the trial is 2-year progression-free survival (53).

## Kidney Cancer

Immune checkpoint inhibition has become a standard of care treatment for patients with metastatic renal cell carcinoma (RCC) (64, 65). Multiple clinical trials are currently evaluating whether the addition of radiotherapy to immunotherapy will further improve outcomes in this disease (**Table 1**). Early results have been presented for the RADVAX RCC single arm phase 2

trial (NCT03065179), in which 25 metastatic RCC patients received nivolumab and ipilimumab (N/I) with SBRT (50 Gy in 5 fractions) between the first and second doses of N/I (59). Partial responses were observed in 14/25 patients, for an objective response rate of 56%, which is higher than the expected response rate of 40%. The regimen was noted to have acceptable safety, although 10 (40%) patients required prednisone for immune-related adverse events. These results are encouraging for further investigation, although the study is limited by its small sample size and single-site design.

Preliminary results of the NIVES single arm phase 2 multicenter study (NCT03469713) have been presented recently, in which patients with metastatic RCC that progressed on up to two prior systemic therapies were treated with nivolumab for 6 months, in combination with SBRT (10 Gy x 3 fractions) to one metastatic lesion given 7 days after initiation of nivolumab (61). At a median follow-up of 15 months, the objective response rate was 17.4% (12/68 patients). Although tolerability was acceptable [most frequent grade 3/4 toxicities were diarrhea (5.8%), elevated amylase/lipase (4.3%), and fatigue (4.3%)], the study did not meet its primary endpoint of improving response rate to 40%.

Overall, the available results for combining immunotherapy with radiotherapy are mixed in RCC. Additional data from ongoing clinical trials are anticipated to clarify whether changing the timing or target site of SBRT will further improve outcomes. For example, to test the strategy of targeting the primary kidney lesion with SBRT rather than targeting metastases in this context, the CYTOSHRINK phase 2 trial (NCT04090710) will randomize up to 78 untreated advanced RCC patients to receive ipilimumab/nivolumab plus SBRT to the primary lesion (30-40 Gy in 5 fractions) between cycles 1 and 2, or ipilimumab/nivolumab alone (62).

## Bladder Cancer

Although muscle-invasive bladder cancer has historically been treated with radical cystectomy, bladder-preserving trimodality therapy consisting of transurethral tumor resection, radiotherapy, and chemotherapy is now considered a standard treatment option according to consensus clinical guidelines (66, 67). Several clinical trials are examining the potential role of adding immunotherapy to further improve outcomes of these patients (**Table 1**). A phase Ib study (NCT02891161) demonstrated the safety of combining the anti-PD-L1 checkpoint inhibitor durvalumab with radiation therapy to the bladder (64.8 Gy in 36 fractions) in 6 patients with locally advanced bladder cancer, with no patients experienced dose limiting toxicity (68). A follow-up randomized phase 2 study (ECOG-ACRIN/NRG 8185; NCT04216290) is examining the addition of durvalumab to chemoradiation therapy in patients with clinically node-positive (N1-2) muscle-invasive bladder cancer. A large cooperative group randomized phase 3 study (SWOG/NRG 1806; NCT03775265) with a planned accrual of 475 patients is investigating the addition of the anti-PD-L1 inhibitor atezolizumab to chemoradiation in patients with localized muscle-invasive bladder cancer. Safety data from the first 73 patients of this study were recently presented, showing no grade 3 or higher immune-related adverse events to date (56). Another study is exploring the potential of this strategy for the management of non-muscle invasive bladder cancer, using the combination of radiotherapy with Bacillus Calmette-Guerin (BCG) and durvalumab (ADAPT-bladder; NCT03317158).

# CONSIDERATIONS SURROUNDING COMBINING RADIOTHERAPY AND IMMUNOTHERAPY

## Sequencing

The optimal timing and sequencing of radiotherapy and immunotherapy for maximum efficacy of combination therapy remain unknown, although these may vary depending on tumor histology and type of immunotherapy (13). Interestingly, in a post-hoc analysis of the PACIFIC trial, patients who received durvalumab within 14 days after completing chemoradiation had better progression free survival than those who received durvalumab after 14 days, suggesting that immunotherapy should be started soon after radiation (69). Similarly, in a retrospective review of 758 patients with a range of cancer diagnoses who received radiotherapy and immunotherapy (either anti-CTLA-4 or anti-PD-1/PD-L1), patients who received concurrent therapy had better overall survival. Moreover, of those who received concurrent therapy, patients who received induction immunotherapy starting more than 30 days before radiation had improved overall survival compared to those who started less than 30 days before radiation (70). These studies suggest that careful consideration needs to be given to timing and sequencing of radiotherapy and immunotherapy in the design of clinical trials.

## Dose and Fractionation

The optimal radiation dosing and fractionation strategy to maximize immunogenicity remains controversial. Most lines of evidence suggest that higher doses of radiation (>6-8 Gy per fraction) are more immunogenic than typical doses used in conventional fractionation (1.8-2 Gy per day) (71–73). Moderately high doses of 8-12 Gy seem to optimally activate the type I interferon response *via* cGAS/STING, while very high doses (20-30 Gy in 1 fraction) result in a decline in radiation-induced STING activation, in part due to negative feedback inhibition by Trex1 exonuclease which reduces accumulation of cytoplasmic DNA (24). Ultimately, the various fractionation schemes incorporated into ongoing clinical trials will yield insights into the optimal radiation dosing and fractionation needed for the effective combination with immunotherapy.

## Biomarkers of Efficacy and Toxicity

The efficacy of immunotherapy varies greatly across patients and cancer types, and biomarkers that can identify the tumors that would be most responsive to specific immunotherapies are an area of active investigation (74). Candidate biomarkers of efficacy including PD-L1 expression, mutational burden, neoantigens, tumor infiltrating lymphocytes, and radiographic characteristics

are under active study (75–79). Whether these same biomarkers will also predict responses to the combination of radiotherapy and immunotherapy remains an open question that should be actively addressed in ongoing and planned clinical trials.

There is also a need for biomarkers that can predict the occurrence of severe toxicity after the combination of immunotherapy and radiotherapy (80). Immune stimulatory drugs can cause immune-related adverse events (IrAEs) including fatigue, rash, skin disorders, and GI issues (81). Several large cohort studies (e.g. NCT03984318) are seeking to discover the underlying mechanisms responsible for severe IrAEs and identify predictive biomarkers. Biomarker candidate for IrAE prediction currently under investigation include cytokines, immune-cell subsets, autoantibodies, human leukocyte antigen haplotype, and radiomic characterization (82). Other studies are investigating the reduction of immunotherapy-related side effects through the use immunosuppressive drugs such as rituximab (anti-CD20) and tocilizumab (anti-IL-6) (NCT04375228). Radiotherapy is associated with its own set of toxicities, but can also cause adverse events similar to IrAEs through non-tumor specific antigens released into the tissue microenvironment by irradiation, potentially priming auto-reactive T cells to attack normal tissue (83). Predictors of these and other adverse events related to the combination of immunotherapy and radiotherapy need further study.

## CONCLUSION

A growing body of preclinical and clinical evidence indicates a potential synergy between radiotherapy and immunotherapy, lending support for the combination of these two treatment approaches. Unanswered questions remain regarding the optimal sequencing of treatment, dose fractionation, and biomarkers of response and toxicity. Within genitourinary cancers, multiple clinical studies are ongoing with early indications of both promising as well as negative results, suggesting that specific details regarding the protocol by which treatment is delivered may impact the overall success of the approach. These efforts are exemplified by the SWOG/NRG 1806 phase 3 study testing the addition of atezolizumab to chemoradiation in muscle-invasive bladder cancer. Should these initial trials show promise, confirmatory trials may be necessary given increased FDA scrutiny of immunotherapy in light of recent voluntary withdrawal of drugs that received accelerated approval in bladder cancer (84). Continued research efforts are needed to fully evaluate and optimize this promising combination of radiotherapy and immunotherapy.

## AUTHOR CONTRIBUTIONS

JU, EK, and DM acquired, analyzed, and interpreted the data. JU, EK, and DM drafted and revised the manuscript for intellectual content. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# The Incorporation of Immunotherapy and Targeted Therapy Into Chemoradiation for Cervical Cancer: A Focused Review

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In 2011 the Food and Drug Administration (FDA) approved anti-vascular endothelial growth factor (VEGF) therapy, bevacizumab, for intractable melanoma. Within the year, immunotherapy modulators inhibiting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) were approved in addition to programmed death-ligand 1 (PD-L1) antibodies in 2012. Since then, research showing the effectiveness of targeted therapies in a wide range of solid tumors has prompted studies incorporating their inclusion as part of upfront management as well as refractory or relapsed disease. For treatment of cervical cancer, which arises from known virus-driven oncogenic pathways, the incorporation of targeted therapy is a particularly attractive prospect. The current standard of care for locally advanced cervical cancer includes concurrent platinum-based chemotherapy with radiation therapy (CRT) including external beam radiation therapy (EBRT) and brachytherapy. Building upon encouraging results from trials testing bevacizumab or immunotherapy in recurrent cervical cancer, these agents have begun to be incorporated into upfront CRT strategies for prospective study. This article will review background data establishing efficacy of angiogenesis inhibitors and immunotherapy in the treatment of cervical cancer as well as results of prospective studies combining targeted therapies with standard CRT with the aim of improving outcomes. In addition, the role of immunotherapy and radiation on the tumor microenvironment (TME) will be discussed.

**Keywords: cervical cancer, radiotherapy, chemotherapy, immunotherapy, angiogenesis inhibitors**

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## INTRODUCTION

Treatment options for early-stage cervical cancer include surgery or primary radiation with or without chemotherapy (1). Surgery in the form of radical hysterectomy is indicated for non-bulky and early stage disease although definitive radiotherapy has similar efficacy. For patients with IB-IIA disease, a randomized trial of 343 women compared surgery versus radiation with initial results showing five-year overall survival (OS) of 83% in both groups (2). Rates of severe morbidity were higher ($p = 0.0004$) in those receiving surgery upfront (28%) compared to radiotherapy (12%), which was attributed to increased use of combination surgery and adjuvant radiation in the surgery arm. Long-term follow up continued to show similar twenty-year OS rates of 72% with surgery and 77% with primary

radiotherapy (p = 0.280) (3). Multivariate analysis identified large tumor size (p = 0.008), adenocarcinoma histology (p = 0.020), and positive lymph node status (p <0.001) as negative risk factors.

For bulky or locally advanced stage disease, the addition of cytotoxic chemotherapy to radiation has been the subject of extensive study. The seminal Gynecological Oncology Group (GOG) 120 trial examined 526 women with untreated stage IIB, III, or IVA cervical cancer. Patients received EBRT with random assignment to one of three concurrent CRT regimens: cisplatin, cisplatin plus 5-fluorouracil, or oral hydroxyurea. Patients receiving either cisplatin-containing arm had improved rates of OS and progression free survival (PFS) (4). In a similar cohort to GOG 120, Radiation Therapy Oncology Group (RTOG) 90-01 examined 403 women with stages IIB–IVA, stages IB to IIA with bulky tumors, or positive pelvic lymph nodes. This randomized study compared extended field radiotherapy (EFRT) alone to CRT consisting of pelvic radiotherapy with concomitant fluorouracil and cisplatin. The 90-01 results met early release criteria due to CRT garnering a significant OS and disease-free survival (DFS) benefit compared to EFRT alone. Long-term follow-up confirmed significantly improved eight-year OS of 67% with CRT compared to 41% with EFRT (p <0.0001) (5). RTOG 90-01 was the tipping point of a culmination of studies that caused a dramatic change in National Institutes of Health recommendations to concurrent CRT as the standard of care for cervical cancer, most notably for stage IB3–IVA disease (4–7). The focus of this review will be to examine studies that are completed or in development combining newer therapeutic agents, including angiogenesis inhibitors and immunotherapy, with CRT in the management of cervical cancer.

# ANGIOGENESIS INHIBITION

## Efficacy of VEGF Inhibitors in Cervical Cancer

There is evidence that VEGF plays a role in human papilloma virus (HPV) mediated oncogenesis of cervical cancer, including through activity of oncoprotein E5 to upregulate the VEGF angiogenesis pathway (1). VEGF is a growth factor responsible for the proliferation, migration, and survival of endothelial cells. Increased levels of VEGF have been associated with advanced stages of cervical cancer, as well as worse PFS and OS (8–10). Bevacizumab is an anti-VEGF monoclonal antibody that binds to VEGF proteins expressed on tumor cells (11, 12). The GOG 227C study evaluated the use of bevacizumab in 46 patients with recurrent cervical cancer (**Table 1**). This Phase II study showed that bevacizumab as monotherapy was tolerable and improved PFS and OS as a second, or third line treatment when compared to historical GOG study controls (13). Few grade 3 or 4 adverse events were reported as well as one grade 5 infection.

Building on these results, GOG 240 was a 2 × 2 phase III randomized trial of the addition of bevacizumab to two different chemotherapy regimens, cisplatin vs paclitaxel-topotecan. The majority (75%) of the 452 patients with recurrent, persistent, or metastatic cervical cancer (**Table 1**) had previously received cisplatin-based CRT. This study showed the addition of bevacizumab to chemotherapy was found to improve median OS from 13.3 to 17.0 months (hazard ratio 0.71 (98% confidence interval (CI), 0.54–0.95; p = 0.004) (14). In a subset of patients who had not received previous radiation, median OS was 24.5 months with bevacizumab added to chemotherapy versus 16.8 months in chemotherapy alone. Bevacizumab was associated with increased risk of grade 2 or higher hypertension (25% versus 2%), although no patients discontinued bevacizumab because of hypertension. In addition, thromboembolic events (grade 3 or higher) were higher with bevacizumab (8% *versus* 1%). Of particular importance is the risk of fistula (grade 3 or higher) with bevacizumab at 6% compared to <1% with chemotherapy alone, and all fistulas occurred in previously radiated patients. Fistula is a consistently reported rare toxicity of CRT regimens with brachytherapy with significant negative effects on quality of life (QOL). In GOG 240 there were no fistula associated surgical emergencies, instances of sepsis or death and although there was a reported decrease in QOL measures in bevacizumab receiving

**TABLE 1** | Clinical trials using anti-vascular endothelial growth factor (anti-VEGF) in cervical cancer with prior or concurrent treatment with chemoradiation.

| Study | Phase Study Population Subject number (n) | Treatment | Results |
|---|---|---|---|
| **GOG 227C** Bevacizumab in the Treatment of Persistent or Recurrent Squamous Cell Carcinoma of the Cervix (13) | Phase II Recurrent, 83% had prior radiation, all had prior chemotherapy n = 46 | Bevacizumab every 3 weeks until disease progression or prohibitive toxicity | Median PFS: 3.40 months (95% CI, 2.53 to 4.53 months) OS: 7.29 months (95% CI, 6.11 to 10.41 months) Adverse Events: grade 3 or 4 Hypertension (n = 7) Thrombo-embolism (n = 5) Gastro-intestinal (n = 4) Grade 5 infection (n = 1) |
| **GOG 240** Incorporation of Bevacizumab in the Treatment of Recurrent and Metastatic Cervical Cancer (14) | Phase III Recurrent, persistent, or metastatic, 75% had prior concurrent cisplatin-radiation n = 452 | 2 × 2 design First randomization: cisplatin + paclitaxel or topotecan + paclitaxel Second randomization: with or without bevacizumab every 3 weeks | Median OS: 16.8 months in chemotherapy + bevacizumab versus 13.3 months in chemotherapy alone (HR 0.77;95% CI 0.62–0.95; p = 0.0068 |
| **RTOG 0417** Efficacy of Bevacizumab in combination with definitive radiation therapy and cisplatin chemotherapy in untreated patients with locally advanced cervical carcinoma (15) | Phase II Newly diagnosed with bulky/locally advanced stage IB-IIIB n = 49 | Bevacizumab every 2 weeks × three cycles concurrent with cisplatin/pelvic radiation then followed by brachytherapy | Results at 3 years OS: 81.3% (95% CI, 67.2–89.8%) LF: 23.2% (95% CI, 11–35.4%) PAF: 8.4% (95% CI, 0.4–16.3%) DFS: 68.7% (95% CI, 53.5–79.8%) |

*GOG, Gynecologic Oncology Group; PFS, progression free survival; CI, confidence interval; OS, overall survival; HR, hazard ratio; LF, locoregional failure; PAF, para-aortic failure; DFS, disease free survival.*

groups, this was non-significant (16). The toxicity profile of bevacizumab for these reasons in the refractory or metastatic setting therefore merits individualized and careful consideration (16, 17). The median post-disease progression-OS was not reduced in the bevacizumab vs chemotherapy-alone group at 8.4 *vs* 7.1 months respectively, lending support to addition of bevacizumab as part of upfront treatment in this setting rather than following next progression. Overall the GOG 240 study results prompted FDA approval in 2014 and established a standard of care for patients with metastatic or recurrent cervical cancer for the addition of bevacizumab to systemic chemotherapy (1).

## Anti-VEGF and Radiation Therapy

While prior radiation was common for patients on GOG 227C (82.6%) and GOG 240 (75% received cisplatin CRT), the unknown effectiveness and toxicity profile of bevacizumab in combination with definitive CRT prompted prospective study on RTOG 0417 (**Table 1**) (13–15). RTOG 0417 was a phase II study combining bevacizumab and CRT in patients with untreated locally advanced cervical carcinoma (15). Unlike GOG 227C, RTOG 0417 was powered to specifically evaluate for toxicity as the primary endpoint. Secondary endpoints included OS, DFS, locoregional failure (LRF) as well as nodal failure associated with radiation and immunotherapy. The study specified the use of 40 mg/m$^2$ weekly cisplatin and standard definitive pelvic radiation therapy with four field high energy photons totaling 45 Gray (Gy) in 25 fractions, 5 days per week to include external iliac lymph nodes. Intensity Modulated Radiation therapy (IMRT) was not permitted. Bevacizumab was given at 10 mg/kg every 2 weeks for three cycles during CRT. Brachytherapy followed at a dose of 40 Gy in one to two low dose rate treatments or 30 Gy in high high dose rate treatments with bevacizumab administered once during brachytherapy course. No maintenance bevacizumab was given. Two of the 46 patients developed grade 3 gastrointestinal (GI) adverse events, with no grade 4 or 5 events. Notably there were no GI fistulas or perforations reported. Hematologic toxicity was the most reported adverse event (nine grade 3, three grade 4). This study showed that the addition of bevacizumab to standard CRT for locally advanced cervical cancer was feasible and safe with respect to protocol-specified treatment related serious adverse events and adverse events. Initial outcomes were encouraging, as incorporation of bevacizumab with CRT resulted in 3-year OS of 81.3%, DFS 68.7% and LRF was 23.2%. An interesting but yet unstudied hypothesis would be to test the efficacy of adjuvant/maintenance bevacizumab following definitive management of locally advanced cervical cancer with CRT, given that in GOG 240 in the recurrent/metastatic setting bevacizumab combined with chemotherapy yielded response rates of 47% (18). We are not aware of an upcoming randomized trial in development evaluating bevacizumab with CRT for cervical cancer in the upfront setting. Possible reasons include toxicity concerns of bevacizumab including risk of fistula as reported in GOG 240, which all cases of fistula were in patients who had previous CRT. However, there were no fistulas reported on RTOG 0417. Also, the OS, DFS, and LRF outcomes on RTOG 0417 were fairly comparable to the CRT arm of RTOG 90-01. Within the NRG cooperative group, the addition of a Ribonucleotide Reductase Inhibitor (Triapine) was selected for randomized study (NRG GY006) given in addition to standard of care CRT for locally advanced cervical and vaginal cancer (19).

# IMMUNOTHERAPY

## PD-L1 Inhibitors for Cervical Cancer Treatment

A majority (>95%) of cervical cancers originate from HPV, an overt carcinogenic factor in cervical cancer development. An increase in PD-L1 expression has been observed in HPV-related head and neck squamous cell carcinoma (SCC) (20). This is likely owing to the upregulation of PD-L1 expression in tumor cells by the E5, E6 and E7 oncoproteins (21). While PD-L1 expression is rare in normal cervical tissue, it is present in about 50% of cervical cancer T-cells, with several studies identifying PD-L1 as a strong prognostic factor as well as a treatment target for cervical cancer (20, 22, 23). Upregulation of PD-L1 on tumor cells leads to increased binding and inhibition of the PD-1 receptor on T-cells. This interaction allows tolerance of tumor antigens presented by major histocompatibility complex molecules and thus turns off the anti-tumor immune response. In addition to deactivation of cytotoxic T-cells, upregulation of PD-L1 causes release of tumor permissive T-helper cell type-2 cytokines in the TME. Blockade of this interaction is a potential treatment strategy that reverses the brakes that upregulation of PD-L1 puts on the immune response.

Pembrolizumab is a highly selective, fully humanized monoclonal antibody that binds to PD-1 and inhibits the PD-L1 pathway. The KEYNOTE-028 study was a phase Ib trial exploring the effects of pembrolizumab in advanced previously treated PD-L1 positive cervical cancer (24). This single arm trial included 24 patients with advanced cervical cancer whose disease failed to respond to prior systemic therapy and whose tumor or stromal tissue had PD-L1 expression of ≥1%. Most patients (62.5%) had received ≥2 previous lines of therapy. Patients received pembrolizumab at 10 mg/kg every 2 weeks for up to 24 months. Pembrolizumab monotherapy had an overall response rate (ORR) of 12.5% at a median follow-up time of 48.9 weeks, as well as no grade 4 adverse events or deaths. In the subsequent phase II study, KEYNOTE-158, patients with advanced cervical cancer were treated with pembrolizumab at 200 mg every 3 weeks, regardless of PD-L1 status (25). The ORR by Response Evaluation Criteria in Solid Tumours (RECIST), (version 1.1), was 12.2% with 10.2 months of follow-up. For patients with longer follow-up (at least 27 weeks) ORR increased to 27%. The results of KEYNOTE-158 prompted FDA accelerated approval of pembrolizumab in the second line treatment of advanced PD-L1 positive cervical cancer (20). It should be noted that many subsequent immunotherapy trials now utilize the immunotherapy-RECIST (iRECIST) criteria for evaluating response to therapy (26).

Nivolumab is another monoclonal antibody with a high affinity to PD-1. It blocks interaction of PD-1 on T-cells with

PD-L1 and programmed death ligand-2 (PD-L2) on tumor cells and allows for tumor antigen-specific T-cell proliferation and cytokine release (27). The CheckMate-358 trial is an ongoing open-label, multicohort, phase I/II study of nivolumab in patients with virus-associated tumors including recurrent or metastatic cervical, vaginal, and vulvar cancers. Patients received nivolumab at 240 mg every two weeks until progression of disease or unacceptable adverse events. Of the 24 patients treated, 19 had cervical cancer. ORR in the phase I cohort was 26.3% for patients with cervical cancer, with a median follow-up of 31 weeks. In all 24 patients, the disease control rate (ORR + stable disease) was 70.8% (28).

## Combination PD-L1 Inhibition and CTLA-4 Inhibition

While PD-1 inhibition has shown promise in cancer therapy, combinatorial approaches that target both PD-1 and CTLA-4 pathways have also been employed. The combination of ipilimumab, a CTLA-4 inhibitor, and nivolumab has shown efficacy and is FDA approved for the treatment of melanoma (29). However, it is not well known how one agent may affect expression of the target for another agent. PD-L1 levels have been evaluated in tumors treated with ipilimumab in metastatic or recurrent cervical cancer patients who had progression after at least one line of platinum chemotherapy with pelvic radiotherapy (30). Thirteen of the 42 total patients had adenocarcinoma versus squamous cell carcinoma and 37/42 were known HPV positive. This study showed that PD-L1 expression at baseline and post immunotherapy did not increase significantly with treatment and was not an indicator of outcome. Median PFS and OS were 2.5 months (95% CI, 2.1–3.2 months) and 8.5 months (95% CI, 3.6 not reached; one patient was still alive) respectively. This study did show evidence of PD-L1 changes with CTLA-4 inhibitor monotherapy in patients with metastatic or recurrent cervical cancer post CRT.

## Combination PD-L1 or PD-1 Inhibition With Radiotherapy

Cemiplimab, a hinge-stabilized immunoglobulin-4 monoclonal antibody to the PD-1 receptor, exhibits a safety profile comparable to other anti PD-L1 agents. During its first in human study of 60 patients with solid tumors deemed to have no standard alternative therapeutic options, nine patients had either partial (7) or complete (2) responses to cemiplimab given concurrently to hypofractionated radiation (31). There were three cervical cancer patients treated, including one of the two patients in the study achieving complete response.

