Radiotherapy driven immunomodulation of the tumor microenvironment and its impact on clinical outcomes: a promising new treatment paradigm

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ABSTRACT
Traditional treatment approaches for advanced malignancies have been associated with limited clinical outcomes necessitating the development of novel therapies. However, the ability of radiotherapy to induce pro-immunogenic changes in tumor immune microenvironment can be leveraged when combined with systemic agents. Radio-immunotherapeutic initiatives employing the use of monoclonal antibodies, genetically engineered T cells, cytokines and virus-vector mediated gene therapies have demonstrated promising potential for the management of various solid malignancies. Future studies incorporating biomarker enrichment strategies and radiobiological variables could pave the way for immune-oncology based personalized medicine approaches to be integrated in standard of care practices for the treatment of challenging clinical populations.

1. Introduction
In an era where conventional treatment for advanced cancers using surgery, radiation and chemotherapy-based modalities are associated with limited efficacy, significant toxicity and drug resistance, there is a great need to devise novel therapeutic strategies. Current research investigations are actively exploring radiotherapy's biological impact on the host Tumor Immune Microenvironment (TIME) which could be leveraged by combining with immunotherapeutic agents. Given the wide array of potential mechanisms, this article will discuss the most promising strategies and therapies that have demonstrated utility in a clinical setting as summarized in Figures 1–3.

2. Exploring radiotherapy driven modulation of the tumor immune microenvironment
2.1. Radiation increases the antigenic load and antigen presentation to dendritic cells (DCs) and T lymphocytes
The classical pathways involving modulation of the immune system in the tumor microenvironment (TME) pertains to direct tumor antigen release via the apoptotic and necrotic processes after a course of radiotherapy [1]. The released antigens are phagocytosed by nearby antigen presenting cells (APCs) such as immature DCs whose proteolytic processing within DC cytosol, endosome-lysosome and endoplasmic reticulum allows it to transform into a mature DC [2]. This pivot towards a mature cell type is marked by phenotypic changes (upregulation of surface maturation ligands CD80, CD83, and CD86) as well as functional enhancements (secretion of immunostimulatory cytokines) [2]. These mature DCs then traffic into the draining lymph nodes which harbor naïve T lymphocytes [1]. Processed antigens are then cross presented by mature DCs by engaging their antigen loaded Major Histocompatibility Complex Class I (MHC I) molecules with naïve T cell receptors [2]. Additional interactions between maturation ligands and secreted cytokines from mature DCs with co-stimulatory receptors in naïve T cells serve as co-stimulatory signals which promote their differentiation into activated CD4+ and CD8+ T cells [1,2]. The activated T lymphocytes are recruited back into the TME where they initiate their anti-tumor activity [1].

Downregulation of MHC I expression is often employed as an immune evasive tactic by cancer cells which can be mitigated by radiotherapy [3]. Radiation increases intratumoral antigenic peptide pool in two phases which serves as the rate limiting step in MHC I formation [4]. In the early phase which occurs within 0-4 h after radiation at 1-25 Gy doses, radiation induces formation of free radicals that damages tumor proteins which are then
Figure 1. Overview of emerging mechanisms pertaining to radiation induced immunomodulation of the tumor microenvironment representing therapeutic opportunities for clinical exploration. Created with Biorender.com.

Figure 2. Overview of intracellular mechanisms of tumor radiosensitization and additional immunomodulation of the tumor microenvironment. Created with Biorender.com.
targeted for proteasomal degradation and loaded onto MHC I molecules [4]. In the late phase which begins 4 h after radiotherapy and lasts for weeks, radiation activates the mammalian target of rapamycin (mTOR) pathway [4]. This pathway promotes translation of proteins where a portion of new proteins is degraded by proteasomes that further raises the intracellular level of antigens and thereby promotes MHC I expression on tumor cells [4]. Therefore, elevated production of protein-based tumor antigens and their presentation to cytotoxic lymphocytes (CTLs) via increased MHC I expression can serve as a potential mechanism of radioimmune sensitization of tumor cells and re-shaping of the TME into an immunogenic milieu.

