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Review

Immunotherapy and Radiation – A New Combined Treatment Approach for Bladder Cancer?

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Abstract. Recently, immunotherapy with checkpoint inhibitors has been showing promise in clinical trials for stage IV bladder cancer. Herein, we review the literature regarding the role for radiation therapy, the role for immunotherapy, and the potential synergy of these treatments combined in bladder cancer. There is ample pre-clinical data in a number of different tumor models, coupled with a growing body of clinical evidence in melanoma and other malignancies to suggest combining radiation and immunotherapy could lead to substantial advances in treatment outcomes for bladder cancer. Yet, these data for bladder cancer remain at the pre-clinical stage, and further study is needed.

Keywords: Immunotherapy, radiation therapy, bladder cancer, checkpoint inhibitor, BCG, bladder-sparing therapy

ROLE OF RADIATION IN BLADDER CANCER TREATMENT

The standard of care for localized muscle-invasive bladder cancer (MIBC) is a consideration of neoadjuvant chemotherapy followed by radical cystectomy [1, 2]. The potential morbidity of this approach is well described [3, 4], and increases with age [5]. The effectiveness of trimodality therapy (transurethral resection of bladder tumor (TURBT) followed by chemoradiation) as a viable alternative to upfront cystectomy for selected patients with MIBC and for those either unwilling or unable to undergo surgery has also been evaluated [6–9]. Despite the lack of completed studies randomizing patients to either trimodality therapy or cystectomy, the available data suggests that outcomes can be similar between the two treatment approaches at comparable stages [10, 11]. A recent RTOG pooled analysis with a median follow-up of 7.8 years among survivors demonstrated a complete response (CR) rate to trimodality therapy of 72% [12], and a Massachusetts General Hospital study looking at long-term outcomes for trimodality therapy in MIBC showed a CR rate in 72% (78% in cT2) [13]. A small study also demonstrated that trimodality therapy for T2 recurrence after bacillus Calmette-Guérin (BCG) failure is viable with actuarial disease-specific survival (DSS) of 77% at 5 years and 70% at 7 years [14]. Furthermore, according to another RTOG pooled analysis, the reported rates of late pelvic toxicity following bladder-sparing therapy are low [15], and a quality of life (QOL) study based on patient reported data demonstrated high QOL among long-term survivors [16]. Indeed, the data seem encouraging that chemoradiation could play an important role in blad-

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der cancer therapy for appropriately selected patients [6], and given these data it would seem only natural to explore new combinations with novel emerging immunotherapeutics.

The current recommendation for non-muscle invasive bladder cancer (NMIBC) including Ta, T1, and carcinoma in-situ (CIS) is a TURBT followed by intravesicular chemotherapy or BCG with BCG recommended as the first line for CIS and a favored option for high-grade Ta and T1 disease [17, 18]. Data from the Surveillance, Epidemiology and End Results (SEER)-Medicare database has shown that BCG reduces bladder cancer deaths by 23% for patients with high-grade tumors [19]. In the absence of adjuvant therapy, the risk of progression to T2 or greater disease at 3 years approaches 50% [20, 21].

Despite the well-established benefit of BCG therapy, a substantial proportion of patients who receive it will still develop BCG refractory disease [21–23]. In these cases the standard recommendation has been cystectomy. As a potential alternative, Weiss et al. demonstrated an 88% complete response rate with radiotherapy following TURBT for high risk T1 disease [24], and currently RTOG 0926 is investigating the effectiveness of trimodality therapy in recurrent BCG refractory T1 disease. In fact, prior to the widespread use of BCG, radical radiotherapy was used to treat T1 disease, but this fell out of favor following a phase III comparison between radiotherapy alone versus conservative measures (including BCG) that showed no difference in overall or progression free survival [25, 26]. NCCN guidelines currently dictate that radiation therapy can be considered for poor surgical candidates with recurrent T1 disease [1]. As will be described later, radiation in combination with other immunomodulatory drugs, in fact, may be the ideal treatment for restimulating an immune system that is no longer functionally responsive to BCG.

IMMUNOTHERAPY FOR BLADDER CANCER

An association between febrile illness and cancer regression has been known for centuries [27]. However, it was not until the 19th century that surgical oncologist William Coley demonstrated, using a systematic approach of injecting a mixture of heat-killed Streptococcus pyogenes and Serratia marcescens into 210 patients with soft-tissue sarcomas, that the immune system's impact could be more accurately quantified. A response was observed in 60 patients, but it would take time before cancer immunotherapy gained broader mainstream acceptance.