There are several cervical-cancer specific, ongoing or newly completed clinical trials exploring the new realm of adding immunotherapy to CRT concurrently, sequentially or both. NRG GY017 is a multi-faceted Phase I clinical trial studying immune activation differences in the timing of anti PD-L1, atezolizumab (**Table 2**) (33). This two-arm study has one arm receiving an upfront single dose of atezolizumab then continues with two treatments of atezolizumab concurrently with extended field CRT and image guided brachytherapy. The second arm receives three doses of atezolizumab concurrently with extended field CRT and image guided brachytherapy. IMRT will be used for its potential reduction in adverse events and regional node recurrence (39). Post-treatment positron emission tomography and computed tomography (PET-CT) scans, an often-utilized post treatment surveillance tool, will also be followed prospectively. Immune expression differences between the arms will be measured *via* clonal expansion of T-cell receptor beta in peripheral blood at baseline and on day 21 of treatment. It is hypothesized that immune responses of increased clonal numbers and in specific tumor associated clones will be shown in the treatment arm with the best clinical outcomes. Baseline and treatment PD-L1 expression in both arms will also be analyzed for outcome predictive value.

An interesting phase II trial of pembrolizumab, NCT02635360 (**Table 2**), is exploring multiple immunological effects of both sequential and concurrent use of pembrolizumab in standard CRT+ brachytherapy (36). Measurements of HPV E2, E7 specific CD8+ T-cells, T-regulatory cells (T-regs), and Plasminogen activator inhibitor-1, a marker of immunosuppressive transforming growth factor-beta and rate of complete metabolic response on PET-CT imaging will be measured at 12 weeks post CRT. Safety, PFS, and OS will be followed to 5 years. The Nivolumab in Association with Radiotherapy and Cisplatin in Locally Advanced Cervical Cancers (NiCOL) trial (**Table 2**), a phase I study that aims to look at dose-limiting toxicity (DLT) of nivolumab as well as ORR and PFS when immunotherapy is continued 5 months post initial treatment with nivolumab + CRT (35). IMRT will be used to deliver pelvic radiotherapy (45 Gy) with simultaneous integrated nodal boost (54 Gy). Nivolumab will be given in a flat dose of 240 mg every 2 weeks or 1 mg/kg every 2 weeks. TME, circulating tumor deoxyribonucleic acid heterogeneity, and Tumor PD-L1 will be measured up to 2 years. The Anti-PD-1, TSR-042, as Maintenance Therapy for Patients with High-risk Locally Advanced Cervical Cancer After Chemo-radiation (ATOMICC) trial is a phase II trial using anti PD-1, TSR-042 as consolidation therapy post standard CRT (**Table 2**) (34). This trial hypothesizes an increased PFS by taking advantages of "the ideal microenvironment" created after radiation. A fixed 500 mg TSR-042 dose every 3 weeks for the first four doses followed by a fixed 1,000 mg dose every 6 weeks will be given for up to 24 months. PFS, OS and multiple quality of life measures will be followed to 30 months.

Atezolizumab is a humanized monoclonal antibody immune checkpoint inhibitor (ICI) that selectively binds to PD-L1 to stop the interaction between PD-1 and B7. The antibody still allows interaction between PD-L2 and PD-1. This antibody is being explored in locally advanced cervical cancer in a randomized phase II trial, the Assessing the Inhibitor PD-L1 Immune Checkpoint Atezolizumab in Locally Advanced Cervical Cancer (ATEZOLACC) trial (**Table 2**) (37). Patients must have bulky disease and/or positive nodes (both pelvic and para-aortic nodes (PAN) allowed). The primary objective is to evaluate clinical benefits of adding atezolizumab concurrently with standard CRT then continued as adjuvant for a total

**TABLE 2 |** Early results and ongoing clinical trials of immunotherapy with chemoradiation in cervical cancer.

| Study | Phase Study Population Subject number (n) | Treatment | Results or Primary/Secondary Outcomes |
|---|---|---|---|
| **GOG 9929** Phase I study of sequential Ipilimumab in the definitive treatment of node positive cervical cancer (32) | Phase I Node positive cervical cancer n =34 (19 patients evaluated for endpoints) | Definitive Cisplatin + EFRT followed by Ipilimumab (CTLA-4 inhibitor) | Results 1 year: Ipilimumab Maximum Tolerated Dose: 10 mg/kg Disease Free Survival: 74% |
| **NRG GY017** Anti-PD-L1 (Azetolizumab) as an Immune Primer and Concurrently with EFRT for Node Positive Locally Advanced Cervical Cancer (33) NCT03738228 | Phase I IB2/IIA with PAN, IIB/IIIB/IVA with Pelvic or PAN n = 40 | Atezolizumab before and/or with standard CRT | Primary outcome: T-cell receptor beta clonal expansion Secondary outcomes: DLT, T-Cell Receptor clonality, PD-L1 expression |
| **(ATOMICC)** A Randomized, Open Label, Phase II Trial of Anti-PD1, TSR-042, as Maintenance Therapy for Patients with High-risk Locally Advanced Cervical Cancer After Chemo-radiation (34) NCT03833479 | Phase II IB2, IIA2, IIB with pelvic nodes and IIIA, IIIB, IVA, or any stage with PAN, post standard CRT + cisplatin with curative intent n =132 | Experimental anti-PD1 (TSR-042) as a maintenance therapy following standard CRT. | Primary outcome: PFS at 30 months Secondary Outcomes: Adverse Events, Overall Survival, Health related quality of life, fatigue, pain |
| **(NiCOL)** A Phase-I Study of Nivolumab in Association with Radiotherapy and Cisplatin in Locally Advanced Cervical Cancers Followed by Adjuvant Nivolumab for up to 6 Months (35) NCT03298893 | Phase I IB2-IVA no PD-L1 expression required n = 21 | Single arm concurrent nivolumab with CRT (IMRT +SIB, no brachytherapy) followed by 5 months of nivolumab alone | Primary Outcome: DLT Secondary Outcomes: ORR, PFS, circulating tumor DNA, Tumor microenvironment, PD-L1 |
| **NCT02635360** Pembrolizumab and Chemoradiation Treatment for Advanced Cervical Cancer (36) | Phase II Stage IB2-IIA with + pelvic lymph nodes, Stage IIB-IVA any nodal status, IVB if metastases to PAN only n = 88 | Pembrolizumab following standard CRT vs concurrently with standard CRT | Primary outcomes: immunologic effects in tumor and peripheral blood mononuclear cells Secondary Outcomes: HPV E2, E7, CD8+ T-cells, FoxP3+ T-regulatory Cells |
| **(ATEZOLACC)** Randomized Phase II Trial Assessing the Inhibitor of Programmed Cell Death Ligand 1 (PD-L1) Immune Checkpoint Atezolizumab in Locally Advanced Cervical Cancer (37) NCT03612791 | Phase II Locally advanced Cervical Cancer n = 190 | Atezolizumab concurrent then continued (max 20 weeks) with standard CRT vs standard CRT alone | Primary outcome: PFS up to 24 months |
| **NCT01711515** Chemoradiation Therapy and Ipilimumab in Treating Patients with Stages IB2-IIB or IIIB-IVA Cervical Cancer (38) | Phase I Stage IB2-IIA with PAN and IIB/IIIB/IVA with positive Lymph nodes n = 34 | Sequential Adjuvant ipilimumab following concurrent weekly cisplatin and EFRT | Primary outcome: Maximum Tolerated Dose Secondary outcomes: DLT, ORR, HPV specific T-cell kinetics and HLA-subtypes |

*GOG, Gynecologic Oncology Group; EFRT, Extended Field Radiation Therapy; NRG (NSABP/RTOG/GOG), National Surgical Adjuvant Breast and Bowel Project/Radiation Therapy Oncology Group/Gynecologic Oncology Group; PD-L1, programmed death ligand 1; PAN, para-aortic nodes; CRT, chemoradiation; DLT, dose limiting toxicities; PD1, programmed death receptor-1; PFS, progression free survival; IMRT, intensity modulated radiation therapy; SIB, simultaneously integrated boost; ORR, overall response rate; DNA, Deoxyribonucleic acid; HPV, human papillomavirus; HLA, human leukocyte antigen.*

maximum 20 cycles. The primary outcome measure is PFS using RECIST (v1.1) or death up to 24 months. Ipilimumab as sequential adjuvant therapy to CRT is being explored in Phase I clinical trial NCT01711515 (**Table 2**) (38). The primary objectives are maximum tolerated dose and DLT following concurrent weekly cisplatin and EFRT in newly diagnosed lymph node positive cervical cancer. Eligible patients include stage IB2/IIA with PAN and stage IIB/IIIB/IVA with any positive lymph nodes (pelvic and/or PAN). Secondary objectives include PFS and evaluation of site of recurrence at 1 year along with chronic toxicities. HPV subtype specific T-cell kinetics, human leukocyte antigen immune markers and PET-CT changes after treatment will also be explored with follow-up to 1 year.

## Combination Anti-VEGF and Anti PD-1 Therapy

There are two ongoing clinical trials evaluating OS when combining anti-VEGF with anti PD-1 immunotherapy. The Efficacy and Safety of BCD-100 (Anti-PD-1) in Combination With Platinum-Based Chemotherapy with and without Bevacizumab as First-Line Treatment of Subjects with Advanced Cervical Cancer, (FERMATA) trial, is a phase III trial combining paclitaxel, platinum-based chemotherapy and an anti-PD-1 (BCD-100) with or without bevacizumab as first line therapy (40). The trial patients include histologically confirmed cervical SCC either progressive/recurrent (previously treated) or initial treatment of advanced stage (IVB) disease. The Platinum Chemotherapy Plus Paclitaxel with Bevacizumab and Atezolizumab in Metastatic carcinoma of the Cervix (BEATcc) Phase III trial is exploring the addition of azetolizumab to platinum chemotherapy, paclitaxel and bevacizumab in 404 patients with Stage IVB, persistent or recurrent cervical cancer (41). Both SCC and adenocarcinoma histology, as well as prior cisplatin-based CRT, will be balanced between the two arms. These trials are set to complete in 2024 and 2023 respectively. Any outcome differences from these combined therapies are anticipated to spur more multi targeted therapy trials.

## CTLA-4 Inhibition in Cervical Cancer

CTLA-4 is a cell marker constitutively expressed on T-reg cells that binds costimulatory molecule B7, thereby suppressing T-cell activity and the subsequent cytokine production required for a full immune response (20, 42). CTLA-4 was identified as a prognostic marker for cervical cancer, with a higher susceptibility in Asian populations, and studies have shown that low T-reg frequencies were associated with longer OS (43–45). Additionally, Qin et al. found that mutations in the CTLA-4 gene were positively associated with tumor mutation burden in

cervical cancer (46). Mutational burden has been shown to correlate with and potentially predict response to immunotherapy (47). This would suggest exploration of CTLA-4 as a meaningful target in cervical cancer.

Concordantly, there are several studies by the Agenus corporation currently examining the role of CTLA-4 inhibitor, or AGEN1884, in cervical cancer. The first of these studies, NCT02694822 is a phase I/II trial assessing the safety, pharmacokinetics, and pharmacodynamics of AGEN1884 in patients with advanced solid cancers or cancers refractory to PD-1/PD-L1 inhibitors (48). This study was subsequently expanded to include cervical solid tumors, NCT03495882 (49). The final AGEN1884 trial, NCT03894215 is a randomized, non-comparative, phase II clinical study observing the efficacy and safety of AGEN2034, a PD-L1 inhibitor versus a placebo, and AGEN2034 + AGEN1884 in subjects with advanced cervical cancer after failed chemotherapy. As of April 2020, the combination of AGEN1884 and AGEN2034 has demonstrated an ORR of 26% in second-line cervical cancer treatment with a median follow-up of 12 months (50). Studies examining second generation CTLA-4 inhibitors are in development which are fragment crystallizable engineered to generate a response in a larger number of patients. Currently, the phase 1 trial using AGEN1181 ± AGEN2034 in advanced solid tumors is open to enrollment in advanced cancers (NCT03860272) (51).

### CTLA-4 Inhibitors and Radiation Therapy

GOG 9929 is a phase I study exploring the use of ipilimumab sequentially after CRT for cervical cancer patients with International Federation of Gynecology and Obstetrics (FIGO) 2009 stages IB2/IIA with PAN and stage IIB/IIIB/IVA with pelvic or PAN (**Table 2**) (32). This high-risk group has a historically poor outcome with CRT alone (52). Lymph node metastasis in cervical cancer has been shown to have a 3-year cause specific survival (CSS) of 29% vs those without lymph node metastasis having CSS of 73% (32, 52, 53). GOG 9929 included concurrent weekly cisplatin, EFRT with nodal boost and intracavitary brachytherapy, followed by four treatments of ipilimumab. Included in GOG 9929 is tracking of immune biomarkers over the course of multimodality treatments. Immune responses including CD4+ and CD8+ T-cell activation *via* expression and activation of Inducible T-cell co-stimulator (ICOS) and PD-1, as well as HPV genotype specific E6/E7 oncogene specific responses were seen following initial CRT (32). These increases in lymphocyte activation appear to show CRT may have a "priming of the immune system" effect. Subsequent administration of ipilimumab sustained the activation of CD8+ T-cells and increased the activation of CD4+ T-cells above initial CRT levels (**Figure 1**). This revealed that in cervical cancer with high risk for recurrence and metastasis, ipilimumab may fortify the patient's own antitumor response once activated by CRT. Preliminary results at the American Society of Clinical Oncology (ASCO) 2017 meeting were presented including 34 patients enrolled of which 19 patients were evaluable. At a median follow-up of 12 months in the patients who received ipilimumab, PFS was 81%, with OS reported as 90%. There were no major toxicities reported. There was suggestion of a significant correlation of increased PFS ($p = 0.049$), (**Table 2**) and OS ($p = 0.036$) for patients with increased activation of CD4+ cells expressing ICOS and PD-1 (32). While this is a possible association with increased immune activation and lower risk of progression and death, these results are preliminary and limited to 19 evaluable patients. Mature results as well as study with larger patient numbers are required to determine if immune-response can be utilized to tailor cervical cancer treatment with CRT.

## SPECIAL CONSIDERATIONS OF IMMUNOTHERAPY

As with all advances in oncology treatment, it is important to not only recognize the potential benefits of highly personalized cancer treatments and immunotherapy, but also the barriers to use and limitations. Cervical cancer presents an enormous burden to women in less developed countries, where the majority of cases present in socially disadvantaged women with advanced stage disease. In these settings there is limited or no access to immunotherapy or the necessary medical environment for implementation (54).

There is also the concern about durable response with the use of ICIs. As of 2018 a publication showed six ICIs had received approval for more than 10 cancer types (55). There are occasions when ICIs are used off-label for patients who have exhausted all other means of treatment, popularly known as "desperation oncology". From 2011–2018 the estimation of the percentage of patients eligible for ICIs has shown a drastic increase from 1.54 to 43.63%. Concordantly, the estimated response to ICIs has increased over the years. With the approval of ipilimumab in 2011, patients with Non-Small Cell Lung Cancer (NSCLC) had an estimated response percentage of 0.14% (95% CI, 0.13–0.15%), which staggered until 2015. During this time nivolumab and pembrolizumab were introduced and the estimated response rate rose to 12.46% (95% CI, 12.37–12.54%) by 2018.

However, further analysis into patient eligibility and the efficacy of ICIs has raised some considerable concerns. Individually, the estimated eligibility and response to ICIs show a positive trend (55). In 2014 the ratio of response to indications peaked and eventually dropped as more ICIs were approved (56). This ultimately widened the gap between patients who are eligible for ICIs and actual benefit or response to the drugs. There is also concern for the under and over estimation of patient eligibility. ICIs are usually not approved as an early treatment option, therefore in settings such as GI cancers which have high mortality rates before later therapies can be used, ICI eligibility is severely miscalculated as it only accounts for a small subset of this population. On the other hand, in the setting of NSCLC, where a significant number of patients have long term survival with chemotherapy, the number of patients eligible for ICIs are underestimated as survivors are not considered in the eligibility criteria. Additionally, with the practice of desperation oncology, a standard does not exist to assess outcomes, which may further underestimate the number of patients affected by ICIs.

Finally, the use of ICIs has shown a correlation with hyperprogressive disease (HPD). There exist various definitions of HPD spanning from doubling of the tumor growth rate to

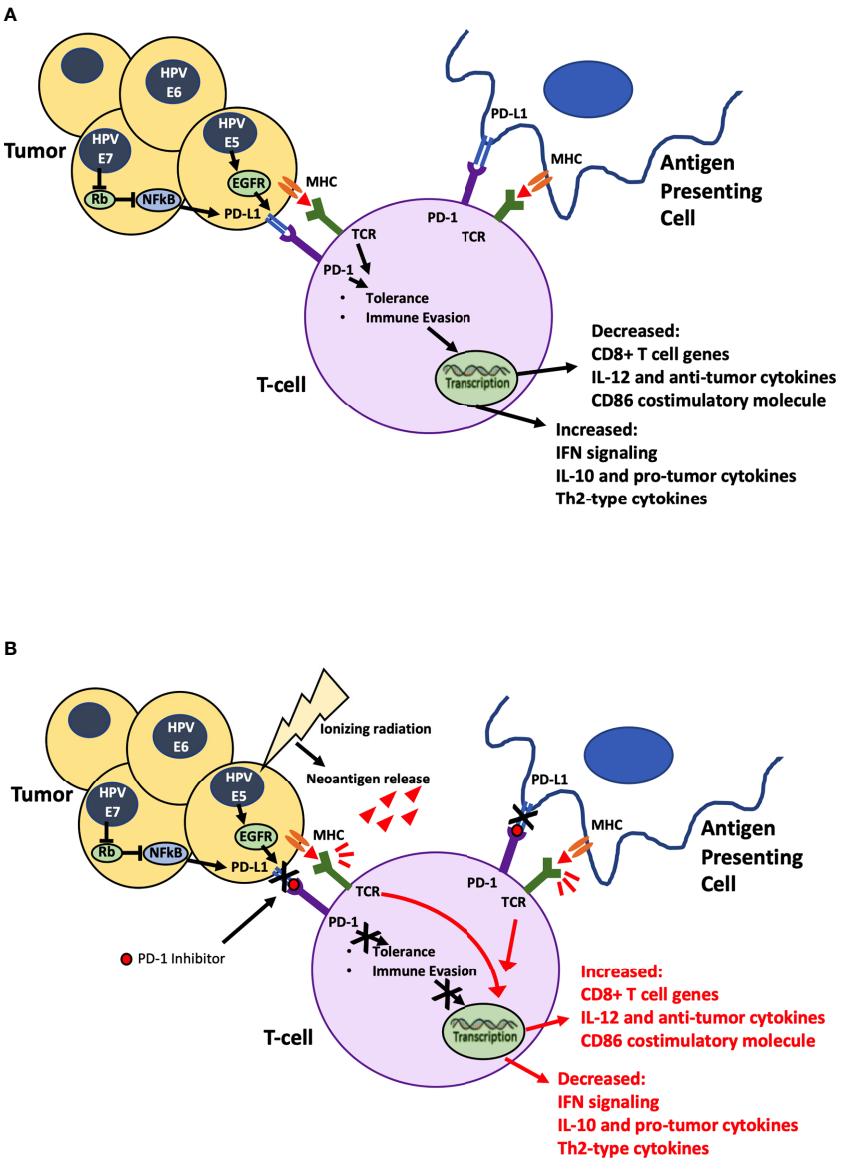


**FIGURE 1** | Ionizing Radiation in Combination with PD-1 inhibitor. **(A)** HPV mediated Oncogenic proteins E5, E6 and E7 hypothesized to cause increase in PD-L1 expression allowing tumor cells to evade identification by immune cells. **(B)** Ionizing radiation damages tumor cells causing neoantigen release, priming the immune system to attack, while PD-1 inhibitor blocks stimulation of immune evasion pathways. Combination of radiation and immunotherapy hypothesized to stimulate robust synergistic attack against tumor cells.

increased tumor burden (57). While HPD is not exclusive to patients receiving ICIs, it occurs at a higher rate in patients who receive them and ultimately leads to poorer patient outcomes.

## TUMOR MICROENVIRONMENT

### Understanding the Effects of Radiotherapy and Chemotherapy on TME

With the promising potential of combining immunotherapy with chemotherapy and radiation, it is important to understand the effects these treatments have on the TME especially when considering concomitant or sequential treatments. Cisplatin has been shown to increase the recruitment of dendritic cells that promote CD8+ T-cells, and stimulate the type I interferon pathway, which ultimately improves host immunity against cancer cells (58). Radiation was shown to increase overall immune tumor response in mice when administered with immunogenic agents including vaccines and Toll like receptor agonists (59). Specifically, one study administered a tumor associated antigen vaccine to mice with carcinoembryonic antigen positive tumors who then received brachytherapy (60).

The results interestingly showed that CD8+ T-cells of mice who received radiation coded for additional tumor antigens not included in the original vaccine. This appeared to define a pathway for the abscopal effect. A study by Nesslinger et al. evaluated the serum concentrations of patients with prostate cancer who received hormone therapy or radiation therapy after radical prostatectomy. Patients who received surgery alone did not generate an immune response, while the highest tumor antibody concentrations (in decreasing order) were for hormone therapy, brachytherapy and finally EBRT (61). Overall, these studies support that radiation has a synergistic immunological effect on the TME with measured tumor specific antigens.

There has been opposition to the therapeutic role of radiation on the immune system with suggestion the stimulatory and functional outcomes of the TME after radiation have yet to be carefully studied (62). There are also studies showing that radiation treatments can decrease the host's immune response. Radiation was found to elicit undesirable immune changes such as decreased reactivity of T-cells to antigenic molecules, and increased expression of PD-L1 on CD4+ T-cells thought to decrease antigenic response (63). Moreover, lymphocyte counts in patients with invasive stage IB1 to IV cervical cancer were still found to be decreased in patients receiving EBRT ± cisplatin. In patients with HPV related cancer, radiation was found to create an adverse ratio of CD8+ T-cells:T-reg cells, in addition to increasing PD-L1 expression on CD4+ tumor cells. Overall, these findings suggest scenarios where radiation may be immunosuppressive and therefore possibly antagonistic to immunotherapy.

However, rather than try to omit radiation therapy, discussions should aim at finding optimal doses of radiation in combination with immunotherapy to yield synergistic effects. A study comparing standard four-field box and anteroposterior–posteroanterior techniques to bone marrow sparing intensity modulated radiation therapy (BMS-IMRT) found that BMS-IMRT can reduce the radiation dose to the lumbosacral spine bone marrow as well as decrease the volume of radiation to the pelvic bone marrow (64). These combined effects of bone marrow sparing constraints can decrease bone marrow suppression and other hematologic toxicities associated with radiation therapy (64, 65).

The synergistic role of radiation when administered with immunotherapy continues to be expounded. Multiple ongoing cervical cancer clinical trials using sequential or concurrent immunotherapy with CRT have included examination of immunological markers, some following changes throughout and beyond treatment (**Table 2**). Increased understanding of how the TME is altered by CRT and immunotherapy will help guide future combinations and timing of immunotherapies to hopefully foster the development of immune-response driven individualized therapy.

## CONCLUSIONS

While the radiotherapeutic management of cervical cancer has advanced with technological advancements, the inclusion of cisplatin-based concurrent chemotherapy has remained largely unchanged. There is significant need for improved outcomes in patients with locally advanced disease. Using anti-VEGF inhibitors to counter the upregulated angiogenesis from HPV-induced E5 oncoproteins in cervical cancer seems a logical consideration. Anti-VEGF therapy, combined with radiation and chemotherapy, has been shown to be effective in initial studies but requires randomized data to determine possible inclusion in standard of care. Immunotherapy targeting the PD-1/PD-L1 pathways has similarly shown promise in treatment of advanced cervical cancers. With increasing evidence of PD-L1 expression from cervical intraepithelial neoplasia to metastatic disease, immunotherapy (with or without additional systemic or local therapy) may potentially have a therapeutic role across several stages of cervical cancer. Preliminary results of CTLA-4 inhibitors in combination with CRT show the ability of radiation to act as an immune primer for further enhancement by immunotherapy. Multiple ongoing studies exploring the concurrent use of immunotherapy with standard of care CRT look to elucidate the importance of therapy timing in addition to provide further definition into the importance of immunological response. Future investigation into the optimal radiotherapy fractionation and sequencing are also required to fully understand the potential synergy of CRT targeted therapies. Of particular interest are studies investigating biomarkers that can potentially be utilized to tailor treatment strategies for individual patients according to tumor and immune response.