2.2. Radiotherapy modulates the adaptive immune system in favor of tumor infiltrating lymphocytes (TILs)

Radiation induced activation of CD4+ T cells can have secondary downstream effects such as cytokine release which fosters an increased presence of effector immune cells within TME [5]. This can occur as a result of Interleukin-2 (IL-2) released primarily from antigen activated CD4+ T cells that engages with IL-2Rz, IL-2Rβ and IL-2Rγ receptors on other antigen activated memory T cells and lymphocytes [6]. Consequently, intracellular transduction of IL-2 signal occurs via Janus kinase/signal transducer and activator of transcription (JAK/STAT), mitogen activated protein kinase/extracellular-signal-regulated kinase (MAPK/ERK), and Phosphoinositide 3-kinases/Akt/mTOR pathways that mediate proliferation and survival of CD4+ T cells as well as differentiation and expansion of CD8+ T cells into memory and cytotoxic T cells [6]. Therefore, prescription of exogenous IL-2 in tandem with radiotherapy provides a therapeutic possibility of enhancing the anti-tumor effect via increased presence of lymphocytes within the TME. Additional alteration of the immune profile in TME following radiotherapy can occur due to the increased expression of checkpoint ligands comprising of Programmed Death-Ligand 1 (PD-L1) on tumors and B7-1/2 on APCs which engage with PD-1 and Cytotoxic T-Lymphocyte-associated Antigen 4 (CTLA-4) receptors on T cells respectively [7,8]. The former mechanism involves an interaction of T cell receptor (TCR) on T cells with peptide-MHC (pMHC) from the tumor cell results in initial T cell activation [9]. Conversely,
negative costimulation resulting from the interaction between PD-1 and PD-L1 leads to inhibition of the T cell activation process [9]. Prescription of anti-PD-1 or anti-PD-L1 antibodies can abrogate this negative signaling resulting in activation of the T cell that can mount an effective immune response [9]. The latter mechanism is based on initial activation of a T cell which requires interaction of TCR with pMHC as well as CD28 with B7-1/2 on APCs [9]. However, CTLA-4 present on T cells competes with CD28 for B7-1/2 ligands which results in attenuation of the T cell activation cascade [9]. Exogenous addition of anti-CTLA-4 antibody can effectively mitigate this competition by binding to CTLA-4, allowing CD28 and B7-1/2 mediated positive costimulation to activate the T cell [9]. Activation of these checkpoint receptors can impair the CD8+ Treg ratio by increasing the number of Tregs and promoting T cell exhaustion comprising of deficient proliferation and effector function of T cells [7]. However, preclinical studies have demonstrated that the addition of CTLA-4 and PD-L1 inhibitors to radiotherapy can effectively restore the balance of CD8+ Tregs via re-invigoration of exhausted CD8+ TILs and reduction in the number of Tregs [7]. Hence, an unintended side-effect of radiotherapy manifesting as an increased number of checkpoint ligands can in fact be leveraged via their inhibition to elevate the number of effector lymphocytes within the TME.

2.3. Enhanced immune-stimulation and tumoricidal activity of immune cells following radiotherapy

Recognition of tumor derived antigens by DCs is not sufficient to induce potent anti-tumor immune responses as they require presence of immune-stimulatory molecules. These can comprise of Damage Associated Molecular Patterns (DAMPs) such as tumor DNA that has been implicated in promoting an immunostimulatory TME via activation of the stimulator of interferon genes (STING) pathway [10]. Initially, radiation induced DNA damage leads to an increase in cytosolic load of tumor DNA which is recognized by cyclic GMP–AMP Synthase (cGAS) that in turn promotes activation of STING protein [10,11]. STING then traffics to perinuclear endosomes to mediate subsequent signaling via phosphorylation of tank-binding kinase 1 (TBK1), Interferon Regulatory Factor 3 (IRF3), and nuclear factor kappa B (NF-kB), which induces transcription of type I interferons (IFN-I) such as interferon beta (IFN-β) [10,11]. IFN-β appears to act via autocrine signaling pathways to enhance MHC I expression among tumor cells to increase antigen presentation to T cells and via paracrine signaling to recruit and activate DCs [10,12]. Moreover, tumor DNA expelled from radiation induced death of cancer cells is detected as a DAMP by these DCs that is endocytosed which then activates the cGAS/STING pathway within DCs to further increase IFN-I secretion [11]. This promotes their optimal activation followed by cross-presentation of CD8+ T cells in order to mount a robust adaptive immune response against tumor cells as comprehensively described by Martínez-Lostao et al. [10,13].