Tumor growth requires modulation and suppression of the immune system. It is known that the incidence of malignancies increases in immunosuppressed individuals [28, 29]. In addition, the immunosurveillance hypothesis, in which organisms are under constant thymic-dependent surveillance for neoplastic growth originally postulated by F. MacFarlane Burnett, has gained added traction with later supporting studies [29–32]. This idea was further modified by Robert Schreiber who described a process of "immunoediting". According to Schreiber, a malignancy undergoes three phases before becoming clinically relevant: 1) the elimination phase or the classical concept of immunosurveillance; 2) the equilibrium phase in which a metastable state is reached between tumor and immune system; and 3) the escape phase in which the tumor has developed immuno-evasive and suppressive mechanisms under the harsh evolutionary pressure of the immune system, thereby allowing it to grow unencumbered [33, 34]. To effectively harness the immune system against cancer these latter evolved barriers must be overcome.

Overcoming the high threshold of tumor immune tolerance is the goal of immunotherapy. There are a number of approaches which range from improving endogenous tumor antigen presentation and lymphocyte activation to ex-vivo manipulation of lymphocytes and re-infusion [35, 36]. Improved antigen presentation occurs with increased tumor antigen liberation and the endogenous production or exogenous addition of adjuvants necessary to fully mature/activate dendritic cells. BCG, a robust adjuvant and a product not dissimilar to the original Coley's toxin, is the forerunner to all other immunotherapy being trialed for NMIBC. Its effective application is already an indication that influencing T-cell responses can impact bladder cancer's risk of recurrence. In fact, in one study it was shown that a febrile response to BCG was a good prognostic factor and correlated with longer recurrence free survival [37]. Urinary IL-2, a potent T-cell mitogen, and IL-2 production by peripheral blood lymphocytes (PBL) also were prognosticators of a good response to BCG. Thalmann et al. demonstrated that patients with elevated urinary IL-18, a molecule critical for effective T_H1 responses, have significantly longer disease free survival [38]. Using a mouse model, Biot et al. suggested that T-cell memory is likely involved in robust anti-tumor activity following intravesicular BCG administration as prior exposure to BCG enhances the anti-tumor immune

response. The authors show that the recruitment of inflammatory monocytes into the bladder was abrogated by CD4 and CD8 T-cell depletion prior to intravesicular BCG instillation. Additionally, repeated BCG instillations also increased the numbers of T-cells infiltrating the bladder. Finally, the authors show in a clinical series of 55 patients, who were stratified by PPD positivity before intravesicular BCG therapy, that prior exposure to mycobacterium confers a significantly better recurrence-free survival. This again indicates that a likely anamnestic response is at work [39]. All these observations are consistent with Sharma et al. who previously demonstrated that higher numbers of $CD8^+$ tumor-infiltrating lymphocytes in muscle invasive disease correlates with superior disease-free and overall survival [40]. Alternatively, other studies have shown that certain genetic signatures can also be predictive of a BCG response [41, 42], specifically polymorphisms in oxidative stress genes [43]. Despite BCG being an established adjuvant for bladder cancer, BCG failure remains a concern.

BCG failure can occur due to patient intolerance to the side effects or tumor resistance to or recurrence following the treatment. BCG intolerance is relatively common, occurring in 20% of patients during maintenance therapy. These patients then require intravesicular therapy with an alternate agent [44, 45]. In contrast, patients who were able to tolerate BCG and had an initial response were reported to have a 38.6% recurrence rate after a median follow-up of 26 months [46]. BCG unresponsiveness is defined as persistent high grade tumor at 6 months or a recurrence within 6 months or less after achieving a disease-free state [47, 48]. Herr and Dalbagni showed that at 6 months 20% of BCG treated patients had persistent or recurrent tumors. In addition to the cytokines described earlier that correlate with a good response to BCG, there is evidence to suggest an aberrant T_H2 response instead of a robust T_H1 response is associated with failure [49, 50].