## AUTHOR CONTRIBUTIONS

All authors contributed to the conception, design, and writing of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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# Radiation in Combination With Targeted Agents and Immunotherapies for Pediatric Central Nervous System Tumors - Progress, Opportunities, and Challenges

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Pediatric brain tumors are the most common solid tumors in children and represent a heterogenous group of diagnoses. While some are treatable with current standard of care, relapsed/refractory disease is common and some high-risk diagnoses remain incurable. A growing number of therapy options are under development for treatment of CNS tumors, including targeted therapies that disrupt key tumor promoting processes and immunotherapies that promote anti-tumor immune function. While these therapies hold promise, it is likely that single agent treatments will not be sufficient for most high-risk patients and combination strategies will be necessary. Given the central role for radiotherapy for many pediatric CNS tumors, we review current strategies that combine radiation with targeted therapies or immunotherapies. To promote the ongoing development of rational combination treatments, we highlight 1) mechanistic connections between molecular drivers of tumorigenesis and radiation response, 2) ways in which molecular alterations in tumor cells shape the immune microenvironment, and 3) how radiotherapy affects the host immune system. In addition to discussing strategies to maximize efficacy, we review principles that inform safety of combination therapies.

**Keywords: radiation therapies, pediatric brain cancer, brain tumor, Immunotherapy, targeted therapeutic, precision oncology, radiation oncology, combination therapy**

## INTRODUCTION

Collectively, central nervous system (CNS) tumors are the most common solid tumors in children. These tumors represent a heterogenous group of diagnoses ranging from low grade lesions that can be observed or cured through surgical resection to aggressive tumors that are uniformly lethal. Insights into diagnosis and prognosis draw from radiographic and histopathologic features. However, these features tell only part of the story, with molecular alterations greatly impacting diagnosis, prognosis, and therapy decisions in many cases. These molecular features include mutations, copy number variations (CNVs),

structural variants (SVs), epigenetic and gene expression changes. Current standard of care for pediatric brain tumors involves molecular profiling of tumor samples to facilitate more precise characterization of this heterogeneous group of tumors, and along with this comes the possibility of treating patients with more individualized regimens. For some, this means tailoring the intensity of treatment through risk stratification. For example, de-intensification of chemotherapy and radiation is being tested in the WNT medulloblastoma subgroup given favorable outcomes with current standard of care, multimodal therapy (NCT 02724579). For high-risk diseases, where standard chemotherapies have historically failed and relapse/refractory disease remains common, an understanding of the specific molecular drivers of malignancy offers the hope of improving outcomes for such patients through a more targeted approach. Ultimately, as new therapies are evaluated, assessment of response in the context of molecular features of a tumor will identify molecular determinants of response.

In the quest to improve outcomes for patients with pediatric brain tumors, the armamentarium has grown to include a spectrum of therapies, including surgery, cytotoxic chemotherapy, radiation, molecularly targeted therapies, and immunotherapies. Radiation has been and continues to be standard of care for many pediatric brain tumors and in some cases, such as diffuse midline gliomas (DMG), is currently the only life-prolonging therapy (1). In contrast, targeted therapy has really only been developed in recent years and refers to agents that directly modify specific cellular processes anticipated to drive cancer. The development of such therapies is driven by an understanding of how genetic alterations in cancer cells promote tumorigenesis and helps form the foundation for precision oncology, where specific agents are chosen for a patient based on the features of their individual cancer. This is in contrast to traditional cytotoxic therapies, which broadly hit rapidly dividing cells indiscriminately (2, 3). In addition, it has been long appreciated that tumor progression is associated with down-regulation of anti-tumor immune responses (4). To this end, immune-based therapies either directly stimulate the immune system or disrupt immunosuppressive pathways to enhance anti-tumor immunity. Both targeted agents and immunotherapies have demonstrated early promise in a number of cancer types and hold promise for the treatment of pediatric CNS tumors; however, the strategies for their application in pediatric neuro-oncology and the acute and long-term side effects of these agents are just beginning to be unraveled. Additionally, although these agents have potential to improve outcomes for the highest risk pediatric brain tumors, single agent therapy is likely to be insufficient for most patients and combination strategies that provide additive or synergistic benefit will likely be necessary.

We are learning that there is substantial overlap and cross talk between the molecular alterations in brain tumors, the immune microenvironment, and the response to DNA damage by radiation (**Figure 1**). The molecular alterations that drive cellular transformation and cancer cell proliferation also shape the immune environment and the response to exogenous sources of DNA damage like chemotherapy and radiation. In addition, radiation-induced DNA damage and cell death modulate the host immune system, and immune function is necessary for full anti-neoplastic efficacy for radiotherapy (5, 6). It is our hope that a holistic understanding of how these therapies interact will translate into rational therapy combinations and improved outcomes for high-risk pediatric brain cancers in which recurrence is common or cure is unavailable. In this review, we will review mechanistic connections between targeted and immune therapies and radiation that impact efficacy and safety of combining these agents and inform how we move forward with combination strategies. Active clinical trials combining radiation with targeted therapies or immunotherapy for pediatric CNS tumors (at the time of publication of this article) are reviewed (**Table 1**).

## CHARACTERISTICS OF PEDIATRIC CNS TUMORS AND EARLY SIGNALS FOR TARGETED AND IMMUNE-BASED THERAPIES

Pediatric CNS tumors represent a spectrum of diagnoses, with imaging, histopathology, and molecular features contributing to an integrated diagnosis (7). The World Health Organization (WHO) classification of CNS tumors, last updated in 2016, incorporated many molecular parameters in defining diagnostic entities, and the pending 2020 update is expected to continue this effort (7). This approach ensures accurate diagnosis and prognosis and can facilitate more targeted approaches to therapy. For pediatric brain tumors, broad histopathologic diagnoses include gliomas and embryonal tumors. Given the frequencies of these diagnoses among pediatric neuro-oncology patients, we will largely focus our review on the efforts to target these challenging tumors.

Pediatric low-grade gliomas (LGG) are the most common pediatric CNS tumors overall, and portend a good overall survival (OS) of approximately 90% (8, 9). Some LGG can be cured by surgical resection alone, but when therapy is indicated for non-resectable cases, standard chemotherapy, targeted therapy, and/or radiation (in select cases) are potential therapy options. Interestingly, LGG tends to be driven single molecular alterations, with activation of the RAS/MAPK pathway being the hallmark alteration in pediatric LGG. These alterations include *BRAF*-rearrangements, gain of function *BRAF* mutations, and loss of function mutations in negative regulators of this pathway, such as *NF1* (10, 11). *BRAF* inhibitors and MEK inhibitors have demonstrated efficacy in patients with LGG and prospective trials are ongoing to compare targeted therapies with traditional cytotoxic chemotherapy regimens in the upfront and recurrent settings (12–15). Given the high overall survival rate in pediatric LGG, we emphasize that a key consideration for therapeutic decision making in this group of patients involves optimizing function and minimizing side effects of therapy. These considerations are built into the Children's Oncology Group (COG) trials comparing cytotoxic chemotherapy (carboplatin, vincristine) with the MEK inhibitor, selumetinib,

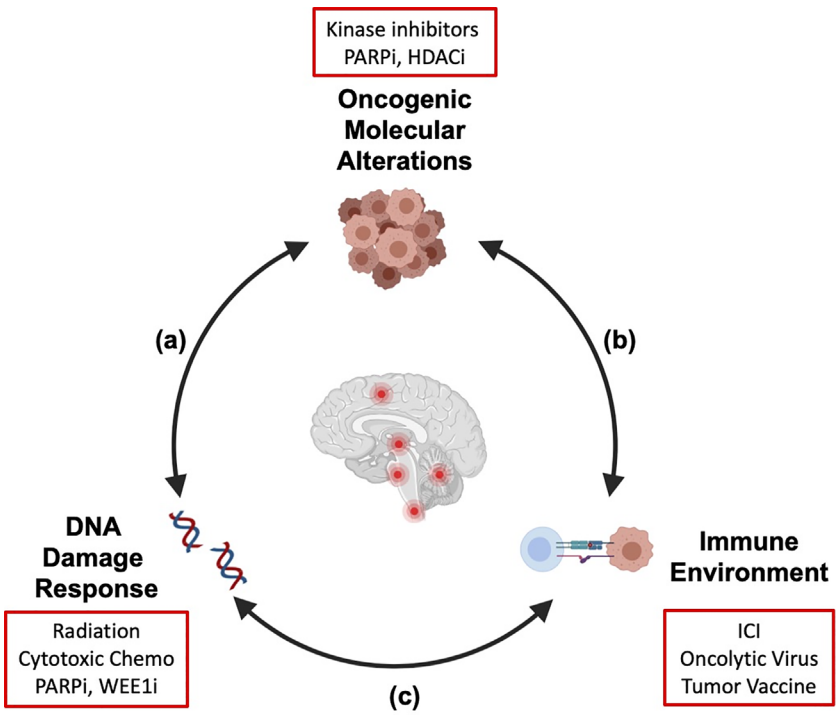


**FIGURE 1** | Crosstalk between key hallmarks of cancer informs combination strategies for treatment of pediatric brain tumors. The molecular alterations that drive pediatric brain tumors modulate the cellular response to DNA damage **(A)** and shape the tumor immune microenvironment **(B)**. Radiation therapy induces DNA damage and remodels the tumor immune microenvironment **(C)**. As targeted therapies and immunotherapies are integrated into treatment regimens for patients with pediatric brain tumors, a systematic understanding of these interactions will be necessary to generate combination strategies that are efficacious and safe. Examples of therapeutic agents discussed in this review are shown (red boxes). We propose that his integrated framework should be considered in preclinical and clinical studies to identify molecular determinants of therapy response and inform rational design for combination strategies. Created with Biorender.

in newly diagnosed LGG without BRAFV600E mutation (NCT04166409). In addition to tumor response, this study will prospectively follow vision, motor function, neurocognitive function, and quality of life. The upcoming European Society for Paediatric Oncology (SIOP-E) trial LOGGIC (Low Grade Glioma in Children) will also prospectively compare cytotoxic chemotherapy versus targeted therapies, with primary outcomes including visual and adaptive behavioral measures alongside disease control endpoints. Further, there are ongoing academic as well as industry efforts to harmonize long-term follow up of patients treated with new signaling inhibitors to capture the impact these therapies might have on the developing CNS.

In contrast to pediatric LGG, pediatric high-grade glioma (pHGG) carry a dismal prognosis, with the primary life prolonging therapies being surgery and radiation (16–18). However, anatomic location often limits the role of surgery within midline structures, such as DMG. For such patients, radiation is the only life-prolonging therapy to date (19). While pediatric and adult HGG both have poor prognoses, recent integrated molecular profiling efforts have demonstrated that these are biologically distinct entities when it comes to molecular drivers of tumorigenesis. For example, pHGG located in midline structures frequently harbor histone mutations in H3.1 (*HIST1H3B*) and 3.3 (*H3F3A*) that are very rarely reported in adult patients. These mutations include a substitution of lysine amino acid at position 27 with methionine (H3 K27M), which are most frequently found in DMG, and glycine at position 34 with arginine or valine (H3 G34V/R), which are found in hemispheric pHGG. Numerous other differences in the molecular drivers of pediatric HGG are well described and make these tumors distinct when comparing to adult counterparts (11, 20).

Despite the overall poor prognosis of pHGG, targeted therapies and immunotherapies have demonstrated early efficacy for select tumors with specific molecular findings. Infant HGGs include patients diagnosed younger than three years of age and are a group of tumors that may carry a better prognosis than pHGG diagnosed at an older age (21). Multiple molecular analyses of pHGG revealed that infant HGGs enrich for single driver, receptor tyrosine kinase (RTK) fusions such as *ALK, ROS1, and NTRK* (20, 22, 23). Multiple prospective "basket trials" that enrolled patients based on presence of RTK fusions across pediatric and adult solid tumor histologies, including CNS tumors, have demonstrated safety and durable responses (24–27). These results led to the FDA approval for larotrectinib for solid tumor patients with *NTRK* fusions and entrectinib for solid tumor patients with *ROS1* or *NTRK* fusions. Within RTK-fusion positive infant HGG, these alterations are likely oncogenic, as demonstrated by preclinical models of tyrosine kinase inhibitor (TKI) efficacy and case reports

**TABLE 1** | Clinical trials evaluating radiation in combination with targeted therapy or immunot.

**Enrollment ongoing or forthcoming**

| NCT Number | Phase | Therapeutic Agent | Radiotherapy | Disease Focus | Primary Endpoints |
|---|---|---|---|---|---|
| NCT03416530 | I | ONC201 | Up-front therapy * | H3K27M Gliomas | Dose finding |
| NCT03690869 | I/II | PD1 inhibitor (cemiplimab) | Up-front conventional and hypo-fractionated regimen, re-irradiation | Newly diagnosed DIPG and newly diagnosed and recurrent non-brainstem HGG | Safety and Efficacy |
| NCT03605550 | Ib | BMI1 inhibitor (PTC596) | Up-front therapy | Newly diagnosed DIPG and non-brainstem HGG | Dose finding, Safety |
| NCT04482933 | II | Oncolytic Herpesvirus (G207) | Single dose | Progressive or recurrent supratentorial brain tumor | Efficacy |

**Enrollment Completed**

| NCT Number | Phase | Therapeutic Agent | Radiotherapy | Disease Focus | Primary Endpoints |
|---|---|---|---|---|---|
| NCT01922076 | I | WEE1 inhibitor (adavosertib) | Up-front therapy | Newly diagnosed DIPG | Dose finding, Safety |
| NCT02502708 | I | IDO1 inhibitor (indoximod) | Up-front therapy | Newly diagnosed DIPG | Safety, Efficacy |
| NCT02457845 | I | Oncolytic Herpesvirus (G207) | Single dose | Progressive or recurrent supratentorial brain tumor | Safety |
| NCT03178032 | I | Oncolytic Adenovirus (DNX-2401) | Upfront therapy following single DNX-2401 injection | Newly diagnosed DIPG | Safety |

**Up-front therapy is considered standard of care in these diseases and anticipated to follow routine dosing schedules.*

of durable responses in patients receiving TKI therapy (20, 22, 23). Prospective studies are underway to evaluate if infantile HGG with such fusion events demonstrate durable response to TKI and if this treatment strategy can be applied to older patients with RTK fusions (NCT04655404, NCT03213704, NCT02576431, NCT02650401). Unfortunately, pHGG affecting older children, including histone mutant cases, are characterized by a combination of molecular alterations that increase the chances that one agent will fail due to resistance or inherent plasticity in oncogenic pathways driving tumor growth (20, 28, 29). In such cases, combinations of drugs and/or radiation offer the potential of increasing therapeutic response and reducing risk of resistance. ACNS1723 is one active phase II clinical trial examining the role for combination maintenance therapy with dabrafenib (BRAFV600E inhibitor) and trametinib (MEK inhibitor) for BRAF V600E mutant pHGG (NCT03919071).

In contrast to the infant HGG, hypermutated HGG that arise in the context of constitutional mismatch repair deficiency (CMMRD) exhibit some of the highest mutation rates in human cancer (30). In the clinical experience with immune checkpoint inhibitors (ICI), tumor mutation burden (TMB) has emerged as a molecular determinant of treatment response and there are now case reports of durable response to ICI in pediatric patients with CMMRD associated hypermutated HGG (30). Unfortunately, the data from the largest prospective trials in newly diagnosed adult HGG, occurring outside the context of CMMRD, revealed no survival difference in patients treated with the ICI nivolumab as maintenance therapy following up front radiation, when compared to bevacizumab, suggesting that single agent ICI was not sufficient to drive a clinically meaningful antitumor immune response in these patients (31). A smaller prospective study in adults with recurrent HGG demonstrated a signal for survival benefit with neo-adjuvant therapy using the ICI pembrolizumab, prior to surgical re-resection, suggesting that timing of immunotherapy may impact the ability to overcome immunosuppressive signals in glioma (32). Collectively, these results demonstrate that single-agent targeted therapies or ICIs may be most effective in specific, rare patients with unique alterations (ie. RTK fusions and hypermutation in setting of CMMRD). For most patients however, the absence of response to single agents illustrates a need to 1) increase our understanding of determinants for response to targeted therapies or immunotherapy and 2) consider combinations of radiation, targeted agents, and immunotherapies (**Figure 1**).

Medulloblastoma is the most common malignant pediatric brain tumor in children and young adults. Standard of care involves multi-modal therapy including maximal safe surgical resection followed by adjuvant chemotherapy and radiation. This regimen is able to cure many patients, but is associated with acute and long-term morbidity due to intensive chemotherapy and craniospinal radiation (33). Recent advances in the molecular profiling of medulloblastoma have revealed distinct biological subgroups with varying pathogenesis and clinical behavior: Wingless (WNT), sonic hedgehog (SHH), group 3, group 4 (33–35). With standard therapy, WNT subgroup patients do quite well, and as a result, clinical trials assessing lower intensity therapies for these patients are under way (NCT 02724579). Relapsed/refractory disease is more common in the remaining subgroups. Accounting for the distinct biology and prognosis of medulloblastoma sub-groups, an open study at St Jude Children's Research Hospital is exploring risk adapted therapy based on disease staging, sub-group assignment, molecular features, and extent of surgical resection

(NCT01878617). Patients with SHH sub-group tumors will also receive vismodegib, a small molecular inhibitor of SHH pathway signaling that targets the G protein coupled receptor Smoothened, with up-front therapy. Phase II data have demonstrated response to vismodegib in a subset of patients with relapsed medulloblastoma and, together with preclinical studies, have shed light on the molecular determinants of response (36–38). In addition, biological sub-types can be identified though integrated molecular analysis with methylation and gene expression profiling, which may further elucidate potential therapeutic targets in these high-risk tumors (39).

Overall, we are beginning to unravel how best to use novel targeted and immune therapies for pediatric brain tumor treatment; however, much work remains on how to best maximize their impact, particularly as part of multi-modal approaches.

## TARGETED THERAPIES AND RADIATION

### DNA Damage Response Pathways – TP53, WEE1, BRCA, PARP

DNA damage is a key mechanism by which both standard chemotherapy and radiation elicit tumor cell death. We are now beginning to understand that underlying genetic drivers of pediatric brain tumors may function as molecular determinants of radiation response. This understanding may facilitate prognostication for patients receiving radiotherapy, but also provides rationale for targeting cellular processes that drive radio-resistance to enhance response. Radiotherapy induced cell death often occurs in a *TP53*-dependent manner. For example, *TP53* mutant or null DIPG demonstrate radio-resistance, as evidenced by *in vitro* assays from cell lines derived from treatment naïve biopsy specimen and in the more rapid development of disease recurrence following radiation in these patients (40). In some instances, tumor cells upregulate DDR and cell cycle checkpoint machinery to tolerate the genomic insults that arise during cellular transformation, which can promote radiation resistance. An example of this is WEE1, a tyrosine kinase involved in the G2/M checkpoint and overexpressed in pHGGs and high risk medulloblastoma (41, 42). Preclinical data in DIPG has demonstrated that concomitant treatment with WEE1 kinase inhibitor, AZD-1775, impairs radiation-induced G2/M cell cycle checkpoint and enhances radiation-induced cell death in pediatric glioma cell lines. Molecular analyses of primary medulloblastoma have also demonstrated *WEE1* overexpression alongside amplification of the MYC family of protooncogenes (*MYC* or *MYCN)*, which characterize high risk disease in patients from SHH, Group 3, and Group 4 sub-groups. Preclinical data indicate that MYC or MYCN overexpression enhances sensitivity to WEE1 inhibition, possibly due to a vulnerability generated by MYC-induced replication stress (42). A phase I/II study of WEE1 inhibitor, AZD1775 (adavosertib), in combination with irinotecan in relapsed refractory pediatric solid tumors, including CNS tumors, has demonstrated tolerability (ADVL 1321) with mainly hematologic and gastrointestinal dose limiting toxicities that are in line with single agent toxicities (43). The phase II expansion of this study included patients with relapsed, refractory medulloblastoma, though these results have not been reported yet. Concurrent chemo/radiotherapy and WEE1 inhibition in newly diagnosed patients was also recently explored in a phase I study of adavosertib in combination with up front radiotherapy in newly diagnosed DIPG (NCT01922076). Interim evaluations have demonstrated safety of this combination, with ongoing analyses pending (44).

Pharmacologic agents that directly target DDR pathways may be capitalized on as a therapeutic strategy, as the molecular alterations that drive tumorigenesis often alter the cellular response to DNA damage and generate vulnerabilities that are not present in normal, non-transformed cells (45). For example, BRCA mutated cancers that are HR-deficient are vulnerable to inhibition of PARP1-mediated base excision repair and NHEJ – an example of synthetic lethality (46). In addition to breast and ovarian cancer, patients with medulloblastoma and glioma can carry germline BRCA1/2 deficiency, making these tumors potentially vulnerable to therapy with PARP inhibitors (47, 48). While the efficacy of PARP inhibitors were first demonstrated in BRCA-deficient cancers, PARP inhibitors have now proven to be effective in select tumors without BRCA mutations. For example, PARP inhibition sensitizes HGG, medulloblastoma, and ependymoma cell lines to ionizing radiation (49). This suggests that BRCA mutation is not the sole molecular determinant for HR-deficiency or vulnerability to PARP inhibition. Oncogenic mutations in the isocitrate dehydrogenase genes (*IDH1* and *IDH2*), found within various human tumors including HGG, are associated with HR-deficiency and also increase sensitivity to PARP-inhibition in the absence of BRCA-mutation (50, 51). In this setting, the impairment in HR machinery is driven by the oncogenic metabolite 2-hydroxyglutarate – a product of mutant IDH enzymes. Based on these findings, an open study investigating the PARP inhibitor BGB-290 in combination with temozolomide (TMZ) in newly diagnosed or recurrent IDH-mutant HGG, with newly diagnosed patients enrolling after completion of radiation is now enrolling (NCT03749187). Ongoing studies exploring mutation signatures that predict HR-deficiency will hopefully identify a greater number of HR deficient tumors that may benefit from PARP inhibition (52–54).

A series of clinical trials are underway to investigate combination therapies with PARP inhibitors and radiation in pediatric and adult HGG and highlight several principles that are relevant to combination of targeted agents with chemo/radio-therapy. A phase I/II study in newly diagnosed DIPG patients was performed by the Pediatric Brain Tumor Consortium (PBTC) to identify a safe dosing regimen for the CNS-penetrant PARP inhibitor veliparib and determine the safety/efficacy of combination with up front radiotherapy and maintenance TMZ (55). This trial stopped early due to no identified survival benefit compared to historical controls (a common design in pediatric CNS tumor trials due to limited

equipoise for side by side comparisons to single-agent strategies) and also poor tolerance of TMZ dose escalation in combination with velapirib. Dose limiting toxicities for the combination were predominantly hematologic, consistent with overlapping toxicities of TMZ and PARP inhibitors. In adult patients, the phase I OPARATIC study in recurrent GBM demonstrated that the PARP inhibitor, olaparib, penetrated to tumor in of 100% of patients on study and identified a safe dosing strategy for intermittent olaparib dosing in combination with continuous TMZ to overcome overlapping hematologic toxicity (56). Currently, a phase I trial is moving this combination up front with radiotherapy in newly diagnosed GBM patients (57). Varied clinical response to PARP inhibitors is impacted by various factors, including: 1) tumor intrinsic features (such as HR-deficiency) and 2) pharmacodynamic properties of distinct PARP inhibitors. Pre-clinical work has demonstrated that anti-tumor activity of PARP inhibitors is not only impacted by inhibition of enzymatic function (suppression of parylation), but also by sequestration ("trapping") of PARP complexes at sites of DNA damage – preventing efficient repair and leading to cell death (58, 59). PARP trapping potency does not always correlate with extent of enzymatic inhibition and different PARP inhibitors are more or less potent at trapping PARP complexes (58). It is not yet clear which of these activities drives anti-tumor activity of PARP inhibitors in patients, but it is plausible that this may be context/tumor specific.