In addition to increasing antigen presentation to T cells, thereby promoting the recruitment and activity of TILs, Tumor Necrosis Factor Alpha (TNF-α) can also elicit direct cytolytic effects on tumor cells [14]. TNF-α can be secreted in the TME by radiation activated lymphocytes which then binds to its receptors TNFRI/2 on tumor cells to trigger the formation of Complex II that contains caspase 8 [15]. Pro-caspase 8 undergoes cleavage to form caspase 8 that binds to ROS modulator-1 (ROMO-1) protein located on the outer mitochondrial membrane [15]. ROMO-1 in turn binds and sequesters B-cell lymphoma-extra large (Bcl-XL) protein resulting in reduction of the mitochondrial potential and activation of c-Jun N-terminal kinase (JNK) which interacts with Sab protein to stimulate production of mitochondrial reactive oxygen species (ROS) [15]. This rise in ROS could essentially enhance the intracellular levels of free radical species during radiotherapy to potentiate DNA damage via a synergistic effect. Moreover, activation of caspase 8 by TNF-α signaling also mediates tumor killing via the apoptotic pathway [15]. Caspase 8 interacts with apoptotic factors to induce mitochondrial outer-membrane permeabilization to release cytochrome c, Second mitochondria-derived activator of caspases (Smac) and Direct IAP-binding protein with low PI (Diablo) that in turn activates caspases 3 and 7 which execute apoptosis [15].

Tumor sensitization afforded by priming of the aforementioned pro-apoptotic pathway mediated by caspase 8, 3 and 7 can be further potentiated by the addition of TNF-related apoptosis-inducing ligand (TRAIL). Pre-clinical studies have demonstrated that TRAIL can be delivered effectively to promote an anti-tumor effect via chimeric antigen receptor (CAR) T-cells [16]. CAR T-cells are modified T cells which are engineered to selectively target tumor antigens such as CD19. This is accomplished via addition of chimeric receptors comprising of an extracellular tumor antigen specific antibody, a transmembrane as well as an intracellular stimulatory component. Together they promote increased
recognition of target antigen expressed on the surface of the cancer cell and stimulates T cell activity independent of antigen presentation by MHC I. However, the ability of CAR T-cells to eliminate cancer cells via classical cytotoxic T cell mechanisms [13] is impaired in a TME containing antigen-negative tumor cells. This clinical problem can be addressed via treatment with low dose radiotherapy which can promote upregulation of pro-apoptotic factors such as caspase 8, 3 and 7 [16]. Prescription of CAR T-cells following radiation first permits their activation by engagement with antigen positive tumor cells, leading to secretion of TRAIL in the TME. This step is then followed by interaction of TRAIL with TRAIL-specific death receptors on the surface of antigen negative tumor cells which promotes their death via induction of the primed apoptotic pathway. Antigen positive tumor cells are also targeted via this TRAIL dependent mechanism in addition to classical cytotoxic T cell mechanisms, further enhancing the anti-tumor efficacy resulting from this combined treatment approach. Therefore, the use of radiotherapy in tandem with immune stimulating or immune-engineered agents provides the possibility to potentiate the anti-tumor effect of our adaptive immune system.