Alternative approaches to cystectomy have been examined following BCG failure. Interferon-α2b (IFN-α2b) in combination with BCG was studied in a multicenter Phase II trial including 231 patients with BCG failure. These patients were treated with a 6-week induction course of BCG and IFN-α, followed by 3 additional treatments. With a median follow-up of 2 years, 57.3% of BCG naïve patients and 42% of the patients with prior BCG failure remained tumor free [51]. However, one randomized trial examining BCG combined with IFN-α2b did not show superiority compared to BCG alone for BCG-naïve patients [52, 53]. Nevertheless, IFN-α2b may still be effective in BCG failure patients because it has been shown in-vitro to inhibit production of IL-10, an immunosuppressive cytokine, allowing potentiation of the T_H1 response [54]. IL-10 has been shown to impair the antitumor activity of intravesicular BCG therapy with IL-10 knockout mice demonstrating enhanced delayed-type hypersensitivity and an enhancement of the antitumor response [55]. Finally combined BCG and IL-12, in an effort to skew the immune response towards a dominant T_H1 phenotype, appears to have some potential efficacy in animal models [56].

Bladder cancer along with melanoma and non-small cell lung cancer represent good candidates for cell-mediated immunotherapy due to their relatively high somatic mutation rates [57]. This generates many novel tumor antigens [57, 58] that are critical for an effective immune response because it creates a variety of epitopes that may differ from native peptides and to which T-cells have not been negatively selected. For effective T-cell activation, T-cells require three signals: 1) effective TCR ligation by its cognate MHC-antigen complex, 2) co-stimulation through CD28 on the T-cell membrane, and 3) a cytokine milieu that determines the T-cells' phenotype. There are also negative regulators of T-cell activation including CTLA-4 and PD-1 which increase the T-cell activation threshold or impair its effector function. Current immunotherapy has focused on disrupting these negative regulators, termed checkpoint blockade, thereby increasing the chance for a functional adaptive immune response. Another group of molecules which include OX40 (CD134) and CD40 are activating receptors on T-cells and antigen presenting cells (APC) respectively. These are also being targeted with agonistic antibodies to promote an anti-tumor response. These biologics are an exciting addition to bladder cancer given the lack of effective therapeutic options for advanced disease [59, 60]. Initial results are encouraging and are examined in more detail here [61].

The newer checkpoint inhibitors improve treatment outcomes for localized as well as metastatic disease. Checkpoint blockade of CTLA-4 using ipilimumab, first approved by the FDA in 2011, has demonstrated encouraging results for unresectable and metastatic melanoma [62, 63]. This drug acts at the initial point of T-cell activation, recruiting T-cells to the immune response that would not normally have received the threshold trigger for activation. Ipilimumab has also been shown to impact regulatory T-cell number and function in a few pre-clinical studies [64, 65]. Carthon et al. at MD Anderson showed in a small dose esca-

lation study of ipilimumab for localized urothelial carcinoma of the bladder that there was limited toxicity. They also noted an increased frequency of $CD4^+$ $ICOS^{high}$ (activated T-cells) in the systemic circulation of these patients [66]. There is currently an on-going phase II clinical trial (NCT01524991) examining the combination of gemcitabine, cisplatin and ipilimumab for metastatic urothelial carcinoma with a primary outcome measurement of one-year overall survival.

The standard of care for stage IV bladder cancer remains platinum-based chemotherapy regimens-methotrexate, vinblastine, doxorubicin, cisplatin (MVAC), high dose MVAC and methotrexate, cisplatin and vinblastin (MCV) -which have demonstrated a survival benefit. The 5-year overall survival for MVAC is 15.3% [67]. Additionally, MVAC is associated with a substantial toxic death rate and toxicities that can reach 4% [68]. Any addition to our armamentarium has the potential for lasting consequences, and disruption of the PD-1/PD-L1 axis appears likely to be one of the most promising recent developments. PD-1, another negative regulator of T-cell activation, can encounter its ligand, PD-L1, in multiple places including tumor cells and tumor-infiltrating immune cells. A phase I trial for lambrolizumab (anti-PD-1) in advanced melanoma enrolled 135 patients, 48 of whom had received prior treatment with ipilimumab. 13% of patients reported grade 3 or 4 toxicity, and the overall response rate (ORR) was 44% which did not vary with prior exposure to ipilimumab. Regressing lesions which were biopsied showed densely infiltrated $CD8^+$ T-cells [69]. Data from Tumeh et al., suggests that pre-existing intratumoral $CD8^+$ T-cells are responsible for tumor regression in advanced melanoma following PD-1 blockade [70]. The authors were able to develop a predictive model for response to PD-1 inhibition based on $CD8^+$ T-cell density at the tumor invasive margin. This was validated in an independent 15 patient cohort. These studies of PD-1/PD-L1 disruption have been extended to bladder cancer.