In addition to combining with chemotherapy and radiation, PARP inhibition in tumor cells can modulate the immune microenvironment through upregulation of tumor cell PD-L1 expression. This upregulation subsequently results in immunosuppressive effects on T cell mediated anti-tumor immunity (60). In this setting, combination of PARP inhibition and anti-PD-L1 therapy improved survival in orthotopic mouse models of high-risk breast cancers. The phase I/II basket trial examining olaparib and the anti-PD-L1 antibody, durvalumab, in patients with germline *BRCA*-mutated metastatic breast cancer (MEDIOLA) demonstrated that this combination therapy was well tolerated with a safety profile similar to individual agents and associated with objective response in 63% of patients (61). This work demonstrates that targeted therapies against tumor cell intrinsic processes exert effects on the tumor microenvironment and highlights thoughtful design of discovery-based combination therapy trials to examine these effects in patients (**Figure 1**).

## MAPK Pathways – BRAF, NF1, PTPN11

A large body of work on MAPK pathway alterations in human cancer has revealed complexities of how this pathway promotes tumor progression (62). Certainly, drugs that inhibit MAPK signaling affect tumor growth by down-regulating mitogenic signals driven by oncogenic alterations in this pathway. Preclinical work indicates that oncogenic MAPK signaling also modulates DDR and response to radiation (**Figure 1**). Dasgupta et al. reported that BRAF inhibitors enhanced radiosensitivity in *BRAF V600E* mutant glioma (63), possibly through disruption of BRAF-mediated upregulation of non-homologous end joining (NHEJ) machinery as seen in radio-resistant papillary thyroid carcinoma (64). Additionally, MAPK signaling in tumor cells elicits cell-extrinsic effects by shaping the tumor microenvironment in ways that can be further exploited therapeutically with immunotherapy combinations. For example, exploratory molecular analysis of patients enrolled in the HERBY phase II study investigating bevacizumab in combination with radiation/temozolomide in pediatric HGG revealed a positive correlation between MAPK pathway activation (alterations in *NF1*, *BRAF*, *PTPN11*, *PTPN12*) and CD8 T cell effector gene expression signature (29). While the net effect of immune signatures on response to immunotherapy is complex, retrospective analyses of adult GBM patients treated with ICI also revealed enrichment of MAPK pathway alterations in responders (65). These data suggest that MAPK-activated high-grade tumors may be more immunogenic and responsive to agents that enhance anti-tumor immune response. Preclinical studies in melanoma have also revealed that combined BRAF and MEK inhibition induces cancer cell death *via* pyroptosis – a highly inflammatory form of programed cell death (66), which triggers an anti-tumor immune response that persists even after drug treatment is completed. Considering these findings in the context of radiation-induced inflammation, there may be opportunities for additive or even synergistic impact when bringing these therapies together in CNS tumors.

## Additional Promising Targeted Therapy and Radiation Combinations in Pediatric CNS Tumors

Activation of the PI3K/AKT pathway and aberrant chromatin regulation are common features of high-risk pediatric brain tumors, including histone mutant DMG, medulloblastoma, and HGG, and may represent additional therapeutic vulnerabilities (20, 39). For instance, the dual histone deacetylase inhibitor (HDAC)/PI3K inhibitor, CUDC-907 (fimepinostat), has enhanced radiation-induced DNA damage and cell death in orthotopic models of HGG and DIPG (67). Building on these findings, a target validation study of fimepinostat in newly diagnosed DIPG, recurrent medulloblastoma, and recurrent HGG is ongoing (NCT03893487). If CNS penetration and safety are demonstrated in this study, prospective studies in combination with up-front radiation for these diagnoses may be the next phase of study for these diseases. Similarly, the polycomb repressive complexes (PRC1 and PRC2), large multimeric protein complexes involved in gene silencing *via* chromatin regulation, are implicated in a variety of human cancers (68). Multiple studies have demonstrated a tumor-promoting function of BMI1, a ubiquitin ligase and PRC1 component, in DIPG (69–71). Preclinical studies demonstrate that inhibition of BMI1 impaired tumor cell proliferation, promoted cell differentiation, and sensitized cells to radiation induced DNA damage (69). Based on these findings, an open phase Ib trial is investigating the BMI1 inhibitor PTC596 in combination with up-front radiation in newly diagnosed DIPG and non-brain stem pHGG (NCT03605550).

ONC201 is another small molecule inhibitor actively undergoing investigation in combination with radiation. The

drug is an imipridone compound that was originally identified as an inducer of TNF-related apoptosis-inducing ligand (TRAIL) expression in cancer cell lines (72). Mechanistic studies have indicated that ONC201 upregulates expression of TRAIL and its receptor DR5 through activation of the integrated stress response pathway – an evolutionarily conserved cellular adaptation that mediates 1) response to nutrient deprivation and 2) cell death in the setting of irremediable cellular stresses (73, 74). Through genetic and chemical approaches, multiple groups have identified the mitochondrial enzyme caseinolytic protease P (ClpP) as a direct target of ONC201 and demonstrated that ONC201-mediated ClpP activity is required for anti-tumor activity (75, 76). ONC201-dependent ClpP activity led to degradation of mitochondrial respiratory chain proteins, generation of mitochondrial reactive oxygen species (ROS), and activation of a cytotoxic integrated stress response (75). In pre-clinical studies, ONC201 was found to enhance radiosensitivity in orthotopic mouse models of glioma (77). Radiation therapy also enhances cellular ROS levels, through direct radiolysis of water molecules and through generation of mitochondrial ROS, suggesting a possible mechanism underlying ONC201-dependent radiosensitization (78, 79). Based on early signals for efficacy in recurrent H3K27M mutant glioma, this agent was explored in an expanded access program for pediatric and adult patients with this diagnosis (80). While the patient cohorts are small, long-term objective responses were reported in several patients, furthering the signal of potential efficacy in this high-risk group of patients. A current phase II study for H3K27M positive pediatric HGG, including brain stem glioma, is now open (NCT03416530), and includes arms for maintenance therapy after standard of care radiation, therapy at time of recurrence, and in combination with up-front radiotherapy. These studies will determine if ONC201-dependent radio-sensitization translates into therapeutic benefit in patients.

## Perspectives on Combining Targeted Therapies With Radiation – Safety and Toxicity

As targeted treatments are developed, the safety profile of combination therapies is a major consideration. When approaching the combination of targeted therapies with radiotherapy, a useful framework accounts for both acute and late effects of each mode of treatment, with an eye toward overlapping toxicities. Consideration of overlapping toxicities has guided the development of current standard of care chemo/radiation regimens, and has informed early experience in the combination of multiple targeted therapies for pediatric brain tumors (81). Proactive consideration of anticipated overlapping toxicities of radiation and targeted therapies will be vital in the design of safe and efficacious combination regimens for pediatric brain tumors. In addition, long term sequelae of exposure to targeted therapies in the developing pediatric CNS must be considered, especially if utilized in combination with radiotherapy, where long term adverse effects are already well documented.

The use of agents targeting the RAS/MAPK pathway combined with radiotherapy has some of the most mature data in this realm. Retrospective experience is available for combination BRAF inhibitors and CNS directed radiotherapy for melanoma patients with brain metastases and may inform the use of such combinations in pediatric CNS tumors. The skin is a key organ system where overlapping toxicities of radiation and BRAF inhibitors must be considered. In patients receiving concurrent BRAF inhibitor with whole brain radiotherapy, the incidence of radiodermatitis was significantly greater in patients receiving combined therapy: reported as 44% for combination compared to 8% receiving radiation alone (82). Stereotactic radiosurgery for melanoma brain metastases appeared to have a lower incidence of such skin toxicities, as the anticipated total dose to normal skin would be smaller (82). Importantly, long-term follow-up revealed that although higher in incidence, acute radiodermatitis was reversible in all cases and did not lead to lasting cutaneous side effects. Cutaneous side effects include cutaneous squamous cell carcinoma, which develops in the context of compensatory signaling through wildtype BRAF and MEK in non-melanoma skin cells. As a result, such toxicities are interestingly mitigated by the addition of MEK inhibitor (83). Thus, it remains to be understood if rates of radiodermatitis with BRAF inhibitor plus radiation are improved with addition of MEK inhibitor. At least one report demonstrated that patients with melanoma brain metastases treated with combination BRAF inhibitor and stereotactic radiotherapy experienced greater rates of intra-tumoral hemorrhage when compared to radiotherapy alone (84). A caveat when extrapolating this experience to the treatment of primary CNS tumors is that the pathophysiology of CNS metastasis and hemorrhage risk is very distinct, with rate of spontaneous intracranial hemorrhage in brain metastases occurring at a much higher rate than in primary CNS tumors, especially in melanoma (85). Still, such findings extrapolated from this combination highlight the need for rigorous adverse event monitoring in patients receiving novel combinations of BRAF inhibitors with radiation. The impact of tissue tolerance toward radiotherapy is further highlighted in the experience treating patients with concurrent EGFR inhibitors and radiotherapy. Schwer et al. reported that concurrent gefitinib and stereotactic radiosurgery in fifteen patients with recurrent glioma was well tolerated (86). On the other hand, experience with extra-cranial radiotherapy with EGFR inhibitors for thoracic tumors demonstrated greater incidence of bystander effect like stomatitis and pneumonitis (87). This likely stems from CNS tissue being largely post-mitotic, as opposed to the continuously renewing mucosal and epithelial tissues.

In addition to injury of neighboring non-tumor tissues, acute toxicities of radiotherapy can derive from achieving tumor cell death and activation of host immune response in the tumor. A commonly encountered outcome of this treatment effect is radiographic and clinical pseudoprogression. On-target, anti-tumor response to radiation can be associated with tumor cell death, immune activation, and edema, leading to the phenomenon of pseudoprogression following radiotherapy. With respect to brain tumors, radiographic pseudoprogression is defined as

increased contrast enhancement and other signs of tissue edema early following radiation therapy, which ultimately subsides without a change in disease directed therapy (88–90). The latter feature distinguishes pseudoprogression from true disease progression, which is inherently challenging to tease out on imaging analyses alone. Radiographic pseudoprogression can be associated with an increase in clinical symptoms, and in such cases, corticosteroids are often utilized as supportive care. However, due to side effects of corticosteroids, the anti-VEGF agent bevacizumab is being increasingly deployed as a steroid sparing agent for such patients (91). We may find that pseudoprogression becomes more prevalent in the setting of combination targeted therapies with radiation, perhaps as a result of additive or synergistic effects of these strategies. Recognition of this potential acute effect must also be considered when determining clinical trial endpoints and imaging measures of response, such as progression-free survival, which could be impacted by erroneous declaration of disease progression (92).

While the acute effects of combination therapy with targeted drugs and radiation are starting to be elucidated, late neurocognitive, neuroendocrine, and neurovascular complications remain to be discovered. Given their contemporary development, long-term side effects of targeted therapies alone are not yet well understood. As discussed above, active prospective trials evaluating the utility of MEK inhibitor for pediatric LGGs include long-term follow up assessments of vision, motor function, neurocognitive function, and quality of life. This understanding will inform the potential long-term toxicities of combination MEK inhibitors and radiotherapy. While this combination is not considered a strategy for LGG treatment, therapy for higher grade lesions may involve such combinations. When these agents are being combined with radiation, where the same organ systems (i.e. vision) can be negatively impacted by each independent strategy, care must be taken to monitor patients closely. Like the strategy for MEK inhibitors in LGG, we emphasize the importance of long-term tracking of functional outcomes in patients receiving any targeted therapy. Additionally, cranial radiotherapy carries a risk of inducing small and large vessel vasculopathy and increased stroke risk (93, 94). Kinase inhibitors against a variety of molecular targets affect angiogenic/vascular signaling pathways as well, with vasculopathy reported most frequently in patients treated with BCR/ABL inhibitors for CML (95). As such, emphasis should be placed on ongoing neurovascular imaging as a routine part of late effect monitoring for patients receiving combination therapies. As the number of targeted therapies and the patients who receive them grows, it will be important to develop and employ long-term follow-up guidelines for adverse event monitoring, especially when given concurrently with radiotherapy.

## IMMUNOTHERAPY AND RADIATION

Cancer immunotherapy refers to treatments that enhance anti-tumor immune function and anti-tumor immune responses, like the response to pathogens, involve a balance of signals that stimulate and restrain immune activation. This balance safeguards against uncontrolled inflammation and autoimmune disorders, but immunosuppressive signals are also co-opted by tumors to escape immune-mediated elimination. Immune-checkpoint signaling restrains T cell function *via* engagement of inhibitory receptors on the T cell surface (including PD-1 and CTLA-4). Ligands for these receptors (including PD-L1 and B7) can be expressed on tumor cells, stroma, or monocytes. ICI therapies function by disrupting these signals (96). In terms of combination strategies, some of the effects of targeted therapies on the immune system were described in the previous section (i.e. PARP inhibitors increasing expression of tumor cell PD-L1). With regard to radiotherapy, the cell-intrinsic effect of radiation on cancer cells is well appreciated, with radiation induced reactive oxygen species eliciting DNA double strand breaks and subsequent cell death or senescence (1). Notably, immune function is also necessary for the anti-neoplastic effect of radiotherapy, including local cytokine production, modulation of tumor associated myeloid cells, cytotoxic T cell infiltration, and enhanced antigen presentation (5, 97, 98). However, radiation-induced inflammation, like other triggers of the immune system, also include inhibitory signals that restrain anti-tumor immune function, including immune checkpoint pathways (99). Thus, there is significant rationale for the combination of immunotherapy with radiation to overcome the immunosuppressive tumor environment.

A goal of immunological therapies is to trigger a local and systemic immune response to eradicate or control the existing tumor. An adaptive immune response also has the potential to promote long term tumor control or prevention of recurrence, even after the patient has completed immunotherapy (100). Preclinical and clinical evidence suggests that radiation can promote such a systemic anti-tumor response (97, 98). This principle is exemplified by the abscopal effect, which corresponds to tumor response at sites of disease beyond the irradiated tumor. Pre-clinical studies have indicated that this effect is at least partly due to systemic anti-tumor immune response following radiation (97, 98). In patients, this is predominantly retrospectively reported and, to date, is unpredictable – likely reflecting variations in antitumor immune status across patients and tumors. ICI and other immunotherapies have the potential to increase the number of patients who might benefit from treatments that trigger a systemic anti-tumor immune response. Within pediatric brain tumors, including DIPG, medulloblastoma, and ependymoma, clinically apparent disease dissemination can be detected on MRI or CSF cytology. However, even in clinically localized DIPG (on MRI and CSF cytology), microscopically disseminated disease is noted in many patients at time of autopsy (101). This dissemination may have occurred following radiotherapy, but there is also the possibility that microscopic disease dissemination occurred before the time of diagnosis, as is the case for microscopic metastatic dissemination for many solid tumors that appear to be localized at diagnosis (102). Focal radiotherapy alone to the primary tumor site thus may not be sufficient to trigger immune surveillance for microscopically disseminated disease. Additional

recent work has implicated a hematogenous route of medulloblastoma dissemination, with subsequent re-seeding of the leptomeningeal space (103). The authors identified circulating tumor cells in newly diagnosed medulloblastoma patients, including those with clinically localized disease, and demonstrated that the hematogenous route can contribute to leptomeningeal dissemination in preclinical disease models. In both of these examples, boosting the systemic anti-tumor immune response may improve the potential for eradicating microscopic disease to prevent relapse or progression.

In this section, we review translational and clinical work around immune regulation and immunotherapy in pediatric brain tumors, with an emphasis on strategies to combine such agents with radiation.

## Immune Environment of Pediatric Brain Tumors

Molecular and histologic profiling of pediatric CNS tumor samples and preclinical disease models have shed light on the immune environment of pediatric brain tumors and provided a glimpse at the molecular determinants of the tumor immune environment. An improved understanding of these determinants will inform patient selection for immunotherapies and the development of rational combination strategies to boost response. Previous immunophenotypic profiling across various types of pediatric brain tumors revealed a spectrum of immune compositions, suggesting that mechanisms shaping the immune environment and extent of immunosuppression are likely distinct across different tumor types (104). Thus, the barrier to overcome tumor-induced immune suppression is likely different for distinct tumor types. For HGG, analyses from the HERBY trial suggested that histone mutant DMG were "immune cold," while MAPK pathway altered pHGG had greater CD8+ effector cell signature (29). For DMG, this "immune cold" transcriptional signature is corroborated by immunohistochemical and flow cytometry based of immune profiling, which demonstrated a very low T cell infiltration (105). These findings suggest that therapeutic agents that enhance cytotoxic T cell function (i.e. ICI) may not be sufficient as single agents and may need to be combined with therapies that enhance cytotoxic T cell infiltration (i.e. radiation). On the other hand, retrospective analyses of adult GBM patients receiving ICI therapy demonstrated that responders to therapy were more likely to exhibit MAPK pathway alterations (106). Given these findings, along with the greater CD8+ effector cell signature noted in MAPK-altered pediatric HGG, these patients may have a lower barrier to overcoming tumor-related immune suppression and may be more amenable to immunotherapies (29). Recent work has also demonstrated that *TP53* mutations, a common feature of many high-risk pediatric brain tumors, impairs anti-tumor immunity through down-regulation of MHC-I in pre-clinical models of medulloblastoma and DIPG (107). These findings serve as initial insight into the intertwined relationship between histologic, molecular, and immune profiles of CNS tumors and potential mechanisms of vulnerability (**Figure 1**).

Another potential determinant of anti-tumor immunity across human cancers is tumor mutational burden (108). One possible mechanism underlying this relationship is increased immunogenicity from tumor-associated neo-antigens generated by somatic mutation (109). The response of hypermutated HGG in the setting of CMMRD to ICI, as described earlier, is a key example of this (30). However, it is also clear that TMB is not the sole determinant of response to ICI for most tumors. Work from Touat et al. suggests that across adult GBM, the path to hypermutation was more likely to impact ICI response, rather than TMB alone (110). The authors identified two primary paths to hypermutation: 1) *de novo* hypermutated gliomas that developed in the setting of CMMRD or DNA polymerase mutations or 2) a much more commonly observed group of acquired mismatch repair deficits following chemotherapy treatment. In the latter group, the mechanism for acquired hypermutation was potentially due to molecular evolution of tumors under selective pressure from TMZ, as acquired mismatch repair deficits arise from this treatment (111). The patients with acquired hypermutation did not exhibit a greater response to ICI than non-hypermutated patients. Single cell sequencing of tumors with acquired hypermutation demonstrated that mutations were sub-clonal, perhaps explaining the absence of a robust boost in anti-tumor immunity following ICI. Contrast this to *de novo* hypermutated glioma, in which mutations (and neoantigens) were more likely to be truncal and subsequently trigger anti-tumor cytotoxic T cells upon ICI therapy. Furthermore, recent preclinical studies in mismatch repair (MMR) deficient tumors report that TMB, and the presumed associated increase in tumor associated antigens, is not sufficient to elicit anti-tumor immunity or predict response to ICI (112, 113). They demonstrate that sensing of cytosolic DNA, which is increased in MMR deficient cells, *via* the c-GAS-STING pathway is necessary for anti-tumor immune response and response to ICI in preclinical tumor models. Furthermore, they demonstrate that in patients with MMR deficient tumors, downregulation of cGAS-STING is associated with a poor prognosis.

Overall, these studies demonstrate that 1) an understanding of the molecular determinants of anti-tumor immunity can help identify patients that may respond to immunotherapy, and 2) combination therapy will likely be indicated to boost anti-tumor immunity or overcome mechanisms of resistance for most patients. In this section, we review various immunotherapies that can be combined with radiation and mechanisms underlying radiation-induced signaling changes within tumor cells and in the microenvironment that provide rationale for such combinations. In addition to highlighting the potential combinations that maximize efficacy, we discuss key factors that will impact the safety of such combinations.

## Systemic Immunotherapies

Experience with immunotherapies for pediatric CNS tumors is an actively evolving field and as such, experiences with

immunotherapy in adult malignancies and other pediatric cancers are pertinent. The CheckMate 143 trial was the first randomized phase III study to evaluate checkpoint blockade in patients with primary brain tumors. This study compared anti-PD-1 inhibition using nivolumab to bevacizumab in adult recurrent GBMs after standard of care surgery and radiation. This study did not find an OS difference in these two groups, but did demonstrate a side effect profile for nivolumab that was similar to those reported when used in other adult malignancies (31). Checkmate 143 included exploratory cohorts that tested combination nivolumab with up front chemo/radiotherapy (114). Preliminary analyses demonstrated that this therapy was well tolerated and prompted ongoing randomized phase III studies examining nivolumab with concurrent chemoradiotherapy in newly diagnosed GBM (Checkmate 498, Checkmate 548). CheckMate 143 also evaluated potential clinical variables that modulate the impact of immune-based therapies. Baseline corticosteroid use is a documented prognostic indicator in patients with GBM (115) and corticosteroid use carries the risk of impairing lymphocyte function. In multi-variate analyses in CheckMate 143, patients with no baseline steroid use had a significantly greater OS when treated with nivolumab, when compared to those on steroids. No significant difference was observed in the bevacizumab group. While potentially subject to confounding factors, this trend suggested the possibility that steroid therapy impaired therapeutic efficacy in the nivolumab cohort. The impact of corticosteroids on lymphocyte count and function is well documented, and may also contribute to immunosuppressive tumor microenvironment in brain tumor patients (116). As such, the use of bevacizumab as a steroid-sparing agent in treatment of pseudoprogression in patients receiving combination radiotherapy and ICI is commonly considered. The safety of bevacizumab plus ICI is demonstrated in other solid tumor therapies and is being actively explored in a phase II study of GBM (NCT03452579) (117, 118).

ICI use in pediatric oncology has ranged from disease-specific application to basket trials across pediatric solid and CNS tumors (NCT02304458). An initial study using the PD-1 inhibitor, pembrolizumab, in progressive DIPG, enrolled only 5 patients before being put on hold due to neurologic deterioration that appeared to be more rapid than historical controls and cautioned enrollment of subjects with late stage recurrent disease in future immunotherapy trials (119). This study has since re-opened and is continuing to enroll across a variety of brain tumor subtypes, outside of DIPG. Since this report, retrospective and prospective experience has demonstrated the safety of ICI in pediatric CNS tumors, including DIPG, at diagnosis and in the setting of recurrence. Single, retrospective institutional experience with nivolumab combined with reirradiation for recurrent DIPG demonstrated overall tolerability of this combination treatment with some potential signal of benefit with the combination approach (OS 22.9 months with nivolumab and reirradiation *vs.* 20.4 months with reirradiation alone) (120). While the small sample size of this retrospective study limited the ability to form conclusions on efficacy, patients on corticosteroids at the start of combination radiotherapy and nivolumab were all able to wean steroids following treatment, providing some signal of therapeutic benefit.

An ongoing prospective efficacy study of the anti-PD-1 agent, REGN210 (cemiplimab), is currently evaluating the combination with radiotherapy in newly diagnosed patients with DIPG and non-brain stem HGG, as well as with re-irradiation in recurrent HGG (NCT03690869). This study is investigating alternative radiation fractionation strategies in combination with ICI. While a discussion around conventional *vs* hypofractionated therapy is outside the scope of this review, we highlight that alternate fractionated strategies are being investigated in combination studies with immunotherapy, based on clinical and preclinical studies showing that alternate radiation strategies have distinct effects on the tumor immune environment and that sub-ablative radiation doses may have immune-priming effects (121–123). Additional benefits of shorter radiation courses include reduced strain on patients and their families, who often need to travel or relocate to medical centers where radiotherapy is provided, and decreased need for daily anesthesia for the youngest patients. To this end, a matched cohort study investigated safety and efficacy of two hypofractionated strategies for newly diagnosed DIPG patients: 39 Gy in 16 fractions or 44.8 Gy in 11 fractions (124). In this study with 27 children, both regimens were well tolerated and OS and progression free survival outcomes were not statistically different from a matched historical cohort (54 Gy in 30 fractions). In addition to exploring how these strategies affect efficacy, it will be necessary to prospectively identify the safety profile of combination strategies as well. The cemiplimab study is not designed to directly compare the two arms of standard *vs*. hypofrationated radiation, but will provide valuable information on radiation schedules in combination with ICI.