3. Evaluating the incorporation of radiation in immunotherapy protocols to improve clinical outcomes

Immunotherapy regimens especially those comprising of immune checkpoint inhibitors (ICI) have garnered a strong interest in the scientific community in recent years leading to approval of several drugs for the treatment of multiple types of cancer. However, their role in combination with radiotherapy is currently an area of active investigation where it is believed that an interplay of multiple mechanisms is responsible for inducing potent anti-tumor responses. Some of the most promising approaches exploiting the aforementioned mechanisms are discussed as follows:

3.1. Immune checkpoint inhibitors

3.1.1. Anti- PD-1 and PD-L1 agents

Pembrolizumab is a monoclonal antibody which inhibits PD-1 on lymphocytes and prevents their co-inhibitory interaction with PD-L1 and PD-L2 on tumor cells to generate a robust adaptive anti-tumor response [17]. The combination of pembrolizumab with fractionated radiotherapy (3000 cGy) has been evaluated for the first time among subjects with metastatic triple negative breast cancer in a prospective study [17]. The overall response rate (ORR) was noted as 17.6% (95% Confidence Interval, 4.7–44.2%), a significant improvement among a heavily pre-treated clinical population with a poor prognosis and where previous patients on pembrolizumab monotherapy have reported an ORR of 4.7%. Complete responses (CRs) were noted in 3 patients which involved complete regression of non-radiated metastatic lymph nodes, highlighting the role of possible abscopal effects mediated by radiotherapy. The combination of radiotherapy with pembrolizumab was also well tolerated since Grade 3 or higher treatment related adverse events (TRAEs) were only noted among 4 out of 17 subjects that comprised of fatigue, lymphopenia and soft tissue infection but no patients discontinued treatment or died on study due to adverse events.

Evaluation of other ICI in additional prospective studies have further lent credence to the utility of including radiotherapy in immunotherapeutic regimens. In this regard, the randomized, placebo controlled, Phase 3 PACIFIC trial has investigated the role of durvalumab, another monoclonal antibody which inhibits PD-L1 on tumor cells among patients with locally advanced, unresectable, non-small cell lung cancer (NSCLC) [18]. These patients had received chemoradiation (mostly ranging from 54 to 66 Gy) within 1–42 days prior to initiating durvalumab or placebo treatment as it was believed that chemoradiation had upregulated the expression of targetable PD-L1 on tumors. The results demonstrated a significant improvement in Overall Survival (OS) outcomes than compared to the placebo group (Median OS = Not reached vs 28.7 months, Hazard Ratio (HR), 0.68 [99.73% CI, 0.47–0.997; \( p = .0025 \)]) irrespective of the patient’s PD-L1 status, underscoring the fact that subjects with limited PD-L1 expression can also derive a clinical benefit. Additionally, patients on the durvalumab arm also availed a significant Progression Free Survival (PFS) advantage than compared to the placebo group (Median PFS = 16.8 months vs 5.6 months, HR, 0.52 [95% CI, 0.42–0.65; \( p < .001 \)]) [19]. This was a remarkable improvement considering that PFS for this patient population following chemoradiation has traditionally been approximated at 8 months [19]. Superior response rates (ORR = 30% vs 17.7%; \( p < .001 \)) were also observed among durvalumab treated patients than compared to the placebo group, where 75.3% of durvalumab responders had an ongoing response for at least 18 months [18]. Durvalumab also had a manageable toxicity profile where the most frequently occurring Grade 3 or higher TRAEs consisted of pneumonitis, pneumonia and anemia [19]. Given the promising efficacy and safety of this treatment approach, durvalumab received FDA approval for the treatment of
patients with unresectable, stage III NSCLC non-progressive disease following concurrent chemoradiation [18].