Recent data from Powles et al. demonstrated in an expanded phase I trial with 67 metastatic bladder cancer patients treated with MPDL3280A (anti-PD-L1) that there was an objective response rate of 11% for patients with PD-L1 negative/low and 43% for PD-L1 intermediate/high expressing tumor-infiltrating immune cells. Interestingly, in this study the response rate was dependent on PD-L1 expression by the tumor infiltrating immune cells (likely myeloid-derived suppressor cells) rather than the tumor cells themselves [61, 71]. This has also been seen in non-small cell lung cancer and renal cell carcinoma [72]. An abstract presented at the European Society for Medical Oncology in 2014 showed encouraging results for pembrolizumab (anti-PD-1) for patients with advanced urothelial tumors [73]. Another recently published study from Harvard, showed that expression of PD-L1 by tumor-infiltrating mononuclear cells (TIMCs) correlated with improved overall survival even in patients treated only with platinum-based chemotherapy. This is consistent with the notion that PD-L1 expression by TIMCs is induced following intratumoral T-cell activation and cytokine production [74]. Of note, it has also been reported that PD-L1 expression is dynamic and PD-L1 upregulation may be an effective surrogate marker for T-cell activation and lytic potential [75]. There are a number of clinical trials (NCT02302807, NCT02108652) still recruiting which are testing the role of MPDL3280A in locally advanced and metastatic bladder carcinoma, in addition to trials testing anti-PD-1 inhibitors (NCT02324582).

Another approach being examined for a variety of metastatic malignancies including bladder cancer is the use of a fusion protein consisting of IL-2 and a humanized soluble T-cell receptor directed against p53 derived epitopes (ALT-801). A number of malignancies, including bladder cancer, overexpress p53 and therefore this approach confers a level of specificity [76, 77]. A phase I trial with a heterogeneous collection of advanced malignancies demonstrated reasonable tolerability and encouraging efficacy [78]. Currently there are trials (NCT01625260) examining ALT-801 in BCG refractory NMIBC or an engineered IL-15 super-agonist (ALT-803) in combination with BCG (NCT02138734) also for NMIBC. Another interesting approach involves an engineered adenovirus, CG0070, which preferentially replicates in RB protein-defective cells and carries a granulocyte macrophage colony-stimulating factor (GM-CSF) gene [79, 80]. This virus is postulated to have direct oncolytic activity in addition to the GM-CSF mediated immunostimulatory effects. In a preliminary phase I trial, patients with BCG failure received single or multiple intravesicular instillations of the virus. An 81.8% response rate was observed in those patients with borderline or high RB phosphorylation who received a multidose regimen. The complete response rate across all cohorts was 48.6% [81]. A phase II/III trial (NCT01438112) evaluating the virus in patients with BCG failure is ongoing. Given these exciting results, it seems reasonable to combine these immunotherapeutics with other immunomodulatory modalities like radiation.

IMMUNOLOGIC EFFECTS OF RADIATION THERAPY IN BLADDER CANCER

Much has been written recently about the immunostimulatory effects of radiation [82–84] (see Fig. 1). Although still somewhat controversial, there is a growing body of evidence that immunogenic cell death induced by radiation liberates tumor antigens along with endogenous adjuvants promoting APC maturation and effective cytotoxic T-cell priming [85]. Radiation induced immunogenic cell death is characterized, in part, by activation of the endoplasmic reticulum (ER) stress pathway and tumor cell surface expression of calreticulin (CRT), a pro-phagocytic molecule, facilitating APC uptake of tumor antigens [86]. In addition, passive secretion of HMGB1, an evolutionarily conserved nuclear protein, by tumor cells undergoing late apoptosis or necrosis can bind toll-like receptor 4 (TLR-4), the lipopolysaccharide (LPS) receptor, on dendritic cells (DC) and lead to DC maturation and effective T-cell priming [87, 88]. Radiation has also been shown to promote effector T-cell recruitment into the tumor through chemokine induction and upregulation of tumor cell MHC-I expression. Prior to RT, many tumors secrete chemokines which recruit immunosuppressive cells including regulatory T-cells [89, 90]. However, radiation in a 4T1 mouse breast cancer model induces CXCL16 production, a chemokine which recruits effector T-cells and whose expression correlates with increased numbers of tumor-infiltrating lymphocytes and improved survival [91, 92]. Radiation also induces a bystander effect influencing tumor cell survival through its effects on stromal components. The bystander effect is largely mediated by stromal cell-cell communication through gap junctions or production of soluble mediators including tumor necrosis factor-α which may help mature APCs for effective antigen presentation [93, 94]. Finally, and perhaps most importantly, there is data that demonstrates radiation and checkpoint inhibitors activate the immune system through two non-redundant mechanisms [65]. Twyman Saint-Victor et al. show that radiation enhances the diversity of the T-cell receptor repertoire intratumorally while anti-CTLA4 promotes expansion of effector T-cells, and anti-PD-L1 reverses T-cell exhaustion. It is these non-overlapping mechanisms that make clinical synergy so exciting.