The timing of immunotherapy may also impact efficacy, as neo-adjuvant pembrolizumab, followed by maintenance therapy, significantly extended OS compared to maintenance therapy alone in patients with GBM (32). This suggests that timing of immunotherapy prior to local control measures (surgery or radiation) may boost anti-tumor immune response, perhaps due to inflammatory signaling elicited by local control treatments. To explore this, a randomized double blind, pilot trial of neoadjuvant checkpoint inhibition in recurrent pediatric or young adult HGG is active (NCT04323046). In this study, patients who are undergoing debulking or re-resection as part of their standard care will receive nivolumab, ipilimumab, or nivolumab plus ipilimumab prior to resection. Through the assessment of CNS tumor tissue following neoadjuvant ICI, this study will also augment our understanding of the impact of neo-adjuvant PD-1 inhibition on the immune micro environment, provide biomarkers of response *vs* resistance, and shed light on future combination strategies to augment PD-1 blockade. Insight gained from this trial may inform investigations of neo-adjuvant ICI in newly diagnosed patients with high-risk brain tumors.

Another immunosuppressive pathway implicated in tumor biology involves the enzyme, indoleamine 2,3 dioxygenase 1

(IDO1). IDO1 is expressed in tumor infiltrating T cells and its enzymatic activity converts tryptophan to kynurenine – a molecule which reduces cytotoxic T and NK cell activity, while promoting the expansion of immunosuppressive regulatory T cell and myeloid derived suppressor cell populations (125). Tumor infiltrating lymphocytes in GBM upregulate IDO1 and greater IDO1 gene expression correlates with worse prognosis in GBM patients (126). This immunosuppressive pathway is active in a number of advanced solid tumors, which led to the development of IDO1 inhibitors. CNS penetrant IDO1 inhibitors have been investigated and demonstrated efficacy in preclinical GBM models when added to radiation and PD-1 blockade (127). Within pediatric brain tumors, a phase I study of the oral IDO1 inhibitor, indoximod, identified a R2PD dose in pediatric patients with progressive high grade brain tumors and demonstrated safety when given concurrently with radiotherapy and temozolomide in newly diagnosed DIPG (NCT02502708) (128). Compared to historical controls, thirteen newly diagnosed patients reported in this study demonstrated median OS of 14.5 months, which is greater than that of historical controls where survival tends to range from 9-12 months, suggesting potential promise of this approach (129, 130). However, biopsy was not a requirement on this study and the absence of prognostically relevant information about tumor biology limits the assessment of treatment efficacy in this small cohort.

Preclinical work has also demonstrated that various pediatric glioma associated antigens can elicit anti-tumor immune responses, which may reflect a novel therapeutic opportunity. Building on these results, a clinical trial assessed sub-cutaneous vaccination with glioma associated antigens (IL-23Ralpha2, EphA2, and survivin) concurrent with up-front radiation or chemo/radiotherapy in newly diagnosed pediatric brain stem glioma and HGG subjects (131). The primary endpoints of this study were safety and assessment for systemic immune response to vaccination. Results demonstrated toleratability and antigen-specific interferon responses in peripheral blood mononuclear cells in the majority of patients. Subjects with DIPG who had evidence of pseudoprogression (4 out of 5) had improved OS compared to those without (median OS 19 versus 11 months). A potential explanation for this is that symptomatic pseudoprogression is associated with a more robust anti-tumor response. Chheda et al. has also demonstrated that H3K27M-specific T cells could be propagated after *in vitro* antigen exposure and that transfer of H3K27M specific T cells led to anti-tumor activity in mouse glioma xenografts, providing a neoantigen target (132). A a phase I/II multi-institutional study is evaluating the combination of a peptide vaccine against H3K27M, alone or in combination with nivolumab, in newly diagnosed patients with H3K27M positive DIPG or DMG (NCT02960230), beginning at 2-8 weeks post initial radiotherapy. In this study, the single agent peptide vaccine was overall well tolerated, with grade 1-2 injection sites reactions being most common (133). Longitudinal immunophenotypic profiling yielded biological correlates to response, including evidence of sustained H3K27M reactive CD8 T cells (39% of patients). Conversely, patients who received dexamethasone therapy, either before or after vaccination, exhibited declining H3K27M specific CD8 cell counts on longitudinal observation and poorer OS. Although steroid dependence is independently associated with worse survival in DIPG patients (134), we highlight again that in the context of immunotherapy, immunosuppressive effects of corticosteroids are likely to impact treatment efficacy and bevacizumab should be considered as a steroid sparing supportive medication in patients receiving immunotherapy (116). Lastly, the combination of tumor vaccine with radiation and/or ICI has the potential to promote a more robust anti-tumor immune response and overcome tumor-related immunosuppression in patients who are immunologic-non-responders to single agent peptide vaccine.

Cytokine release instigated from ionizing radiation induces a local inflammatory environment that further shapes local immune response. The use of exogenous cytokine therapy as an immune adjuvant in combination with radiotherapy has been explored for various malignancies (135). Specifically, TNF-dependent regulation of pathogen or cancer associated immune responses has been the subject of long standing research (136). Preclinical work has identified that combined TNF and immune checkpoint blockade is sufficient to overcome the immunosuppressive tumor microenvironment in two high risk pediatric brain tumors – *TP53*-mutant SHH medulloblastoma and DIPG. A recurrent mechanism of tumor immune escape is down-regulation of surface MHC-I, which is necessary for presentation of tumor associated antigens (TAAs). The authors of this study demonstrated that mutant *TP53* was sufficient to drive immune escape in mouse models of medulloblastoma and DIPG and that this immunosuppression was dependent on *TP53*-mediated down-regulation of MHC-I. In orthotopic tumor models, systemic TNF alpha was sufficient to restore MHC-I expression on tumor cells and enhance response to ICI in mouse models, leading to tumor regression that was associated with lasting systemic anti-tumor immunity that prevented engraftment on repeated tumor challenge. This work demonstrates that *TP53* alterations may serve as key biomarker for tumor-related immunosuppression when compared to *TP53* wildtype counterparts and may require combination strategies. Previous clinical studies with systemic single-agent TNF alpha demonstrated significant dose-limiting acute, systemic toxicities due to inflammatory signaling (137, 138). Notably, in the work summarized above, low doses of TNF that were tolerable for weeks were sufficient to upregulate tumor cell MHC-I and enhance ICI efficacy. These findings suggest that low dose TNF alpha plus ICI may be a viable therapy option in patients with *TP53* mutated brain tumors and highlights the principle that synergistic anti-tumor effects of combination therapies might be obtainable with lower doses than those identified in studies with single agent treatments (i.e. below the maximum tolerated dose for single agents). This strategy is going to be tested in an upcoming trial combining TNF and nivolumab. Notably, radiotherapy is also sufficient to increase local TNF and enhance tumor MHC-I expression, possibly with less systemic toxicity than systemic exogenous TNF (139). It will be of interest

to determine if radiation plus ICI would provide similar effects in high risk *TP53* mutant tumors.

## Local Immunotherapies

To overcome tumor associated immunosuppression, local agents have been utilized to stimulate the immune system. Advantages for local immunotherapy deliver include: direct inoculation to overcome the blood brain barrier and limiting systemic toxicities through local injection. An example of this is intra-tumoral injection of unmethylated cytosine-guanosine motifs (CpG-ODN), which are not present in mammalian cells, but correspond to a pathogen associated molecular pattern (PAMP) found in bacterial and viral genetic material (140). Immune responses to CpG are mediated by Toll-Like Receptor 9 (TLR9), which is located primarily on antigen presenting cells, including dendritic cells and CNS microglia, as well as glioma cells (141). Engagement of TLR9 results in inflammatory cytokine production and enhances antigen presentation to CD8 T cells. A phase II randomized study combined CpG injection into the tumor bed at the time of up-front resection in newly diagnosed GBM (142). This therapy was found to be safe but did not enhance survival in this study. Side effects included greater risk of post-operative fever and injection site hematoma, but no severe or lasting adverse events were noted. The combination of CpG with other modalities that enhance immune activation, including radiation and ICI, is now being evaluated (140). Rodent glioma models demonstrated a survival benefit of combined CpG and XRT, an effect that required T cell function (143). These results suggest that local CpG plus radiotherapy provide additive or synergistic benefit to overcoming tumor-induced immune suppression. In high risk pediatric tumors, intra-tumoral delivery of local therapies is employed in various settings, including oncolytic virus injection (discussed in next section) and in novel catheter-based infusion strategies that deliver anti-neoplastic agents *via* convection enhanced delivery (144, 145). Thus, the clinical systems for delivery of local immune-adjuvants are in place and can be explored as another strategy for overcoming tumor associated immunosuppression, in combination with radiation or systemic therapies.

Oncolytic viruses are another avenue for local immunotherapy. These viruses exert anti-tumor activity through several possible mechanisms: direct tumor cell killing, increased tumor associated antigen presentation, and stimulation of a local pro-inflammatory environment. Multiple oncolytic viruses with tropism for CNS tumors have reached the clinic and have demonstrated safety when injected locally as a single agent. For example, oncolytic herpes simplex viruses (oHSV) have a natural tropism for neural tissue and have been modified to restrain viral replication in normal neural tissue while permitting replication in tumor cells (146). In preclinical models, single doses of radiation enhanced oHSV replication and viral-associated tumor cell killing (147). In addition, oncolytic viruses have demonstrated radio-sensitizing effects, with a potential mechanism involving viral-mediated impairment of DNA repair pathways (148). These findings led to combination oHSV and radiotherapy in glioma patients. Intra-tumoral injection of the oHSV G207 with 5Gy single dose focal irradiation has now been found to be safe and result in stable disease or partial reponse in a cohort of nine adult patients with HGG (149). A phase I study investigating the safety profile of delivering oHSV *via* surgically implanted catheters in 12 pediatric patients with recurrent or refractory supratentorial pHGG found that oHSV ($10^7$ or $10^8$ plaque forming units, alone or in combination with a single 5 Gy dose of focal radiotherapy) was well tolerated and resulted in no identified peripheral blood virus shedding (150). The authors reported a median OS of 12.2 months (95% CI 8 to 16.4), which is longer than the median OS of 5.6 months in historical cohorts. This study also highlighted the challenges of post-therapy clinical and imaging follow up after local immunotherapy for pediatric CNS tumor therapy. Several patients underwent repeat tissue biopsy per standard care (due to indeterminant MRI findings or new onset neurologic symptoms), facilitating histologic assessment of local immune response to therapy. Immunohistochemistry revealed presence of tumor infiltrating lymphocytes, suggesting that oHSV therapy may help overcome the "immune cold" nature of pHGG. Lastly, the authors found that HSV serologies may serve as a biomarker for G207 therapy benefit, with inferior OS in patients who were HSV seropositive at baseline (median OS 5.1 months) and improved OS in patients who seroconverted during therapy (median OS 18.3 months). A forthcoming phase II trial will assess efficacy of oHSV ($10^8$ plaque forming units with 5 Gy radiation) in a larger cohort of relapsed or refractory supratentorial pHGG and provide additional prospective information on determinant of immune and tumor response to therapy (NCT04482933).

As oHSV trials proceed, preclinical efforts are underway to identify combination therapies with oHSV to promote anti-tumor immune response, including concomitant ICI or exogenous expression of pro-inflammatory cytokines *via* the modified oHSV. In a mouse glioma model, combination of oHSV with PD-1 and CTLA-4 blockade led to tumor regression in most mice and prevented tumor engraftment on tumor re-challenge in mice with initial tumor regression, suggesting that this combination therapy generated a lasting anti-tumor immune response (151). In advanced melanoma patients, a randomized phase II reported improved objective response rate to 39% from 18% when oHSV engineered to express GM-CSF was added to anti-CTLA4 therapy (odds ratio, 2.9; 95% CI, 1.5 to 5.5; P = .002) (152). Interestingly, responses were not limited to the injection site (i.e. abscopal effect), suggesting that a systemic anti-tumor immune response was elicited. The most common side effects were very similar to those seen with each single agent.

Adenovirus, poliovirus and measles virus are additional oncolytic viral therapies being studied in the context of pediatric and adult brain tumors. In adults with recurrent GBM, A phase I dose escalation study of single intratumoral injection of DNX-2401, a modified oncolytic adenovirus, found no dose limiting toxicities and noted objective responses in the majority of patients in this cohort (153). Another subset of patients underwent planned tumor re-resection fourteen days following adenovirus injection. Pathological assessments of

resected tumors demonstrated immunohistochemical markers of active viral replication and CD8 T cell infiltration. Compared to baseline tissue samples, post-DNX-2401 injection specimens exhibited upregulation of the co-inhibitory TIM3 protein in T cells, but no change in PD-1, PD-L1, or IDO-1 expression. This trial highlights potential benefit of neo-adjuvant immunotherapy prior to planned standard of care re-resection. Such investigational approaches provide valuable assessment of *in vivo* responses to immunotherapy, which helps evaluate the accuracy of pre-clinical models and provide hypothesis generating information to inform future investigations. Another single institution pediatric trial for DNX-2401 in newly diagnosed DIPG patients is currently evaluating the safety of a single virus injection after biopsy and preceding standard of care radiotherapy (NCT03178032). In this study, radiotherapy is initiated three to four weeks following DNX 2401 injection. An interval report describing the first eight patients on study reported no evidence of dose limiting toxicities and indicated that patients were able to discharge from the hospital three to four days post-injection (154). Based on the data from DNX-2401 in adult GBM, it is expected that actively replicating virus should be present in the DIPG tumors at the initiation of radiotherapy and that the immune-stimulating effects of the virus and radiation therapy were active concurrently in these patients.

Poliovirus is another virotherapy, which demonstrates tropism for surface CD155, a marker expressed on many solid tumors including glioma and on antigen presenting cells (APCs) (155). Preclinical data demonstrated that anti-tumor immune activity was driven by direct tumor cytotoxicity and by APC-dependent cytokine release, local inflammation, and T cell stimulation (156). A phase I study with a dose expansion phase treated 61 adult patients with recurrent WHO grade IV glioma with intra-tumoral attenuated poliovirus, delivered by catheter-based convection enhanced delivery (155). Therapy was generally well tolerated and no cases of disseminated encephalitis or meningitis were identified. A dose limiting toxicity was observed in one patient who experienced an intratumoral hemorrhage that the authors attribute to the catheter procedure, rather than local inflammatory effects of the virus. Median OS for study patients was not significantly different from a historical control cohort. However, OS did reach a plateau of 21% in study patients at 24 months, which was sustained at 36 months. While duration of follow-up limited statistical analyses at the time of the report, the historical control group did not exhibit this pattern of sustained OS at these time points. The biological determinants underlying the response in these patients is not understood. An early phase trial for CED-based delivery of this attenuated poliovirus in pediatric patients with recurrent HGG (WHO grade III and IV) is active (NCT03043391). If this therapy is found to be well tolerated, follow-up studies may consider combination therapy with radiation to enhance anti-tumor immunity and overcome tumor-related immunosuppression.

An attenuated measles virus is also under clinical investigation for children and young adults with recurrent medulloblastoma or atypical teratoid rhabdoid tumor (ATRT), which express the CD46 surface marker that mediate measles virus entry (NCT02962167). This trial employs local injection of virus at the time of planned surgical resection for localized recurrence or injection into the subarachnoid space *via* lumbar puncture for patients with disseminated disease at relapse. Both approaches have demonstrated safety and efficacy in preclinical models (157, 158). A study investigating local injection of modified measles virus in adult patients with recurrent GBM has completed enrollment and is pending analysis (NCT00390299). While patients with medulloblastoma and ATRT generally receive craniospinal irradiation with a focal boost to the tumor bed, focal re-irradiation is often considered at the time of relapse (159, 160). If these approaches demonstrate safety, strategies to combine oncolytic measles virus with local radiotherapy can be explored to enhance anti-tumor immune response and offer abscopal benefit for patients with disseminated disease.

## Perspectives on Combining Immunotherapy With Radiation – Safety and Toxicity

When evaluating the safety of immunotherapies in pediatric brain tumors, especially in combination with radiotherapy, treatment related inflammation and edema due to immune-mediated tumor cell death must be considered. As described above, clinical and radiographic pseudoprogression can occur in patients undergoing radiotherapy for the treatment of brain tumors, but the overall tolerability in the published experience with combined radiotherapy and ICI in pediatric CNS tumors is reassuring. However, as more patients are treated with these combinations, the incidence of these acute toxicities may become more apparent. Another consideration in evaluating response to combined immunotherapy and radiotherapy is the complexity of interpreting radiographic changes following therapy and potential pseudoprogression, which may make it challenging to ascertain disease progression versus treatment response.

As far as direct CNS toxicity with immune-based therapies, the greatest amount of literature is available for ICI. Anti-CTLA4 therapy is associated with auto-immune hypophysitis in 13% of patients, more so than PD-1 or PD-L1 blockade. Auto-immune thyroiditis is also reported in patient receiving ICI (161). Fortunately, endocrine dysfunction in patients affected by auto-immune hypophysitis or thyroiditis is transient and typically responds to corticosteroids. In contrast to acute neuroendocrine injury seen with ICI, radiation related neuroendocrine dysfunction is a late effect. Long term follow-up studies in patients receiving combined ICI and radiotherapy will be necessary to determine if risk of long-term neuroendocrine dysfunction is affected by this combination. Mechanistically, ICI-related hypophysitis and thyroiditis likely emerge due to on-target engagement of ICI therapy, which boosts systemic immune activation. Interestingly, analyses of adverse events in metastatic melanoma patients receiving ICI has revealed a positive correlation between development of vitiligo, an autoimmune attack of normal melanocytes, and treatment response (162). However, incidence of auto-immune

injury of other organ systems did not exhibit this correlation. Hypotheses for this phenomenon include the shared immuno reactivity of anti-tumor T cells toward antigens in the normal melanocytes. It remains to be determined if similar autoimmune phenomenon will be observed in brain tumor patients receiving immunotherapy. This highlights the importance of treating patients on clinical trials with thorough adverse event monitoring can occur.

As investigations around immunotherapy for pediatric CNS tumors continues, efforts are underway to identify biomarkers that predict response to agents like ICI. However, much remains unknown about the molecular determinants of immune environment and subsequently on the potential response to immunotherapies. As summarized above, some understanding is beginning to emerge. For example, tumors harboring *TP53* mutations are likely associated with greater immune suppression, while tumors with MAPK pathway activation are associated with a more immunogenic environment. As a result, different patients likely require different levels of immunotherapy to achieve therapeutic response. This highlights the need to prospectively investigate immunotherapy on clinical trials where correlative studies, such as pre- and post-treatment biopsies, can be performed to provide hypothesis generating data on determinants of response. Such data will aid in identifying novel drug combinations that can overcome the immunosuppressive environment.

## DISCUSSION

Given crosstalk between the mechanisms underlying various cancer therapies and the ongoing need for better therapies for many CNS tumors affecting children and young adults, it is vital to continue exploring novel combination strategies of radiation, targeted agents, and immune-based therapies (**Figure 1**). Each of these singular approaches offers potential clinical benefit to patients, but by bringing these interventions together, benefit will ideally be augmented. Enhancing our understanding of the molecular and immune drivers of pediatric CNS tumors, will lead to improved translation of novel combination therapy strategies to clinical practice. Such combination approaches will hopefully take advantage of some of the vulnerabilities described in this report and provide new, multi-modal approaches to target high-risk tumors like DMG and recurrent medulloblastoma. In exploring these approaches, potential areas of resistance, as determined by intrinsic patient or tumor characteristics, will need to be considered to ensure selection of patients with the greatest potential to receive clinical benefit. Safety and tolerability will remain of key importance as well, given that combination strategies may confer additive clinical benefit, but could come at a cost of additive toxicity. Within the pediatric context it will also be critical to establish measures that will allow researchers to collect long-term functional outcomes such as endocrine function and cognitive measures, as the impact of these new strategies on the developing brain remain poorly understood. Lastly, the importance of collecting informative biologic specimens will be necessary to provide insight into further patient stratification for combination therapies, validate hypotheses generated from preclinical work, and provide new hypotheses based on pathways of response or resistance. Such efforts will provide foundation from which we can make progress towards improved survival for patients with some of the greatest clinical need.

## AUTHOR CONTRIBUTIONS

BQ: conceptualization; writing, original draft; writing, review and editing. CK: conceptualization; writing, review and editing. SM: conceptualization; writing, review and editing. All authors contributed to the article and approved the submitted version.

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MINI REVIEW
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# Harnessing Lactate Metabolism for Radiosensitization

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Cancer cells rewire their metabolism to promote cell proliferation, invasion, and metastasis. Alterations in the lactate pathway have been characterized in diverse cancers, correlate with outcomes, and lead to many downstream effects, including decreasing oxidative stress, promoting an immunosuppressive tumor microenvironment, lipid synthesis, and building chemo- or radio-resistance. Radiotherapy is a key modality of treatment for many cancers and approximately 50% of patients with cancer will receive radiation for cure or palliation; thus, overcoming radio-resistance is important for improving outcomes. Growing research suggests that important molecular controls of the lactate pathway may serve as novel therapeutic targets and in particular, radiosensitizers. In this mini-review, we will provide an overview of lactate metabolism in cancer, discuss three important contributors to lactate metabolism (lactate dehydrogenase, monocarboxylate transporters, and mitochondrial pyruvate carrier), and present data that inhibition of these three pathways can lead to radiosensitization. Future research is needed to further understand critical regulators of lactate metabolism and explore clinical safety and efficacy of inhibitors of lactate dehydrogenase, monocarboxylate transporters, and mitochondrial pyruvate carrier alone and in combination with radiation.

**Keywords: lactate metabolism, Warburg phenomenon, radiation therapy, radiosensitization, synergistic effects**

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## INTRODUCTION

At the most fundamental level, cells must be self-reliant—producing sufficient ATP and biosynthetic compounds to fuel their ongoing survival and proliferation (1). However, the ability of tumor cells to reprogram their metabolic activity in order to promote their own survival is one of the defining hallmarks of cancer (2). Dating all the way back to the 1920s and the pioneering work of Otto Warburg, lactate has long been identified as a major player in cancer metabolism (3). In his work, Dr. Warburg noted that many cancer cells uptake large amounts of glucose and preferentially produce lactate through glycolytic pathways, even in the presence of oxygen (4). This phenomenon has been observed across many different neoplasms and serves as the basis for tumor detection using glucose tracers with positron emission tomography (PET) (5–9). Recent studies have further shown that lactate plays a critical role in fueling tumor progression, remodeling the tumor microenvironment (TME), and inducing treatment resistance (10). Even more so, research has

begun to reveal how lactate metabolism may be used to influence the radiosensitivity of tumors (11–13). This mini-review will provide a general overview of lactate metabolism and its role within diverse cancers, and specifically, summarize recent studies that suggest an interplay between lactate metabolism and response to radiation.

## LACTATE PATHWAY

In normal human cellular physiology, glucose serves as a major source of lactate production (**Figure 1**). Glucose is most commonly taken into cells *via* facilitated diffusions through glucose transporter proteins (GLUT) (14). Once inside, glucose is phosphorylated to glucose-6-phosphate by hexokinase, effectively entrapping it in the cell (15). In the cytoplasm, glucose-6-phosphate is routed through several oxygen-independent glycolytic reactions to generate two ATP and two molecules of pyruvate, among other products (15). Under normal aerobic conditions, the majority of this pyruvate is then transported into the mitochondria *via* either mitochondrial pyruvate carrier (MPC) or after conversion to lactate *via* monocarboxylate transporters (MCTs), and undergoes oxidative phosphorylation, generating another 32 to 34 ATP per glucose molecule *via* the tricarboxylic acid cycle and electron transport chain (16, 17). However, under anaerobic conditions, cells are unable to rely on oxidative phosphorylation to balance their redox state, and pyruvate is preferentially converted to lactic acid through an enzymatic reaction catalyzed by cytosolic lactate dehydrogenase (LDH) (18). Within cells, lactic acid almost completely dissociates to lactate and $H^+$, and lactic acid accumulation leads to acidification of the cytoplasm and potent inhibition of further glycolysis (18, 19). As such, proper physiological functioning depends on the efflux of lactate out of the cell, and transport across mitochondrial and cellular membranes requires MCTs. Once outside of a glycolytic cell, the excreted lactate is ultimately destined for one of several possible fates. Under physiological conditions, tissues like the heart, brain, and skeletal muscles can use lactate as a fuel source, while the liver can convert circulating lactate into glucose through the Cori cycle (20, 21). Indeed, a growing body of literature has shown that such "shuttling of lactate" between organs plays an important role in the overall regulation of metabolism (22).