An important consideration in a combinatorial approach is the treatment sequence where relevant reports are currently lacking and have mixed results. However, retrospective studies appear to suggest that a concurrent treatment approach is associated with greater therapeutic efficacy as demonstrated with the use of PD-1 inhibitors in tandem with Stereotactic Radiosurgery (SRS) among lung cancer patients with brain metastasis [20]. SRS is a modified radiotherapy technique involving prescription of high radiation doses with fewer treatment sessions than conventional radiotherapy. Radiation is delivered from various different angles with a high degree of precision which obviates the need to perform a surgical incision within critical organs such as the brain. The researchers of this study found that concurrent prescription of SRS (mostly single fraction of 18 Gy) within a span of 1 month of PD-1 inhibitor use was associated with improved OS and reduced distant brain failure (DBF) when compared against SRS use before or after administration of PD-1 inhibitors (1-year OS, 87.3% vs 70.0% vs 0%, \( p = .008 \); 1-year DBF, 38.5% vs 65.8% vs 100%, \( p = .042 \)). A mechanistic explanation of this finding in the concurrent setting could be attributed to an increase in local and systemic neoantigen load resulting from radiotherapy which can be efficiently recognized within a specific timeframe during PD-1 blockade, resulting in effective anti-tumor responses.

### 3.1.2. Anti-CTLA-4 agents

Immune checkpoint inhibitors involving CTLA-4 blocking monoclonal antibodies in combination with radiation is also another emerging radio-immunotherapeutic modality. A prospective study demonstrated that the combined use of radiotherapy and ipilimumab among heavily pre-treated metastatic melanoma patients confers a durable clinical benefit among 50% of the patients, with responses ranging from 29–65 weeks [21]. Three CRs were observed only among patients who received low dose radiation (ranging from 4 Gy to 12.5 Gy per fraction), which suggests that low dose fractionation schemes are suitable to produce an antigenic load sufficient enough to produce an anti-tumor immune response during immune checkpoint blockade without injuring immune cells via exposure to higher radiation doses, an observation which has also been noted by other investigators using low dose radiation and CTLA-4 blockade [21,22]. The addition of ipilimumab to radiotherapy did not induce supra-additive toxicity, since the rate of Grade 3 or higher TRAEs noted here was only 14% which is lower than historical rates of 7–19.9% associated with ipilimumab monotherapy. These TRAEs consisted of colitis as well as hypophysitis only among complete responders, which reflects the robustness of the immune response among these patients that concurred with correlative studies depicting elevated levels of systemic IL-2 producing CD8+ T cells and central memory CD8+ T cells.

Additionally, a similar Phase I/II study involving lung cancer patients has further elucidated the mechanisms behind this combined treatment approach [23]. With respect to the correlative component of this study, responding patients had increased clonal expansion of T cells which was attributed to enhanced recognition of mutant proteins derived from radiated tumor cells, such as karyopherin \( \alpha_2 \) whose expression is upregulated following radiotherapy. Furthermore, responders also had elevated levels of IFN-\( \beta \) which was attributed to the optimal functionality of cGAS/STING pathway. Interestingly, lower IFN-\( \beta \) levels among non-responders were believed to result from a reduced induction of the cGAS/STING pathway. This could be due to the limited availability of cytosolic tumor DNA resulting from enhanced exonuclease Trex1 activity, which is activated at varying radiation dose thresholds in different cancers [23,24]. Therefore, additional studies are required to clarify optimal fraction sizes that can be employed to maximize synergistic anti-tumor activity by combining radiation with immune checkpoint inhibitors.

### 3.2. CAR T-cell therapy

The use of CAR T-cell therapy is gaining increasing momentum especially in the domain of hematologic malignancies. This treatment approach involves extraction of circulating T cells from a patient through a process called leukapheresis which are then engineered to selectively target tumor antigens. The engineered CAR T-cells are then amplified in the laboratory and re-infused back into the patient after the patient has undergone lymphodepletive chemotherapy to enhance the efficacy of CAR T-cell therapy. The treatment is prescribed in a single session but the entire manufacturing process can take several weeks, which offers a window of opportunity to explore the addition of another treatment modality in the interim to further improve treatment outcomes. Such addition is warranted since treatment toxicity is high and PFS outcomes are limited [25]. Furthermore, there is the added challenge of patients developing rapid disease progression while waiting to be re-infused.