In the specific context of urinary bladder carcinoma, O'Toole et al. used an interesting technique to evaluate the effect of tumor irradiation on PBL cytotoxicity. In this study, patients with T1-T4 bladder cancer received radiation using a three-field technique (two anterior wedge fields and an open posterior field). Prior to, during, and following radiation, patient PBL were collected and cultured with target cells to assess cytotoxicity, and it was shown that two of three patients with T3 cancer who were negative for PBL cytotoxic activity prior to radiation were positive afterwards [95]. In a follow-up study, the authors demonstrated that the patients who were clinically tumor free 5 years after treatment all had a more rapid post-radiotherapy increase in lymphocyte numbers than the patients who recurred again suggesting that adaptive immunity plays a role in bladder cancer control [96].

There is a subsequent report that irradiation of bladder carcinoma cell lines may enhance their susceptibility to NK cell mediated killing. Mizutani et al. showed that T24, a known NK cell resistant bladder cancer cell line, was increasingly sensitive to lysis following as little as 1 Gy of radiation. This effect is likely not antigen specific, but further suggests the immunoenhancing effects of ionizing radiation in bladder cancer [97]. More recently, Demaria et al. demonstrated in a pre-clinical model that if a DC growth factor was administered to mice bearing a syngeneic mammary carcinoma, there was regression of tumor cells outside of the radiation field (abscopal effect) [98]. In a follow-up "proof-of principle" clinical trial, 14 patients with advanced metastatic disease, including bladder cancer, were treated with GM-CSF and received radiation to one lesion at 3.5 Gy $\times$ 10 fractions over 2 weeks. The overall results were encouraging with 30% achieving an abscopal response, while others showed a decreased SUV of non-irradiated lesions [99].

Admittedly, at this time most studies examining this exciting synergy between radiation and immunotherapy are not focused on urinary bladder cancer (Fig. 1). In patients with metastatic cutaneous melonoma there have been a number of case studies reporting encouraging results after receiving a CTLA-4 antagonist and radiation [100, 101]. A report out of Memorial Sloan Kettering followed a 39 year old woman with diffuse disease who received palliative radiation to a paraspinal metastasis while also receiving ipilimumab. Four months following treatment, imaging showed regression of both the irradiated lesion and unirradiated distant metastases [101]. NCT01689974 is a phase II trial evaluating ipilimumab alone versus ipilimumab and radiation in metastatic melanoma. A number of clinical trials are investigating this interaction in a variety of other malignancies including non-small cell lung cancer (NCT02221739) and head and neck cancer (NCT01935921). NCT00861614 is a