## LACTATE AND CANCER

In blood and healthy tissues, the physiological concentration of lactate is roughly 1.0 to 3.0 mmol/L. However, in cancer cells,

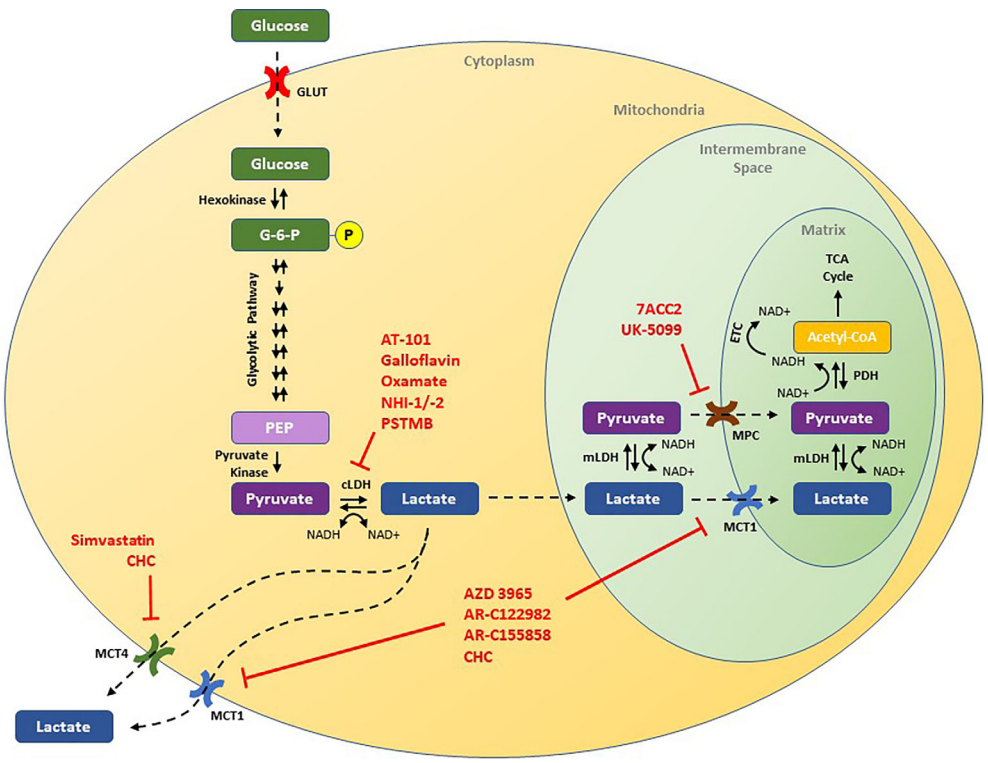


**FIGURE 1** | Schematic of the lactate pathway and illustration of novel therapeutic strategies that have been shown to decrease tumor growth in preclinical studies. Drugs discussed in this review are presented in red and are shown by their putative target of action. ECT, electron transport chain; G-6-P, glucose-6-phosphate; GLUT, glucose transporter; cLDH, cytosolic lactate dehydrogenase; mLDH, mitochondrial lactate dehydrogenase; MCT1, monocarboxylate transporter 1; MCT4, monocarboxylate transporter 4; MPC, mitochondrial pyruvate carrier; NAD+, nicotinamide adenine dinucleotide (oxidized); NADH, nicotinamide adenine dinucleotide (reduced); PEP, phosphoenolpyruvate; PDH, pyruvate dehydrogenase.

lactate concentrations may be up to an order of magnitude higher. Such high concentrations of lactate have been shown to arise primarily from enhanced rates of glycolysis (23). Interestingly, despite its inherent inefficiency in terms of ATP production, high rates of glycolysis have been observed in many cancer cells, even under fully aerobic conditions and with intact oxidative phosphorylation function (23). There are two major theories regarding the preferential dependence of cancer cells on glycolysis. First, the rate of ATP production through glycolysis is much more rapid than oxidative phosphorylation allowing cells to meet changing energy requirements, and second, glycolysis produces many intermediate biosynthetic molecules required by rapidly proliferating cells (24, 25). Regardless of teleology, there are a number of adaptive enzymatic alterations that lead to this so-called, "Warburg phenotype," including changes in the function of hexokinase 2 (HK2), pyruvate kinase type M2 (PKM2), GLUT1, LDH, MCTs, and pyruvate dehydrogenase (PDH) (26–32). The resulting high concentrations of lactate have been further implicated in a wide range of tumoral aberrations, including changes in the TME, immune suppression, and metastasis—where increasing tumoral lactate concentrations are associated with an increased risk of metastatic dissemination (10, 33–39).

Radiotherapy is a curative treatment modality in diverse cancer types, including breast cancer (40, 41), head and neck cancers (42), brain cancer (43), and many pediatric solid tumors (44–46). Furthermore, radiotherapy remains an essential option for palliation such that approximately 50% of patients with cancer will receive radiation treatments during their disease course (47, 48). Recent studies have found that high rates of glycolysis and the build-up of lactate likely contribute to radioresistance in many tumor types through diverse mechanisms including antioxidant protective effects and promoting an immunosuppressive TME (39, 49–53). Interestingly, through impacts on several cellular processes including LDH and PDH, radiation itself can also promote lactate production, which, in turn, may drive a degree of radioresistance (39, 54–56). Within the extensive cellular machinery involved with lactate metabolism, three promising targets, LDH, MCT1/4, and MPC, have been shown to modulate radiosensitivity.

## LACTATE DEHYDROGENASE (LDH)

LDH is a nicotinamide adenine dinucleotide (NAD+) oxidoreductase enzyme that catalyzes the conversion between pyruvate and lactate (57). While constitutively active in aerobic conditions, LDH expression is upregulated in hypoxic environments *via* HIF-1$\alpha$ (58). LDH is a tetrameric enzyme comprised of 2 subunits that can combine in any of 5 combinations. The most common subtype, known as LDHA, preferentially reduces pyruvate to lactate, and is frequently over-expressed in many tumors (10). In addition, LDHA has been shown to catalyze a number of "non-canonical" reactions, including the formation of an "onco-metabolite"—2-hydroxyglutarate—in acidic and anaerobic environments, thus promoting oncogenesis (59–65). Recent studies demonstrate that 2-hydroxyglutarate can promote a transcriptional program of genes that regulate proliferation and growth by inhibiting histone demethylation and TET-mediated DNA demethylation (59–65). Clinically, increased tumoral LDHA expression is associated with poorer clinical outcomes and recent studies suggest it may serve as a prognostic biomarker (66–68).

Knockdown studies of both PKM2 and LDHA have been shown to reduce ATP production, inhibit cell growth, decrease invasiveness, and induce oxidative stress and radiosensitivity in cancer cells (39, 69, 70). Several clinical and pre-clinical studies have further analyzed the effects of both selective and non-selective inhibitors of LDHA on cancer cells. AT-101, a naturally occurring compound derived from cottonseed, is an oral non-selective inhibitor of LDH that additionally inhibits the anti-apoptotic proteins Bcl-2, Bcl-xL, Bcl-W, and Mcl-1 while simultaneously stimulating pro-apoptotic signaling (71, 72). A study of AT-101 monotherapy in 23 men with metastatic castrate-resistant prostate cancer showed that a dose of 20 mg/day was well tolerated and led to a >50% decrease in PSA in roughly 9% of patients (71). Heist and colleagues found that while AT-101 administered concurrently with topotecan was safe for patients with small cell lung cancer (SCLC) who had failed prior platinum-based chemotherapy, this regimen failed to show significant activity with only 8% of patients experiencing a partial response (73). Similarly, Baggstrom et al. failed to demonstrate efficacy in patients with chemo-sensitive recurrent SCLC (74). However, in pre-clinical studies, FX11—a derivative of AT-101 that selectively inhibits LDHA over LDHB—effectively inhibited tumorigenesis *in vivo* using human lymphoma and pancreatic tumor xenograft models (38). When combined with a small molecule inhibitor of NAD+ synthesis, FX11 was further able to induce tumor regression in the lymphoma xenograft model (38). Another study found that both galloflavin, a polyphenol inhibitor of LDH, and oxamate, a competitive analogue of pyruvate, disrupted the heat shock response in cultured hepatocellular carcinoma (HCC) cells and induced cellular senescence (75). Furthermore, two recent studies found that inhibition of LDHA in glioblastoma cell lines with either oxamate or the selective inhibitors, NHI-1 and NHI-2, improved chemotherapy and radiation sensitivity and triggered apoptosis and differentiation of cancer stem cells (76, 77). More recently, PSTMB—a novel allosteric inhibitor of LDH—was found to reduce cellular proliferation in *in vitro* models of lung cancer, breast cancer, melanoma, HCC, and colon cancer (78). In cultured colon cancer cells, PSTMB reduced LDH activity in both aerobic and anaerobic conditions without altering LDH expression, and increased reactive oxygen species (ROS) formation (78).

Despite clinical and pre-clinical interest in LDH inhibitors, there have been few studies of these agents in combination with radiotherapy. Koukourakis, et al. assessed the effects of LDH blockade on the treatment sensitivity of 2 glioblastoma cell lines, U87MG and the more radio-resistant T98G. Silencing LDHA gene expression or inhibiting LDH with oxamate led to enhanced sensitivity to both radiation and temozolomide, with more pronounced effects observed in the T98G cell line (76). Another study by Zhai and colleagues showed that oxamate increased radiation sensitivity primarily by enhancing mitochondrial ROS generation, which in turn promoted

apoptosis in two nasopharyngeal cancer cell lines (79). Yang et al. found that radiotherapy increased lactate concentrations in the TME which then led to localized immunosuppression *via* MDSCs in murine models with explanted human pancreatic cancer cells, and administration of the selective LDHA inhibitor GSK2837808A concurrently with radiation improved antitumoral T-cell response and reduced tumor progression (39). These results suggest that lactate is at least partially responsible for the observed radiotherapy-induced immunosuppression. In a different tact, Judge et al. observed that high lactate concentrations activated latent TGF-β, leading to excessive fibrosis and found that increased LDHA expression correlated with higher rates of pulmonary fibrosis in patients treated with radiotherapy (80). Treatment with AT-101 four weeks after exposing C57BL/6 mice to total-body and thoracic radiation showed significantly decreased TGF-β expression and rates of pulmonary fibrosis (80). Two early phase clinical trials are examining the safety of concurrent chemoradiation with AT-101 in glioblastoma and esophageal and esophagogastric junction cancers (**Table 1**). Overall, these results highlight many of the potential advantages of LDH inhibitors in combination with radiotherapy; however, significant work still remains in order to determine clinical utility.

## MONOCARBOXYLATE TRANSPORTERS

MCTs constitute 14 isoforms of membrane transport proteins that aid in the absorption and efflux of a wide range of biological compounds (83). Ubiquitously expressed, MCT1 primarily mediates import of lactate along with other monocarboxylates (84–87). On the other hand, MCT4 expression is regulated by hypoxia through a HIF-1$\alpha$-dependent mechanism. MCT4 has lower affinity for lactate compared to MCT1, and predominantly participates in lactate efflux (84–87). MCT1 and MCT4 are overexpressed in many cancer types, and their upregulation correlates with worse overall prognosis (17, 88–91). MCT1 and MCT4 may help not only maintain metabolic balance for oxidative and glycolytic cancer cells, respectively, but may also promote an immunosuppressive milieu by increasing the acidity of the TME secondary to the accumulation of lactate (92, 93). Increased acidity has been found to decrease CD8+ T-cell cytotoxicity and CD8+ T-cell-mediated cytokine release; and induce macrophage polarization to an immunosuppressive M2 state (3, 94). Thus, studies have investigated MCTs as a therapeutic target and in particular, a radiosensitizer, in various cancers (81, 82, 89, 95–98).

Numerous studies have found that knockdown or inhibition of MCT1 and/or MCT4 decreases lactate levels and tumor cell growth, migration, and invasion *in vitro* and *in vivo* for diverse cancers, including bladder cancer, breast cancer, colon cancer, glioblastoma, and liver cancer (89, 95–100). Interestingly, in a model of Burkitt's lymphoma, a selective small molecule inhibitor of MCT1, AZD3965, also decreased lipid biosynthesis after lactate build-up, and specifically, levels of phosphocholine were significantly decreased by inhibition of choline kinase α expression and *de novo* phosphocholine synthesis. Furthermore, in the TME, AZD3965-treated tumors also displayed greater interaction with dendritic cells—increasing tumor antigen presentation—and natural killer cells—leading to direct killing of tumor cells (101).

**TABLE 1** | Preclinical studies and clinical trials exploring lactate pathway targets with radiotherapy in cancer.

| Preclinical Studies | | | | |
|---|---|---|---|---|
| **Target** | **Inhibitors** | ***In vitro*/*In vivo* model** | **Results** | **Reference** |
| LDH | Oxamate | *In vitro:* U87MG and T98G glioblastoma cell lines | Oxamate and radiation decreased RD50 | (76) |
| LDH | Oxamate | *In vitro:* CNE-1 and CNE-2 nasopharyngeal carcinoma cell lines<br>*In vivo:* CNE-1 xenograft tumors | Oxamate and radiation increased apoptosis at 24 hours after radiation and increased radiation-induced inhibition of clonogenic survival. Oxamate and radiation decreased tumor growth in *vivo* | (79) |
| LDH | GSK2837808A | *In vivo:* Panc-02-luciferase orthotopic tumors | GSK2837808A and radiation decreased tumor growth and MDSC activation, and increased cytotoxic CD8+ T cells within the tumor in *vivo* | (39) |
| MCT | AR-C122982, AR-C155858, simvastatin, 2-cyano-3-(4-hydroxyphenyl)-2-propenoic acid (CHC) | *In vitro:* CAL27 oral squamous cell carcinoma cell line | AR-C122982, simvastatin, or CHC and radiation decreased cell proliferation | (81) |
| MCT | AZD3965 | *In vitro:* H526 small cell lung cancer cell line<br>*In vivo:* H526 small cell lung cancer xenograft tumors | AZD3965 and radiation increased intracellular lactate concentration, and decreased tumor growth and improved survival in *vivo* | (82) |
| MPC | 7-aminocarboxycoumarin 2 (7ACC2), UK-5099 | *In vivo:* SiHa cervical cancer xenograft tumors | 7ACC2 or UK-5099 and radiation decreased tumor growth | (11) |
| **Clinical studies** | | | | |
| **Target** | **Treatment regimen** | **Diagnosis** | **Results** | **Reference** |
| LDH | AT-101 and chemoradiation with docetaxel and 5-fluorouracil (NCT00561197) | Locally advanced esophageal or gastroesophageal junction cancer | Ongoing trial | |
| LDH | AT-101 and chemoradiation with temozolomide or temozolomide alone (NCT00390403) | Glioblastoma Multiforme | Ongoing trial | |

*MDSC, myeloid-derived suppressor cells; RD50, radiation dose resulting in 50% cell viability.*

Two studies have examined the combination of MCT inhibition and radiation in tumor models. Brandstetter et al. studied the effects of multiple MCT inhibitors with or without radiation on the oral squamous cell carcinoma (SCC) cell line CAL27 *in vitro*. Specifically, they analyzed MCT1 inhibitors, AR-C122982 and AR-C155858, the MCT4 inhibitor, simvastatin, and the non-specific MCT inhibitor, 2-cyano-3-(4-hydroxyphenyl)-2-propenoic acid (CHC) (81). Though the MCT inhibitors differed in their potency and efficacy, treatment decreased cell proliferation, viability, and wound healing (81). The combination of radiation with specific MCT inhibitors further resulted in enhanced anti-proliferative activity (81). Currently, it is not known whether these other pathways may further contribute to the effects seen in this study.

Many studies have found that AZD3965, a selective small molecule inhibitor of MCT1, is effective in inhibiting tumor growth in many different preclinical models of cancer (82, 99–104). Two studies demonstrated that AZD3965 inhibited bidirectional lactate transport leading to both accumulation of intracellular lactate (with greater effects observed in hypoxic conditions), and antitumor activity in SCLC models *in vitro* and in xenograft models (82, 99). Bola et al. found that radiation alone did not affect intracellular lactate in H526 SCLC cells, but when delivered in combination with AZD3965, intracellular lactate concentration significantly increased (82). Furthermore, compared with radiation alone, AZD3965 for seven days with concurrent radiation delivered on days 3-5 significantly decreased tumor growth and improved survival with one mouse showing no tumor recurrence (82). Unfortunately, MCT1 inhibitors are ineffective in tumor cells that highly express MCT4, which suggests that MCT4 expression may serve as a biomarker for patient selection and predictor of response to anti-MCT1 therapy (98, 99).

Of the MCT1 inhibitors, AZD3965 has entered early phase clinical trials. Preliminary results from a phase I study investigating the safety of AZD3965 in patients with refractory advanced solid malignancies found that AZD3965 was well tolerated. While the most common side effects were nausea and fatigue, patients also experienced expected on-target effects of retinal electroretinographic changes that were dose-limiting at 20 mg daily and increased urinary ketones (105). One patient had exacerbation of previously undiagnosed tumor-associated lactic acidosis, which was dose-limiting (105). Future research is still needed to explore the tolerability and efficacy of AZD3965 and other MCT inhibitors alone and in combination with radiation.

## MITOCHONDRIAL PYRUVATE CARRIER

MPC is formed by two proteins encoded by genes *MPC1* and *MPC2*. It transports pyruvate from the cytoplasm into mitochondria, and sits at the crossroads of glycolysis, mitochondrial oxidative phosphorylation, and lactate production (106, 107). In highly glycolytic tumors, decreased MPC expression can lead to aerobic glycolysis and shunting to glutaminolysis, ultimately leading to greater tumor proliferation. On the other hand, tumors that are more dependent on oxidative phosphorylation may be more sensitive to alterations in MPC-mediated pyruvate transport (106). Recent studies have also found that lactate accumulation in tumors can promote the synthesis of intermediates of the tricarboxylic acid cycle, further supporting cell proliferation (12, 108, 109). Thus, there is growing interest in MPC as a regulator of both oxidative phosphorylation and lactate production in tumorigenesis.

Recently, 7-aminocarboxycoumarin 2 (7ACC2) was identified as a novel MPC inhibitor that led to downstream reductions in lactate influx and delays in tumor growth within *in vitro* models of cervical cancer, colorectal cancer, breast cancer, hypopharyngeal SCC, and pancreatic cancer (11, 110–112). Corbet et al. found that 7ACC2 blocked MPC activity, thereby inhibiting pyruvate metabolism and subsequently blocking lactate influx consistent with another known MPC inhibitor, UK-5099 (11). In a spheroid model using FaDu hypopharyngeal SCC cells, treatment with MPC inhibitors produced cytotoxic effects and led to decreased hypoxia in the spheroids (11). In SiHa cervical cancer xenograft models, the combination of 7AAC2 with radiation using either 16 Gy in one fraction or 20 Gy in five fractions, led to significantly decreased tumor growth compared with 7AAC2 or radiation monotherapy. Similar results were also observed *in vivo* using shRNA targeting MPC1 or UK-5099 (11). These preclinical data suggest that MPC represents a novel target warranting further clinical investigation both alone and in combination with radiation.

## FUTURE DIRECTIONS

Many preclinical studies have identified multiple targets within the lactate metabolic pathway that play a role in radiosensitization, and future research is ongoing to identify novel targets for lactate metabolism. Studies exploring safety of these targets are still needed, particularly for patients at risk for metabolic acidosis either from co-morbidities or prior cancer therapy. Furthermore, it remains important to note that different solid tumors may have unique alterations in lactate metabolism and intratumoral metabolic heterogeneity may also cause differential response to inhibition of lactate metabolism (10, 108, 113, 114). These characteristics are important considerations for future studies and increasingly support identifying tumor types in which harnessing radiosensitizing properties through lactate metabolism inhibition has the greatest therapeutic benefit. For example, additional imaging techniques, such as $^{13}C$ magnetic resonance spectroscopy, can better provide dynamic imaging of lactate metabolic reprogramming (115–117). A recent clinical trial explored de-escalation of radiation to 30 Gy for patients with human papillomavirus-associated oropharyngeal tumors who had no hypoxia at baseline using dynamic fluorine-18-labeled fluoromisonidazole PET or resolution of hypoxia during intratreatment PET; while patients with persistent hypoxia received 70 Gy (118). Identifying patients with hypoxic tumors

or tumors with specific alterations of lactate metabolism may allow for improved patient selection for future clinical trials involving radiation and inhibitors of lactate metabolic pathways.

## CONCLUSION

A growing body of evidence has shown that, in addition to its use as a fuel source, lactate also promotes tumor growth. Interestingly, elevated lactate levels and lactate-mediated downstream pathways can cause changes in transcriptional programming (59–65), tumor immune microenvironment (10, 39), lipid synthesis (101), among others (3). The effects of these downstream changes, particularly with regards to decreasing the levels of ROS, can contribute to radio-resistance (3). Recent studies have found that inhibitors of LDH, MCT, and MPC can serve as radiosensitizers in models of glioblastoma, pancreatic cancer, SCLC and cervical cancer (11, 39, 76, 82). There remains limited clinical investigation of these inhibitors with radiation as only two early phase clinical trials are studying AT-101 in combination with radiation (**Table 1**). Future research is needed to understand the mechanisms by which regulators of lactate metabolism promote tumorigenesis, identify tumor subtypes that are uniquely dependent on lactate pathways, and to further explore targeted inhibitors of this pathway in preclinical and clinical studies.

## AUTHOR CONTRIBUTIONS

KL, SP, DH-K, and MM contributed to the conception and design of this study. KL, EE, and MM wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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# The Evolving Role of Radiotherapy for Pediatric Cancers With Advancements in Molecular Tumor Characterization and Targeted Therapies

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Ongoing rapid advances in molecular diagnostics, precision imaging, and development of targeted therapies have resulted in a constantly evolving landscape for treatment of pediatric cancers. Radiotherapy remains a critical element of the therapeutic toolbox, and its role in the era of precision medicine continues to adapt and undergo re-evaluation. Here, we review emerging strategies for combining radiotherapy with novel targeted systemic therapies (for example, for pediatric gliomas or soft tissue sarcomas), modifying use or intensity of radiotherapy when appropriate *via* molecular diagnostics that allow better characterization and individualization of each patient's treatments (for example, de-intensification of radiotherapy in WNT subgroup medulloblastoma), as well as exploring more effective targeted systemic therapies that may allow omission or delay of radiotherapy. Many of these strategies are still under investigation but highlight the importance of continued pre-clinical and clinical studies evaluating the role of radiotherapy in this era of precision oncology.

**Keywords: precision medicine & genomics, pediatric cancer, targeted therapies, molecular diagnostics, radiation therapy (radiotherapy), pediatric glioma, medulloblastoma, pediatric sarcomas**

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## INTRODUCTION

In the early history of pediatric cancer treatment, surgical resection and then radiation therapy served as the primary treatment modalities (1, 2). Subsequent introduction of chemotherapy regimens resulted in combination therapies with reduction in radiotherapy dose in many cases (3, 4). Further refinement of chemotherapy regimens and significant advancements in radiotherapy techniques have led to improvements in disease outcomes while limiting late toxicities, critical for treatment of childhood cancers. Recently, dramatic and rapid advancements in precision medicine, which we define here as more precise genomic and molecular characterization of individual tumors, development of targeted anti-tumor drugs, and improved accuracy and conformality of radiotherapy, have enabled treatment approaches that may be better tailored to each patient (5–8). Radiotherapy has remained a mainstay and one of the most effective anti-cancer treatments;

however, these advances in precision medicine require constant re-evaluation of the role of radiotherapy in this evolving landscape. A critical goal in the treatment of pediatric malignancies is to maintain effective cancer control while minimizing late toxicities as much as possible. On one hand, it can be tempting to try to omit or limit the use of radiotherapy for childhood cancers given potential late effects in an era of improvements in targeted systemic therapies. In some cases, this may be appropriate for select patients, as long as disease control can be maintained. On the other hand, the potential for radiotherapy to synergize with targeted drugs should be explored and fully utilized. Significant advancements in radiotherapy techniques have also been made in this era of precision medicine, *via* improvements in conformality with intensity-modulated radiotherapy (IMRT) and proton therapy, better precision with image guidance, and reductions of dose and treatment volumes where appropriate, allowing for reduced toxicity and an improved therapeutic ratio with radiotherapy.