Radiation can potentially address these issues and can also provide additional clinical benefit to
patients with a high tumor burden or chemorefractory lesions to ensure disease stabilization or to provide palliative relief prior to CAR T-cell infusion. This ‘bridging radiation’ treatment has been explored only in emerging retrospective studies among subjects with non-Hodgkin lymphoma. Investigators at MD Anderson Cancer Center have demonstrated that relapsed or refractory patients \( (N = 11) \) receiving only bridging radiation prior to CAR T-cell therapy had improved ORR (100% vs 67%, \( p = .03 \)) and CR rate (82% vs 38%, \( p = .01 \)) than compared to subjects receiving a systemic bridging treatment such as steroids, chemotherapy or targeted agents [26]. Such patients had received a median dose of 35.2 Gy delivered at 2.5 Gy per fraction and also had better PFS outcomes than subjects receiving systemic bridging therapy (Median PFS = 8.9 months vs 4.7 months, \( p = .05 \)). It is also important to note that addition of radiation to CAR T-cell therapy is also associated with improved clinical outcomes than CAR T-cell therapy monotherapy, where ORR, CR rate and median PFS are typically limited to 74%, 54% and 5.9 months respectively [25]. The bridging radiation regimen also appears to have a higher safety profile than CAR T-cell therapy alone, such as lack of Grade 3 or higher cytokine release syndrome (CRS) \( (0\% \text{ vs } 11\%) \) and toxicity associated deaths \( (0\% \text{ vs } 4\%) \) [25,26].

Another consideration is the timing of delivering radiation with respect to CAR T-cell therapy in order to avoid additive toxicities while maintaining therapeutic efficacy. Researchers from University of Pennsylvania have compared patients with relapsed or refractory aggressive B-cell lymphoma receiving either bridging radiation alone within 30 days before CAR T-cell infusion \( (N = 3) \) or non-bridging radiation comprising of either radiation received greater than 30 days before CAR T-cell infusion or subjects with no prior history of radiation \( (N = 26) \) [27]. The study showed subjects on the bridging radiation alone arm did not experience Grade 3 or higher radiation or CAR T-cell related toxicities. However, Grade 3 or higher CRS and neurotoxicity was observed at a rate of 23% and 15% respectively in the non-bridging radiation cohort. Additionally, post CAR T-cell hospitalization rates were better for bridging radiation alone recipients than compared to patients on the non-bridging radiation cohort \( (0\% \text{ vs } 35\%) \). A possible explanation for a higher safety profile among bridging radiation alone subjects include a reduction in tumor burden due to radiotherapy, leading to moderation of the systemic antigenic load and subsequently limiting the intensity of CRS while maintaining treatment efficacy as seen by 2 out of the 3 subjects achieving CRs. In the non-bridging radiation cohort, 8 out of 21 subjects attained CR and it is possible that responses were dampened due to delivery of radiation prior to leukapheresis. This highlights the importance of selecting CAR T-cells that can remain viable and functionally competent after infusion. It is likely that radiation prior to leukapheresis could hamper this process by inducing a state of lymphopenia especially if prescribed using larger radiation fields among lymphopoietic organs such as the bone marrow and thymus [28,29]. The current preliminary data supports the use of bridging radiation after leukapheresis albeit limited by sample size differences. Therefore, larger prospective trials considering prescription of radiation before or after leukapheresis are warranted to clarify the ideal timing of radiotherapy to optimize the efficacy of subsequent CAR T-cell therapy.

### 3.3. Cytokine therapy

#### 3.3.1. IL-2 Immunotherapy

The clinical utility of a combined IL-2 and Stereotactic Body Radiotherapy (SBRT) approach (20 Gy in 1–3 fractions) as a first line treatment of metastatic melanoma and renal cell carcinoma has been demonstrated in a prospective study [5]. The ORR was reported as 66% comprising of 1 CR and 7 PRs from a pool of 11 patients, where durable responses were maintained among 6 patients for a median follow-up of 480 days. These notable responses could be attributed to SBRT use, where a high radiation dose resulted in copious release of antigens that would be recognized by the local tumor immune system. Moreover, SBRT offers a higher level of precision than conventional radiotherapy, thereby mitigating the bystander effect to spare CD8+ and CD4+ T cells whose anti-tumor activity was pronounced due to the presence of IL-2. This was evidenced by the amplification of CD4+ and CD8+ early TEm phenotypes that are associated with an endogenous memory response in responding patients. No dose limiting AEs were associated with SBRT use and anticipated toxicities associated with IL-2 administration were resolved after treatment completion.

#### 3.3.2. TNF-α gene therapy

Another therapeutic strategy to boost the anti-tumor effect of lymphocytes involves a novel gene therapy approach to increase the concentration of TNF-α in the TME [30]. Multiple studies have employed the use of TNFerade, which involves incorporation of TNF-α gene linked to a radiation inducible early growth response 1 (EGR-1) promoter into a non-replicative adenovirus [14,30]. Consequently, intratumoral administration of TNFerade followed...
by radiotherapy allows for transcription, production and secretion of TNF-\(\alpha\) within a localized region that can enhance the therapeutic index by increasing tumor radiosensitivity and prevent systemic toxicity associated with TNF-\(\alpha\) use [14,30].

The synergistic effect of combining conventional radiotherapy with TNFerade has demonstrated therapeutic advantage among advanced solid tumor patients refractory to standard of care regimens. An ORR of 43% including both CRs and PRs has been noted, with melanoma patients deriving the greatest clinical benefit with DOR extending to over 24 months [14,30]. Other investigators have demonstrated that TNFerade can be incorporated with chemoradiotherapy to improve OS outcomes among select patient populations [31]. For instance, the combined treatment can extend median OS from 24.1 months to 47.8 months among subjects with esophageal or gastroesophageal junction carcinoma but not among pancreatic cancer patients [31,32]. No DLTs were observed in these studies whereas common AEs included fatigue, fever, nausea, vomiting, esophagitis, chills, fever, injection site pain and pain in other sites [14,31,32]. Therefore, concomitant administration of TNFerade and radiotherapy can extend clinical benefits to specific clinical populations with locally advanced disease.

4. Emerging biomarkers to aid patient stratification for treatment with radio-immunotherapeutics

A key aim of ongoing immune-oncology initiatives is to identify ideal patients who are best suited to a systemic immunotherapy of interest. In this regard, tumor mutational burden [33], mismatch repair deficiency [34] and PD-L1 expression levels [35] have been reported to predict the efficacy of ICI and are often taken into account by clinicians when considering a patient’s placement on a specific ICI. However, clinically validated biomarkers aiding patient selection for treatment with specific radio-immunotherapeutics are currently lacking. Nevertheless, recent data from prospective clinical trials indicates that imaging and blood-based methods can help us identify such molecular markers which hold promise for possible translation into the clinical as validated predictive biomarkers. Major emerging biomarkers are discussed as follows:

4.1. PD-L1

Researchers have demonstrated the predictive value of PD-L1 expression levels by analyzing pre-treated tumor cells using the FDA approved VENTANA PD-L1 immunohistochemistry assay from subjects treated with chemoradiation and durvalumab as part of the PACIFIC trial [36]. They showed that patients in the PD-L1 tumor cell (TC) \(\geq 25\%\) cohort had better PFS outcomes (Median PFS = 17.8 months) than the PD-L1 TC <1% group (Median PFS = 10.7 months). However, the median PFS among PD-L1 TC 1–24% patients was not reached, highlighting that even PD-L1 low patients can expect to derive a PFS benefit. Higher PD-L1 expression levels were also associated with improved OS outcomes, where PD-L1 TC \(\geq 25\%\), 1–24% and <1% patients had a median OS of not reached, 43.3 and 33.1 months respectively. The rate of Grade 3 or higher AEs in the PD-L1 TC \(\geq 25\%\), 1–24% and <1% groups were 37.4%, 29.6% and 30.8%. This highlights the notion that there is a trade-off in selecting patients with PD-L1 TC \(\geq 25\%\) for this treatment, since PFS and OS benefits are counter-balanced with a slightly higher risk of toxicity. Therefore, such patients could be referred for this treatment if they are physically fit since frail subjects or those with pre-existing co-morbidities might be placed at a greater risk for toxicity using this combined treatment approach.