## ROLE OF RADIOTHERAPY WITH ADVANCES IN TARGETED SYSTEMIC THERAPIES

Better molecular and genomic characterization of tumors, along with advances in targeted drug development, have resulted in more specific systemic therapies for pediatric tumors, which in some cases may have better anti-tumor efficacy and in many cases are associated with less toxicity compared to standard chemotherapy regimens. In some cases, these targeted systemic therapies can be used upfront, delaying local radiotherapy and reserving it for progression, while in others, these targeted therapies may be given concurrently with or following radiotherapy, or in the recurrent or metastatic setting.

### Management of Pediatric Low-Grade Gliomas With Advances in Targeted Therapies

Low-grade gliomas (LGG) are among the pediatric tumor types for which novel targeted agents have demonstrated promising potential. While malignant progression is rare in pediatric LGG (in contrast to adult LGG) and 5-year overall survival is greater than 90% (9), patients whose tumors cannot be fully resected often end up requiring multiple courses of therapy, with associated late effects and long-term reduction in quality of life (10). For LGG that cannot be managed by surgery alone, current management is controversial: conventional cytotoxic chemotherapy is typically the recommended initial approach for pediatric patients, deferring radiotherapy to limit late toxicities (11). However, advances in radiotherapy techniques that can reduce late toxicities, including IMRT and proton therapy, may make radiotherapy a more viable earlier-line option. Further, it is now fairly established that the majority of pediatric LGG arise from an alteration in the mitogen-activated protein kinase (MAPK) signaling pathway, including BRAF mutation (most commonly V600E point mutation) or fusion (most commonly BRAF : KIAA1549), NF1 mutation, NTRK family fusion, and FGFR1 mutation or rearrangement, along with other less common alterations (**Figures 1**, **2**) (5, 6, 13–16). Thus, targeted agents including MEK1/2 (an upstream kinase of MAPK), BRAF, and TRK inhibitors have been evaluated and have demonstrated promising activity in pediatric gliomas (17–22).

The most mature data in this setting exist for the MEK1/2 inhibitor selumetinib. In a multicenter phase 2 study by the Pediatric Brain Tumor Consortium, pediatric patients with recurrent, refractory, or progressive LGG after at least one line of standard therapy were treated with selumetinib (18). Response and survival outcomes compare favorably to prior studies of recurrent or progressive pediatric LGG treated with chemotherapy regimens including carboplatin/vincristine and vinblastine monotherapy (**Table 1**) (18, 23–27). We note that data regarding the efficacy of selumetinib for patients without NF1- or BRAF alteration-associated LGG from this study are still pending, and prior studies of chemotherapy did not stratify or have information regarding NF1 or BRAF status. Nonetheless, these promising results have led to the current Children's Oncology Group (COG) randomized studies ACNS1831 [NCT03871257] and ACNS1833 [NCT04166409], which are evaluating selumetinib *versus* standard carboplatin/vincristine chemotherapy in the upfront setting for patients with NF1-associated or non-NF1-associated low grade gliomas, respectively.

Studies of other targeted agents are also complete or underway, including a phase 2 study (TRAM-01, NCT03363217) of the MEK1/2 inhibitor trametinib (the first FDA-approved MEK inhibitor) in patients with progressing/refractory LGG or plexiform neurofibroma with activation of the MAPK pathway (28), and a phase 1/2 study of the BRAF V600 inhibitor dabrafenib in pediatric patients with BRAF V600-mutant relapsed or refractory LGG (**Table 1**) (19, 29). BRAF V600E mutation has been identified in nearly 20% of pediatric LGG across a range of histologies and sites and confers a worse prognosis than BRAF wild-type tumors when treated with conventional adjuvant therapies (including chemotherapy and radiotherapy) (15). While TRK fusions are less commonly identified in pediatric gliomas, robust responses to TRK kinase inhibitors have been seen in pediatric solid tumors harboring TRK fusions, including high grade gliomas (20, 21, 30). Thus, when feasible, pediatric LGG should be evaluated for potentially targetable alterations, as MEK1/2, BRAF, and TRK inhibitors have demonstrated promising activity in pediatric gliomas and can be considered for patients who have failed upfront chemotherapy.

The timing of use of radiotherapy for LGG is controversial and continues to evolve with developments in targeted systemic therapies and radiotherapy techniques. Radiotherapy has for years demonstrated effective control of unresectable, progressive LGG, with 10-year PFS and overall survival (OS) of approximately 70% and 80%, respectively (31–33). However, concerns of late toxicity, including neurocognitive deficits, stroke, endocrine dysfunction, and secondary malignancy, especially in younger patients treated with radiotherapy (32–35), led to a shift toward initial treatment with systemic therapy and avoidance or delay of

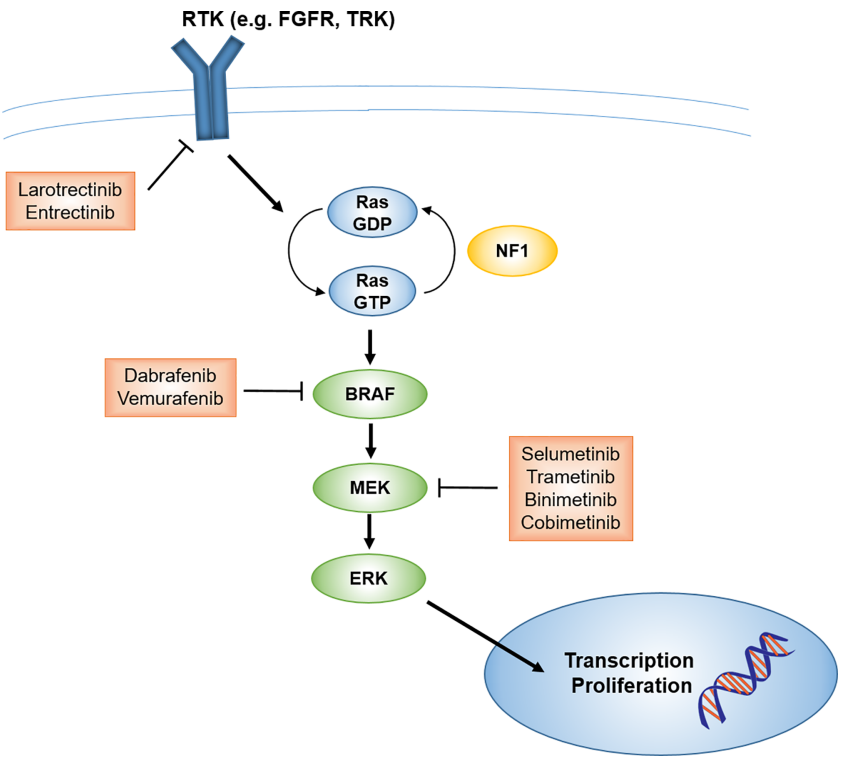


**FIGURE 1** | Schematic of MAPK signaling pathway and potential targets and therapeutics for pediatric LGG. FGFR, fibroblast growth factor receptor; LGG, low-grade glioma; MAPK, mitogen-activated protein kinase; RTK, receptor tyrosine kinase; TRK, tropomyosin receptor kinase.

radiotherapy (36–38). In many cases, treatment with multiple lines of systemic therapy, deferring radiotherapy, has resulted in significant morbidity from tumor progression (39). Advances in radiotherapy techniques since the 1990s have allowed for more precise and conformal delivery of radiotherapy, maintaining tumor control while reducing normal tissue toxicity (**Table 2**). An early study of stereotactic radiotherapy for pediatric low-grade gliomas in the 1990s at the Dana Farber Cancer Institute used magnetic resonance imaging (MRI)-based treatment planning and smaller radiotherapy target margins and demonstrated maintained PFS and OS (65% and 82%, respectively, at 8 years), with no marginal failures (40). A subsequent phase 2 trial was conducted at the St. Jude Children's Research Hospital of conformal radiotherapy for pediatric low-grade gliomas using primarily 3-dimensional conformal radiotherapy (3D-CRT) with a 10mm clinical target volume (CTV) margin and MRI-based planning. Disease control was similarly maintained, with 10-year EFS and OS of 74% and 96%, respectively (41). Late effects were overall limited compared to patients treated with less conformal techniques, although cognitive deficits and risk of vasculopathy were greater in patients younger than age 5 at the time of treatment (41, 45). More recently, the COG study ACNS0221 (2006–2010) evaluated conformal radiotherapy for pediatric LGG, using a smaller 5mm CTV margin with the majority (71%) of patients receiving IMRT, the current standard radiotherapy technique. This study also demonstrated favorable disease control (5-year PFS and OS of 71% and 93%, respectively) with limited toxicity (42). Finally, treatment with proton therapy, which can often further spare normal tissues for pediatric brain tumors compared to IMRT (46), has demonstrated reduced toxicity while maintaining excellent disease control for pediatric LGG. A study from the Massachusetts General Hospital demonstrated 8-year PFS and OS of 83% and 100%, respectively, and no significant declines in intelligence quotient (IQ), although a subset analysis suggested more neurocognitive decline in patients <7 years and those with significant dose to the left temporal lobe/hippocampus (43). More recently, a report on a large series of patients (n=174) treated with proton therapy for LGG at the University of Florida Health Proton Therapy Institute also demonstrated excellent disease control (5-year PFS and OS of 84% and 92%, respectively), with <5% developing serious late toxicity at a median follow-up of 4.4 years (44).

In this context of reduced toxicity from newer radiotherapy techniques, recent studies suggest that delayed radiotherapy may be associated with worse outcomes in some patients with pediatric LGG. A study of pediatric patients treated with radiotherapy for optic pathway and hypothalamic LGG at St. Jude found that receipt of chemotherapy prior to radiotherapy was associated with worse EFS (hazard ratio 3.1, 95% CI: 1.4-7.0, P=0.007) and that younger age <6 years at the time of radiotherapy (patients who were typically treated first with chemotherapy) had worse EFS and OS (32). A very recent study by investigators at St. Jude reviewed pediatric patients

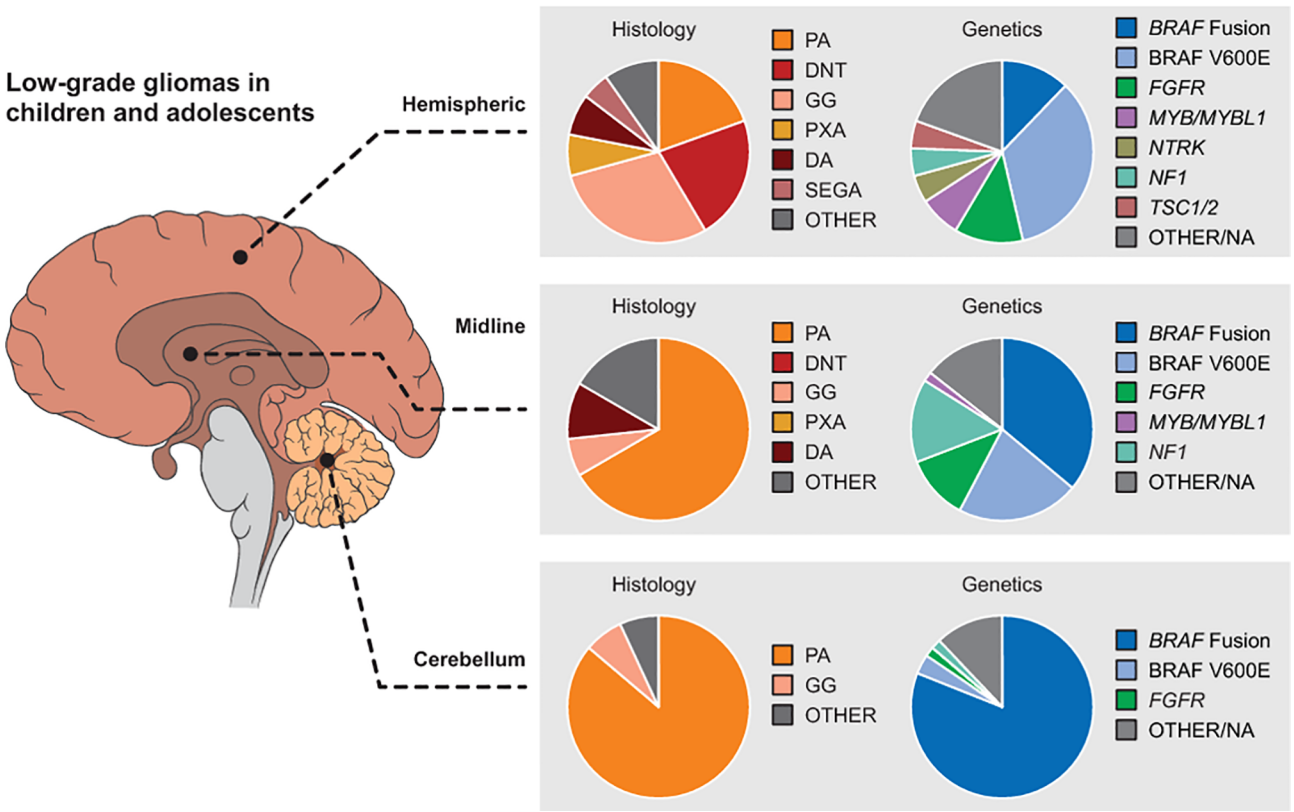


**FIGURE 2** | Distribution of pediatric LGG histologies and genetic alterations by location in the brain. Reproduced with permission from Filbin and Sturm (12). DA, diffuse astrocytoma; DNT, dysembryoplastic neuroepithelial tumors; GG, ganglioglioma; LGG, low-grade glioma; PA, pilocytic astrocytoma; PXA, pleomorphic xanthoastrocytomas; SEGA, subependymal giant cell astrocytoma.

with unresectable LGG treated with radiotherapy and identified low- and high-risk groups based on OS [10-year OS of 96% (95% CI: 89-98%) *versus* 76% (95% CI: 59-87%) respectively] (47). Within the high-risk group, which included diffuse astrocytoma or location within the thalamus/midbrain, delayed radiotherapy (after at least one line of chemotherapy) was associated with worse PFS (hazard ratio 2.5, 95% CI: 1.4-4.4, P=0.001). Thus, early radiotherapy should be considered for LGG patients with higher risk disease, those at risk of functional impairment with progression, older patients, and those without targetable alterations.

Several questions arise from these studies regarding the management of pediatric LGG: can novel targeted agents be combined with radiotherapy, and can modifications in radiotherapy dose be considered? The studies of MEK1/2 and BRAF inhibitors for pediatric LGG have been for recurrent, refractory, or progressive disease and not in combination (whether concurrent or sequential) with radiotherapy. Pre-clinical data have suggested synergy between MEK1/2 and BRAF inhibitors with radiotherapy for pediatric gliomas (48–50), but concerns regarding toxicity of concurrent treatment exist (51, 52). The standard radiotherapy dose for pediatric LGG (~54 Gy) is largely derived from adult studies, where dose escalation above 45-50 Gy has not been associated with improved outcomes in randomized trials, but retrospective data in both adult and pediatric studies suggest better survival with treatment to ≥53 Gy (44, 53–55). As recent studies of radiotherapy for pediatric LGG have focused on reduced margins and more conformal delivery techniques (reviewed above), the standard dose has remained ~54 Gy. While improvements in conformality may lessen the benefits of dose reduction for LGG, there would likely still be significant benefit for patients with larger tumors or those near critical structures such as the hippocampi (56). Further, combination with MEK1/2 and/or BRAF inhibitors may allow for reduction of radiotherapy dose while maintaining tumor control. Future investigations could evaluate these combinations, with standard *versus* reduced-dose radiotherapy and with targeted therapy and radiotherapy delivered concurrently *versus* sequentially as in ACNS1723 for high-grade glioma (discussed in the next section) to minimize toxicities of combined therapy.

## Management of Pediatric High-Grade Gliomas With Advances in Targeted Therapies

While pediatric high-grade gliomas (HGG) are standardly treated with conventional radiotherapy and temozolomide chemotherapy based on adult data (57), this treatment approach as studied in

**TABLE 1** | Prospective studies of systemic therapies for recurrent/progressive/refractory pediatric low-grade glioma.

| Study (Accrual years) | Number of patients | Patient population | Study type | Systemic therapy agent(s) | ORR | EFS/PFS | OS |
|---|---|---|---|---|---|---|---|
| Packer et al. (23) (accrual years not reported) | N=23 (recurrent)<br>N=37 (newly diagnosed) | Age <21 years with recurrent LGG or age <5 years with progressive, newly diagnosed LGG | Single arm (multi-center) | CARBO/VCR | Recurrent: 52 ± 10%<br>Newly diagnosed: 62 ± 8% | NR | NR |
| Gururangan et al. (24)(1993-2000) | N=81 | Age ≤18 years with progressive LGG | Single arm phase 2 (multi-center) | CARBO | 28% (95% CI 18-38%) | 3-year FFS: 64% (95% CI 54-76%) | 3-year OS: 84% (95% CI 76-93%) |
| Ater et al. (25) (1997-2005) | N=274 | Age <10 years with progressive or residual LGG | Randomized (COG) | CARBO/VCR *vs*. TPCV | CARBO/VCR: 50%<br>TPCV: 52% | 5-year EFS (all patients): 45 ± 3% (difference between arms NS) | 5-year OS (all patients): 86 ± 2% |
| Bouffet et al. (26) (2002-2006) | N=51 | Age <21 years with recurrent or refractory LGG | Single arm phase 2 (multi-center) | Vinblastine | 36% | 5-year EFS: 42 ± 7% | 5-year OS: 93 ± 4% |
| Fangusaro et al. (18)(2013-2015) | N=25 (Stratum 1: pilocytic astrocytoma with BRAF aberration)<br>N=25 (Stratum 3: NF1-associated LGG) | Age 3-21 years with recurrent, refractory, or progressive LGG (≥1 prior line of therapy) | Single arm phase 2 (PBTC) | Selumetinib | Stratum 1: 36%<br>Stratum 3: 40% | 2-year PFS:<br>Stratum 1: 70% (95% CI 47-85%)<br>Stratum 3: 96% (95% CI 74–99% | NR |
| Hargrave et al. (19) (2013-2015) | N=32 | Age <18 years with BRAF V600-mutant recurrent, refractory, or progressive LGG (≥1 prior line of therapy) | Single arm phase 1/2a (multi-center) | Dabrafenib | 44% (95% CI 26-62%) | 1-year PFS: 85% (95% CI 64-94%) | NR |

*COG, Children's Oncology Group; CARBO, carboplatin; CI, confidence interval; EFS, event-free survival; FFS, failure-free survival; LGG, low-grade glioma; NR, not reported; NS, non-significant; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PBTC, Pediatric Brain Tumor Consortium; TPCV, thioguanine, procarbazine, lomustine (CCNU), vincristine; VCR, vincristine.*

**TABLE 2** | Studies of advanced radiotherapy techniques for pediatric low-grade glioma.

| Study (Treatment years) | Number of patients | Patient population | Radiotherapy dose, technique, and margin | Sites of failure | EFS/PFS | OS |
|---|---|---|---|---|---|---|
| Marcus et al. (40) (1992-1998) | N=50 | Age 2-26 years with LGG | Dose: Mean 52.2 Gy (range 50.4-58 Gy)<br>Technique: Stereotactic RT, MRI-based planning<br>CTV margin: 0mm<br>PTV margin: 2mm | Marginal: 0<br>In-field: 6<br>Distant: 5 | 8-year PFS: 65% | 8-year OS: 82% |
| Merchant et al. (41) (1997-2006) | N=78 | Age 2-19 years with LGG | Dose: 54 Gy<br>Technique: 3D-CRT, MRI-based planning<br>CTV margin: 10mm<br>PTV margin: 3-5mm | Marginal: 1<br>In-field: 8<br>Distant: 4 | 10-year EFS: 74 ± 15% | 10-year OS: 96% ± 6% |
| Cherlow et al. (42) ACNS0221 (2006-2010) | N=85 | Age 3-21 years with unresectable progressive, residual, recurrent LGG | Dose: 54 Gy<br>Technique: IMRT (71%), MRI-based planning<br>CTV margin: 5mm<br>PTV margin: 3-5mm | Marginal: 0<br>In-field: 19<br>Distant: 4 | 5-year PFS: 71% ± 6% | 5-year OS: 93% ± 4% |
| Greenberger et al. (43) (1995-2007) | N=32 | Age 2-21 years with LGG | Dose: Median 52.2 GyRBE (range 48.6-54 GyRBE)<br>Technique: Proton RT, MRI-based planning<br>CTV margin: 3-5mm<br>PTV margin: N/A | Marginal vs. in-field (not specified): 4<br>Distant: 1 | 8-year PFS: 83% | 8-year OS: 100% |
| Indelicato et al. (44) (2007-2017) | N=174 | Age 2-21 years with LGG | Dose: 54 GyRBE (74%)<br>Technique: Proton RT, MRI-based planning<br>CTV margin: 5mm<br>PTV margin: 3mm | Marginal: 0<br>In-field: 21<br>Distant: 2<br>In-field + distant: 1 | 5-year PFS: 84% (95% CI 77-89%) | 5-year OS: 92% (95% CI 85-95%) |

*3D-CRT, 3-dimensional conformal radiotherapy; CI, confidence interval; CTV, clinical target volume; EFS, event-free survival; Gy, Gray; IMRT, intensity-modulated radiotherapy; LGG, low-grade glioma; MRI, magnetic resonance imaging; OS, overall survival; PFS, progression-free survival; PTV, planning target volume; RBE, relative biological effectiveness; RT, radiotherapy.*

ACNS0126 and ACNS0423 did not improve outcomes in children with HGG compared to prior treatments with radiotherapy and other chemotherapy regimens (58–60). Pediatric diffuse midline gliomas, including diffuse intrinsic pontine glioma (DIPG), are typically considered high grade given aggressive behavior even with lower grade histology (61) and are treated with radiotherapy and best supportive care. Outcomes overall are still very poor for these tumors, and thus novel treatment approaches are desperately needed. Multiple studies have now established a different molecular genetic profile underlying pediatric HGG compared to adult disease, with frequent somatic mutations in histone H3 genes, TP53, and ATRX; focal amplification of PDGFRA; chromosome 1q gain; NTRK and other targetable gene fusions in infant HGG; and infrequent IDH1 hotspot mutations (14, 21, 62–65). Approximately 5-10% of pediatric HGGs harbor BRAF V600E mutations and have a slightly better clinical outcome, potentially accounting for some of the long-term survivors in pediatric HGG trials (66, 67).

Therapeutically, these advances in molecular characterization will allow tailoring of treatment approaches for pediatric HGG instead of a single standard paradigm for all patients. Unfortunately, in contrast to LGG, a single drug is unlikely to benefit a large number of patients given the heterogeneity of these tumors, and radiotherapy will likely remain a critical component of upfront treatment for these patients. Infant HGG may be one subset where targeted therapies are used upfront, deferring radiotherapy, as these tumors more frequently exhibit targetable MAPK alterations and gene fusions targeting ALK, NTRK, ROS1, and MET (14, 21) and have demonstrated rapid clinical responses to targeted therapies in case reports (20, 68). For older children with HGG, two ongoing COG trials are evaluating novel systemic therapies together with radiotherapy depending on tumor molecular features: for patients with BRAF V600 mutant-HGG, ACNS1723 [NCT03919071] is a phase 2 trial evaluating treatment with the BRAF V600 inhibitor dabrafenib and MEK 1/2 inhibitor trametinib following radiotherapy. For those without BRAF V600 or H3 K27M mutations, ACNS1721 [NCT03581292] is a phase 2 trial evaluating concurrent radiotherapy with the poly (ADP-ribose) polymerase (PARP) inhibitor veliparib, followed by maintenance chemotherapy with veliparib and temozolomide. PARP inhibitors, as DNA damage response inhibitors, can effectively synergize with radiotherapy (69, 70) and have demonstrated radio- and chemo-sensitization in pre-clinical studies of glioblastoma (71). PARP inhibition has been evaluated clinically in combination with temozolomide in recurrent adult glioblastoma and recurrent pediatric brain tumors (72, 73), as well as in combination with radiation and temozolomide in the Pediatric Brain Tumor Consortium (PBTC) study PBTC-033 for newly diagnosed DIPG but did not improve survival compared to historical series (74) (thus patients with H3 K27M mutations are excluded from ACNS1721). Along similar lines, Wee1 is a cell cycle regulator that is also involved in the DNA damage repair pathway. Based on promising pre-clinical data (75), the COG is conducting a phase 1 trial of the Wee1 inhibitor adavosertib with radiotherapy for newly diagnosed DIPG (COG-ADVL1217, NCT01922076).