However, additional prospective data highlights the variability in interpreting outcomes associated with specific PD-L1 TC cut-off scores, making it challenging to apply standardized cut-off scores across the spectrum of cancer histologies. This is evidenced from results of the Phase 3 JAVELIN trial involving treatment with avelumab (PD-L1 inhibitor) plus standard-of-care chemoradiotherapy versus chemoradiotherapy alone in patients with locally advanced squamous cell carcinoma of the head and neck (SCCHN) [37]. Contrary to the PACIFIC trial which demonstrated a PFS benefit even in PD-L1 TC 1–24% and <1% subgroups treated with durvalumab over the placebo group, the JAVELIN investigators did not note a PFS benefit in PD-L1 TC low (<25%) patients on the investigational arm (HR, 1.37 [95% CI, 1.00–1.88; \(p = .003\)]) [37]. This suggests that addition of avelumab to chemoradiotherapy might in fact be detrimental in PD-L1 TC low SCCHN subjects who are likely to benefit by receiving standard-of-care chemoradiotherapy only to potentially spare additional toxicities associated with avelumab use.

4.2. IL-2 Producing CD8+ T cells and Central memory CD8+ T (TCM) cells

In addition to the expanding role of tumor specific markers in predicting treatment specific outcomes, researchers are also evaluating peripheral blood mononuclear cells for this purpose through correlative studies in recent clinical trials. In the
aforementioned study by Hiniker et al., the investigators noted a significant association between treatment responders and elevated baseline levels of IL-2 producing CD8+ T cells (p < .01) and TCM cells (p < .05) in peripheral blood versus patients who progressed on the radiation and ipilimumab therapy [21]. However, production of cytokines was not different in TCM among responders versus progressors, indicating that responders may have a higher frequency of systemic TCM cells but these may not be functionally different. Perhaps having a greater number of TCM cells allowed responding subjects to mount effector CD8+ T cells responses following CTLA-4 blockade of negative signaling [21]. Further exploration via multiple, large scale prospectively designed studies can validate the role of these two immune cells in serving as a predictive biomarker of treatment response following radio-immunotherapy.

4.3. Lactate dehydrogenase (LDH)

Prospective data has demonstrated that decreasing levels of LDH from baseline are associated with improved disease control and OS outcomes following ICI therapy [38]. However, the utility of LDH from peripheral blood as a potential immunological marker predicting clinical outcomes by combining immunotherapy with radiation still remains to be seen. The previously discussed study by Ho et al. has shed some light on this issue, where although LDH was not consistently associated with ORR but higher LDH levels were associated with worse PFS outcomes at week 7 and 13 after commencing treatment (p = .039 and p = .043, respectively) [17]. Future clinical trials with a larger sample size can help clarify the role of LDH in aiding patient selection for combined ICI and radiotherapy.

5. Concluding remarks

Radio-immunotherapy is a relatively nascent field that holds significant promise and appears to be gaining momentum as evidenced by over 200 ongoing clinical trials registered on ClinicalTrials.gov. However, it is vital to conduct additional radiobiologically guided studies to aid our mechanistic understanding of combining these two treatment modalities in addition to configuring the ideal dose, radiation quality, fractionation schedule, sequence of treatments, treatment duration and previous treatment lines especially those pertaining to immunotherapeutic regimens. Other radiation specific parameters which warrant additional investigation comprise of assessing the utility of dose heterogeneity via spatial fractionation to prime immune cells or to target immunosuppressive cells and outcomes associated with effective cross-priming of T cells by avoiding prophylactic radiation of clinically uninvolved lymph nodes [39]. From a clinical standpoint, candidate selection would be governed by an assessment of cancer subtype considering the tumor immune microenvironment among patients with an immuno-competent clinical status. Ongoing clinical trials integrating translational studies can further aid the identification of predictive biomarkers to ensure selection of appropriate patients in order to truly optimize clinical outcomes.

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