## Management of Pediatric Sarcomas and Other Extracranial Solid Tumors With Advances in Targeted Therapies

Outside of the central nervous system (CNS), targeted systemic therapies are increasingly incorporated in the treatment of pediatric sarcomas, as well as other tumors based on specific molecular and genetic alterations. These are typically included concurrently with radiotherapy as part of definitive treatment, or following standard of care therapy in the recurrent or refractory setting. Based on clinical efficacy in the treatment of adult soft tissue sarcoma (STS) and renal cell carcinoma, pazopanib, a multikinase angiogenesis inhibitor targeting vascular endothelial growth factor receptors (VEGFR), c-kit, and platelet-derived growth factor receptors (PDGFR), was initially evaluated in a phase 1 trial by the COG for children with STS and other refractory solid tumors. This study demonstrated pazopanib was well tolerated in children, had evidence of anti-angiogenic effect, and had potential clinical benefit in pediatric sarcoma (76). Subsequently, the COG together with the adult cooperative group NRG Oncology conducted a randomized phase 2 trial, ARST1321, evaluating the addition of pazopanib to pre-operative chemoradiotherapy for children and adults with large, unresectable, intermediate- or high-grade STS. Initial results after the second interim analysis have recently been published and demonstrated improvement in the pathological near-complete response rate with addition of pazopanib (≥90% pathological response in 58% of patients in the pazopanib group *versus* 22% of patients in the control group) (77). Longer-term follow-up will be required to compare survival outcomes.

Targeted therapy is also being evaluated for newly diagnosed metastatic Ewing sarcoma. Prior phase 1 and phase 2 studies demonstrated favorable responses to ganitumab, an insulin-like growth factor receptor (IGFR) inhibitor, in patients with relapsed or refractory Ewing sarcoma (78, 79). Based on these data, the COG randomized phase 3 trial AEWS1221 is evaluating addition of ganitumab to standard multi-agent chemotherapy for newly diagnosed metastatic Ewing sarcoma [NCT02306161]. Local control with surgery and/or radiotherapy after induction chemotherapy, as well as metastatic site radiotherapy following consolidation chemotherapy, remain components of treatment on this study.

In the relapsed or refractory setting, multiple agents targeting VEGFR, PDGFR, mechanistic target of rapamycin (mTOR), and IGFR, among others, are being evaluated for pediatric sarcomas (80, 81). While multi-agent chemotherapy regimens are standard for rhabdomyosarcoma (RMS) and Ewing sarcoma, targeted therapies are increasingly being evaluated for recurrent or refractory disease. For example, a phase 1/2 trial conducted by the National Cancer Institute is evaluating the IGF-1R antibody ganitumab in combination with the Src family kinase inhibitor dasatinib in patients with embryonal or alveolar RMS refractory to other standard treatments [NCT03041701]. For patients with relapsed or refractory Ewing sarcoma, a prior phase 2 trial demonstrated partial response or stable disease following treatment with ganitumab in 55% of patients (79), and a phase 2 trial is currently being conducted to evaluate ganitumab in combination with the cyclin-dependent kinase (CDK) 4/6 inhibitor palbociclib [NCT04129151].

Desmoplastic small round cell tumor (DSRCT), a rare and aggressive STS that is characterized by translocation between EWSR1 and WT1, is typically treated with intensive multimodal therapy including alkylator-based chemotherapy, cytoreductive surgery with or without hyperthermic intraperitoneal chemotherapy (HIPEC), and whole abdominopelvic radiotherapy (82). However, survival outcomes remain dismal (5-year OS ~25%) (82), and novel therapeutic approaches are critically needed. Currently, targeted systemic therapies are usually considered at progression after first- or second-line chemotherapy, and data are limited to small case series or trials of Ewing sarcoma that include DSRCT (83). Pazopanib is one of the agents with more clinical experience that has demonstrated clinical activity in DSRCT, with partial response observed in a small subset of patients and at least stable disease observed in the majority of patients in the largest study of 22 patients with heavily pre-treated DSRCT (76, 84). Other reports have shown stable response to mTOR inhibitors and other PDGFR and VEGFR inhibitors, and a few ongoing studies are evaluating therapies targeting these and other pathways (83).

Advances in molecular and genetic tumor evaluation have allowed identification of a small subset of pediatric solid tumors that harbor targetable NTRK gene fusions and BRAF alterations (introduced above) (16, 85). A phase 1 study of the TRK kinase inhibitor larotrectinib for pediatric solid tumors harboring NTRK gene fusions demonstrated an ORR of 93% with predominantly grade 1 adverse events. Tumors included infantile fibrosarcoma, other STS, and papillary thyroid cancer (30, 86). Five patients on this phase 1 study were treated preoperatively with larotrectinib for locally advanced sarcomas, and all had radiographic partial response. Three of the five patients had R0 resections and complete or near-complete pathological responses (87). Thus, robust responses to these agents have led to their incorporation primarily for recurrent, refractory, or metastatic disease but may also be considered earlier in the course of treatment and, in rare cases, may provide an alternative to pre- or post-operative radiotherapy for management of pediatric sarcomas.

The incorporation of hypofractionated radiotherapy for local control in advanced disease settings is evolving. Stereotactic body radiotherapy (SBRT) is increasingly being utilized and studied for oligometastatic and recurrent disease, as a more convenient treatment that can minimize interruption of systemic therapy, and with possibly less toxicity than conventional radiotherapy. For sarcomas, which are typically more radioresistant, SBRT may also offer increased local control efficacy. However, the relevance and success of SBRT, which delivers high biologically effective doses to focal areas of disease, relies on improvements in micrometastatic disease control with systemic therapy. Thus, SBRT may become increasingly relevant with effective targeted systemic therapies. Several retrospective and early phase prospective studies (summarized in **Table 3**) have evaluated SBRT for metastatic and recurrent sarcomas (88–91). These have generally shown good local control outcomes, but increased toxicity when given with

**TABLE 3** | Studies of stereotactic body radiotherapy for pediatric sarcomas.

| Study (Treatment years) | Number of patients | Patient population | Radiotherapy site and dose | Systemic therapy | Local control and survival | Toxicity |
|---|---|---|---|---|---|---|
| Brown et al. (88) (2008-2012) | N=14 (27 lesions) | Retrospective study of pediatric and adult patients with metastatic or recurrent ES or osteosarcoma | Sites: Bone and lung/mediastinal<br>Dose: Median 40 Gy in 5 fractions (range, 16-60 Gy in 1-10 fractions) | Concurrent chemotherapy in 50% of patients (n=4 with I/E, n=2 with VCR, topotecan, CP, n=1 with GEM/docetaxel, n=1 with CP/topotecan) | 2-year LC: 85% | No grade ≥3 acute toxicity<br>N=3 grade ≥2 late toxicity: sacral plexopathy (re-RT), myonecrosis (concurrent GEM), pathologic fracture/AVN |
| Liu et al. (89) (2017-2018) | N=5 (8 lesions) | Phase I/II study of patients ≤21 years with lung metastases from sarcoma | Sites: Lung metastases<br>Dose: 30 Gy in 3 fractions | Concurrent systemic therapy in 1 patient (nivolumab) | 2-year LC: 60% | No grade ≥3 adverse events<br>N=1 grade ≥2 pneumonitis (concurrent nivolumab) |
| Elledge et al. (90) (2014-2018) | N=14 (37 lesions) | Phase II study of patients age 4-25 years with unresected, osseous metastatic non-RMS sarcomas of soft tissue and bone | Sites: Osseous metastases (spine, extremity, pelvis, skull)<br>Dose: 40 Gy in 5 fractions | No systemic therapy within 2 weeks before or after SBRT | 2-year LC: 89% (patient), 95% (lesion)<br>Median PFS: 6 months<br>Median OS: 24 months | N=2 grade ≥3 toxicity: esophagitis, osteoporosis/necrosis |
| Parsai et al. (91) (2014-2019) | N=31 (88 lesions) | Retrospective study of patients age 4-29 with recurrent or metastatic sarcomas | Sites: Osseous, pulmonary, soft tissue, hepatic<br>Dose: Median 30 Gy in 5 fractions (range, 16-60 Gy in 1-5 fractions) | Concurrent systemic therapy with treatment of 56% of lesions (multiple agents) | 1-year LC: 83% | No grade ≥3 acute toxicity<br>N=2 grade ≥2 late toxicity: radiation enteritis (re-RT and concurrent I/E/CARBO), pain (concurrent Ra-223/sorafenib)<br>N=2 radiation recall: dermatitis (I, mesna), myositis (paclitaxel, GEM, BEV) |

*AVN, avascular necrosis; BEV, bevacizumab; CARBO, carboplatin; CP, cyclophosphamide; E, etoposide; ES, Ewing sarcoma; GEM, gemcitabine; Gy, Gray; I, ifosfamide; LC, local control; OS, overall survival; PFS, progression-free survival; re-RT, re-irradiation; RMS, rhabdomyosarcoma; SBRT, stereotactic body radiotherapy; VCR, vincristine.*

concurrent systemic therapy or in the re-irradiation setting. The prospective phase II study by Elledge et al. importantly suggested that survival outcomes may be improved with consolidation of all known metastatic sites with SBRT (90), consistent with data from the EURO-EWING trial indicating improved EFS with local therapy to primary and metastatic sites (92). Current COG trial AEWS1221 is evaluating SBRT for treatment of osseous metastatic sites, to a dose of 40 Gy in 5 fractions [NCT02306161]. Additional data are still needed to evaluate the safety of SBRT with newer targeted systemic therapies.

## Role of Immunotherapy in Management of Pediatric Cancers

While immunotherapy has revolutionized the treatment of several adult cancers, its role in pediatric malignancies has thus far been limited, in large part due to how most pediatric cancers arise: typically from embryonal cells through transcriptional abnormalities, chromosomal rearrangements, and copy number variants, as opposed to accumulation of genetic mutations in epithelial cells (93, 94). Thus, most pediatric tumors have low mutational burden and limited neoantigen expression and are non- or weakly immunogenic, with the rare exception of cancers arising from mismatch repair deficiencies (94, 95). However, a few immunotherapies have been FDA-approved for treatment of pediatric cancers. Blinatumomab, a bispecific antibody targeting the B lymphocyte antigen CD19, and tisagenlecleucel, a chimeric antigen receptor (CAR)-T cell therapy targeting CD19, are approved for treatment of relapsed/refractory B-cell acute lymphoblastic leukemia (93, 96, 97). Dinutuximab is an antibody specific for disialoganglioside (GD2), a glycolipid antigen highly expressed on the surface of neuroblastoma and other embryonal tumors. The Fc portion of anti-GD2 antibodies engages receptors on monocytes, macrophages, neutrophils, and natural killer cells, which then triggers antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (93, 98). Based on promising initial phase I data of dinutuximab alone and in combination with granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin-2 (IL-2) to enhance ADCC (99–101), the COG conducted the randomized phase 3 study ANBL0032 to evaluate the addition of dinutuximab with GM-CSF and IL-2 to standard isotretinoin post-consolidation therapy for high-risk neuroblastoma patients. The study was stopped early due to the superiority of the dinutuximab arm at 2 years, with significant improvements in EFS (66 ± 5% *vs.* 46 ± 5%, $P$=0.01) and OS (86 ± 4% *vs.* 75 ± 5%, $P$=0.02) (102). Thus, dinutuximab is FDA-approved for treatment of high-risk neuroblastoma patients with response to frontline multi-modal therapy (including consolidative radiotherapy) and is a standard component of post-consolidation therapy on the current COG trial ANBL1531 [NCT03126916] (93). Finally, immune checkpoint inhibitors, which have had significant success in the treatment of adult cancers, have not yet been widely adopted in the pediatric setting. Pembrolizumab, an antibody specific for programmed cell death protein 1 (PD-1) expressed on activated T and B lymphocytes, is approved for the treatment of refractory or relapsed Hodgkin lymphoma based on data extrapolated from

adult studies (93, 103). Ipilimumab, an antibody targeting cytotoxic T lymphocyte antigen 4 (CTLA-4), is approved for treatment of unresectable or metastatic melanoma in pediatric patients ≥12 years of age (104, 105).

Ongoing clinical trials evaluating various immunotherapies (including immune checkpoint inhibitors, CAR-T cell therapies, cancer vaccines, and oncolytic virus therapies, among others) across a spectrum of pediatric cancers are summarized in Hutzen et al. (93) A handful of trials incorporate radiotherapy, either in combination with immunotherapy or as consolidative therapy after upfront systemic therapy. For patients ≥12 years of age with newly diagnosed stage III-IV classic Hodgkin lymphoma, a randomized phase 3 trial is evaluating immunotherapy (nivolumab, an anti-PD-1 antibody, *versus* brentuximab vedotin, an antibody-drug conjugate targeting CD30 on the surface of Hodgkin lymphoma cells) with standard combination chemotherapy followed by consolidative radiotherapy as clinically indicated [SWOG S1826, NCT03907488]. A few studies are investigating combinations of immunotherapy and radiotherapy for progressive or recurrent primary brain tumors. Indoximod is an inhibitor of the indoleamine 2,3-dioxygenase (IDO) pathway, which serves multiple immunomodulatory functions but ultimately results in immune tolerance to tumor antigens (106). A phase 1 trial of indoximod combined with temozolomide or radiotherapy for pediatric patients with progressive brain tumors (or with radiotherapy for patients with newly diagnosed DIPG) has completed enrollment [NCT02502708], and a phase 2 trial is now underway [NCT04049669]. Other studies are investigating intratumoral virus injection together with radiotherapy for malignant gliomas or recurrent ependymomas [NCT02457845, NCT00634231], as well as adoptive cellular therapy with radiotherapy (with or without temozolomide) for patients with brainstem gliomas [NCT03396575]. Finally, based on pre-clinical and clinical data suggesting more robust systemic immune responses to combinations of focal radiotherapy and immunotherapy (107–113), a few early studies are evaluating this combination in extracranial solid tumors and lymphomas [NCT03445858].

# TAILORING RADIOTHERAPY WITH ADVANCEMENTS IN MOLECULAR CHARACTERIZATION

## Dose-Reduced Radiotherapy for Patients With Low Risk Medulloblastoma

Medulloblastoma is standardly treated with an aggressive multi-modal regimen of maximal safe resection followed by post-operative craniospinal irradiation (CSI) and multi-agent chemotherapy. However, as the median age at diagnosis is ~6 years of age and the majority of patients are long-term survivors (5-year OS ~80% for patients with standard risk disease and ~60% for patients with high risk disease), all patients experience late toxicities, including neurocognitive impairment, neuroendocrine dysfunction, impact on growth, infertility, and secondary malignancies, and strategies to decrease late effects from treatment while maintaining survival rates are constantly being evaluated (114). Patients with standard risk disease per traditional definitions (≤1.5cm$^2$ residual disease, ≥3 years old, and no metastatic disease) are treated with lower dose CSI (23.4 Gy) with an involved field boost to 54 Gy total, while patients with high risk disease per traditional definitions (>1.5cm$^2$ residual disease or metastatic disease present) are treated with higher dose CSI (36 Gy) with a posterior fossa boost to 54 Gy total and metastatic site boost to 45-54 Gy total. CSI has been an essential component of treatment for medulloblastoma, as cure was rare before the use of CSI, and early efforts to omit or reduce the dose of CSI resulted in worse outcomes (115, 116). Based on early studies of medulloblastoma demonstrating significant and often unacceptable neurocognitive deficits attributed to high dose radiotherapy in children under the age of 3 (1, 117, 118), radiotherapy is typically delayed for infants and young children with medulloblastoma until age 3 or older. Surgical resection is usually followed by adjuvant chemotherapy, delaying radiotherapy until progression (the "acceptable" age for proceeding with CSI varies across studies, from 18 months to 6 years) (119–121). The COG trial ACNS0334 [NCT00336024] is evaluating two high-dose chemotherapy regimens followed by peripheral blood stem cell rescue for infants up to age 2 with high-risk medulloblastoma or CNS embryonal tumors, and preliminary results suggest that while focal radiotherapy may be reasonable upfront for select patients, omission of CSI upfront does not appear to compromise survival (122).

Newer radiotherapy techniques, including IMRT and proton therapy, as well as reduction in the boost margin, have resulted in steadily lower doses to normal tissues without compromising disease control (123–125). Specifically for proton therapy, dosimetric studies indicate reduction of dose to anterior organs, including heart, gastrointestinal tract, lungs, kidneys, and thyroid, with proton CSI (126), and evaluation of long-term toxicity of proton therapy for medulloblastoma suggests decreased cardiac, pulmonary, and gastrointestinal toxicity compared to photon-based treatments (127). While neurocognitive impairment will always occur with CSI regardless of treatment modality, especially with younger age at the time of treatment (128), a recent study suggests that better intellectual outcomes may still be achieved with proton *versus* photon radiotherapy for medulloblastoma based on the boost treatment (129). Thus, even with standard-dose radiotherapy for medulloblastoma, advancements in radiotherapy techniques are resulting in improvements in the late toxicity profile.

More recently, the management of medulloblastoma has been revolutionized by advancements in tumor molecular characterization, moving from previous risk definitions based on amount of residual disease, age, and presence of metastatic disease to current stratifications based on molecular subgroups: WNT, sonic hedgehog (SHH), Group 3, and Group 4. With standard treatments, the WNT subgroup is most favorable, with >90% 5-year PFS, followed by intermediate outcomes in the SHH and Group 4 subgroups (5-year PFS of 70-80%), and poor outcomes for Group 3 (5-year PFS of 50-60%) (8, 114, 130). Thus, current studies are evaluating whether patients in low risk

subgroups may be eligible for de-intensified treatment regimens, whether avoiding radiotherapy altogether or reducing the dose or volume of radiotherapy (**Figure 3**) (7, 130). COG study ACNS1422 [NCT02724579] is evaluating whether both chemotherapy intensity and CSI dose (18 Gy) can be reduced in patients with average risk WNT-driven tumors who have positive β-catenin and presence of *CTNNB1* [exon 3] mutation and without large cell/anaplastic medulloblastoma or *MYC/MYCN* amplification. SJMB12 [NCT01878617] is evaluating a reduced CSI dose of 15 Gy in the same population. However, a pilot study omitting CSI entirely for WNT-driven medulloblastoma has closed due to inferior outcomes [NCT02212574]. In Europe, the ongoing International Society of Paediatric Oncology (SIOP) PNET-5 study is investigating the possibility to deliver, within a combined modality approach, a reduced CSI dose of 18 Gy to a selected subgroup of children with a low-risk biological profile [NCT02066220]. At the same time, SJMB12 is investigating intensified treatment regimens for patients in higher risk subgroups, including the addition of gemcitabine and pemetrexed for those with high risk Group 3 or Group 4 medulloblastoma and targeted SHH inhibitor therapy for those with SHH-medulloblastoma (**Figure 3**).

## Risk-Adapted Radiotherapy for Patients With Rhabdomyosarcoma

Rhabdomyosarcoma is standardly treated with a combined modality regimen of surgery (if resectable), multi-agent chemotherapy, and radiotherapy. Use and dose of radiotherapy for rhabdomyosarcoma is typically based on clinical group, FOXO1 fusion status, and site (primary/metastatic). Patients with clinical group I, FOXO1 negative or indeterminate tumors do not receive radiotherapy, while all others receive radiotherapy with dose based on the factors above. Given the young age of many of these patients and significant risk of late toxicity from radiotherapy (131–134), there is always a question of whether radiotherapy can be safely omitted or reduced and thereby minimize treatment-related toxicity for appropriately selected patients. An analysis of ARST0331 and ARST0531 suggests worse local control and survival outcomes when "individualized local therapy" (typically omission or delay of radiotherapy) as opposed to protocol-specified radiotherapy is given to infants with rhabdomyosarcoma (135). Thus, attempting to select for more favorable risk patients, the current protocol ARST1431 [NCT02567435] permits deviations for patients ≤2 years of age only if they are FOXO1 fusion negative. Histologic and radiographic response to initial chemotherapy is another measure that has been used to guide radiotherapy usage and dose (used in D9602/D9803 and ARST0331/ARST0531, as well as in ARST1431). Second-look procedures after initial chemotherapy largely correlate with clinical/radiographic complete response; however, ~40% of patients without clinical/radiographic complete response have no viable tumor histologically, and thus post-chemotherapy biopsies/DPE may be helpful for selecting patients for radiotherapy dose reduction (136). On the other end, ARST1431 is evaluating higher doses of radiotherapy in patients at greater risk of local failure by increasing the boost dose to 59.4 Gy total for tumors >5cm at diagnosis.

Future studies will need to incorporate our evolving understanding of molecular and genetic features of rhabdomyosarcoma that are associated with favorable or adverse outcomes, such that patients can be appropriately selected for potential treatment de-escalation or escalation. For instance, recent histological and molecular analysis of infant rhabdomyosarcoma suggests favorable prognosis of the spindle cell subtype associated with alterations in VGLL2, NTRK, and BRAF, and potential consideration of de-intensified treatment for this subset of patients (137). Conversely, MYOD1-mutant spindle cell and sclerosing rhabdomyosarcoma is associated with

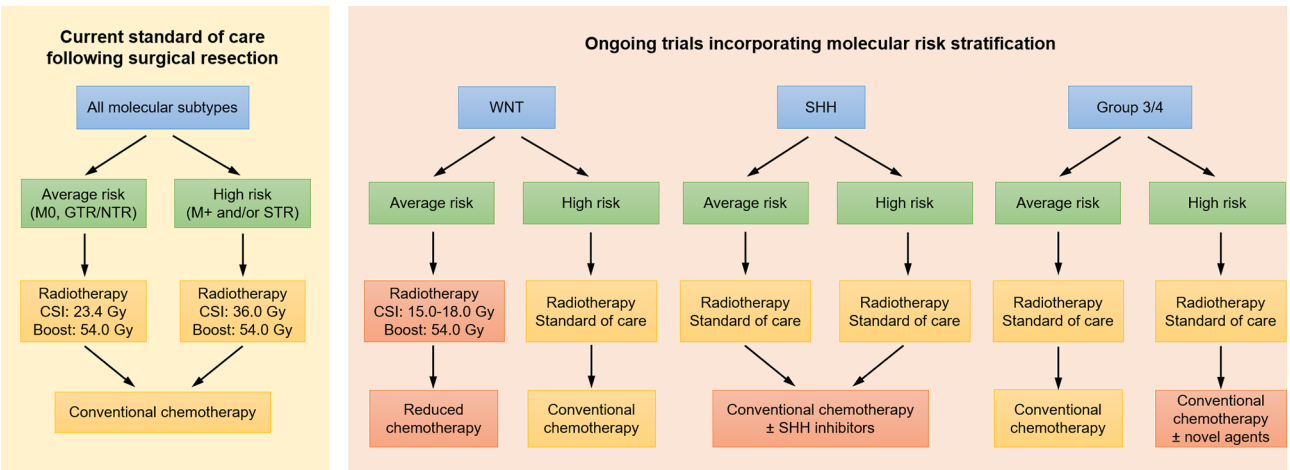


**FIGURE 3** | Current treatment paradigms for medulloblastoma, as well as approaches under investigation in clinical trials incorporating molecular risk stratification. Investigational approaches are indicated in red. CSI, craniospinal irradiation; GTR, gross total resection; NTR, near total resection; SHH, sonic hedgehog; STR, subtotal resection.

an aggressive clinical course and poor outcomes (138, 139) and, together with tumors with anaplasia and TP53 mutation, should be excluded from consideration of de-escalated therapy and perhaps considered for augmented therapy.

## CONCLUSION

Radiotherapy has remained an integral component in the treatment of pediatric cancers over several decades. However, its role has continued to evolve with the introduction of chemotherapy regimens and now molecularly targeted therapies in an era of rapid advances in precision medicine. In particular, MEK1/2, BRAF, and TRK inhibitors have demonstrated significant promise in pediatric gliomas and extracranial solid tumors harboring these alterations and warrant further investigation in larger trials, as well as clinical consideration when these alterations are present. Developments in molecular diagnostics and targeted systemic therapies are providing opportunities for potentially more effective and specific but less toxic therapies, critical for treatment of pediatric patients. At the same time, advances in radiotherapy techniques are improving the precision and conformality of local therapy. Together, these developments are leading to novel synergistic combinations of radiotherapy and systemic therapy, as well as potential avenues to select patients for treatment de-escalation, leading to more tailored treatments with improved therapeutic ratio for pediatric cancer patients.

## AUTHOR CONTRIBUTIONS

CS and ST contributed to conception of the manuscript. CS wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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