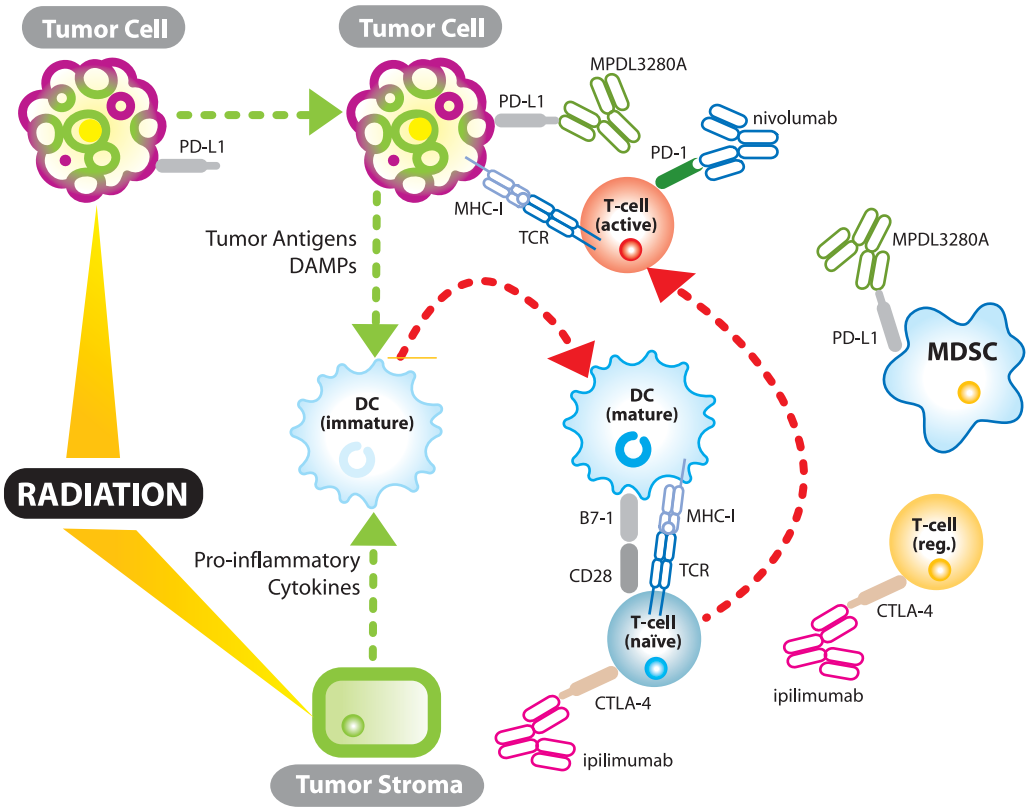

Fig. 1. The synergistic relationship between immunotherapy and radiation. TCR – T-cell receptor; MHC-I – major histocompatability complex 1; DC – dendritic cell; MDSC – myeloid derived suppressor cell; DAMPs- damage associated molecular patterns (endogenous adjuvants which mature dendritic cells); T-cell (reg.) – regulatory T-cell.

completed phase 3 trial evaluating radiation and ipilimumab for castration-resistant prostate cancer. The primary outcome measure was overall survival and it compared ipilimumab plus radiation to radiation alone for patients that had received prior treatment with docetaxel. Results published in 2014 demonstrated no statistically significant (p-value = 0.053) difference between the ipilimumab and placebo groups, however, the authors do state the overall survival hazard ratio decreased over time and ipilimumab at later time points correlated with improved survival relative to placebo [102]. Additionally, a subgroup analysis of patients with more favorable prognostic features including an alkaline phosphatase less than 1.5 times the upper limit of normal, a hemoglobin concentration of 110 g/l or higher and no visceral metastases showed a median overall survival with ipilimumab of 22.7 months compared to 15.8 months with placebo and a p-value of 0.0038.

The PD-1 axis inhibitors and radiation are being evaluated in pre-clinical models with data suggesting improved local tumor control [103] and this interaction is also being investigated in some upcoming clinical trials (Table 1). As a parallel, there have also have been a number of trials demonstrating an improved response rate when multiple immunomodulatory drugs are combined in renal cell carcinoma and melanoma-perhaps physiologically analogous to a combined radiation/immunotherapeutic approach [104, 105]. Results from clinical trials evaluating radiation therapy and PD-1/PD-L1 blockade are still outstanding.

There is no overriding consensus about the timing of radiation relative to immunotherapy although initial data seems to support concurrent administration. In a pre-clinical trial, commencing anti-PD-L1 administration on either the first or the last day of radiation had equivalent overall survival, but both were superior to commencing PD-L1 blockade 7 days after radiation completion [106]. Administering radiation following immunotherapy could potentially blunt an already active response [84]. There is also limited data with regards to optimal dose and fractionation of the radiation with immunotherapy. For induction of an abscopal effect, preclinical data suggests a fractionated regimen is superior to a single dose: 24 Gy in 3 is superior

Table 1
A sampling of combination trials

Clinical trials.gov identifier	Sponsor	Immunotherapy	Radiotherapy	Treatment timing	Phase	Condition
NCT01436968	Advantagene, Inc	ProstAtak (AdV-tk) injected into prostate	Standard of care	Radiation 0–3 days after second AdV-tk injection	3	Prostate Cancer
NCT00751270	Advantagene, Inc	AdV-tk injected into tumor/ tumor bed	Standard of care	Radiation 3–7 days following AdV-tk injection	1b	Malignant Glioma
NCT00589875	Advantagene, Inc	AdV-tk injected into tumor bed	Standard of care	Radiation 3–7 days following AdV-tk injection	2a	Malignant Glioma
NCT00634231	Advantagene, Inc	AdV-tk injected into tumor/ tumor bed	Standard of care	Radiation 3–7 days following AdV-tk injection	1	Pediatric Brain Tumors
NCT00589875	Advantagene. Inc	AdV-tk injected into tumor bed	Standard of care	Radiation 3–7 days following AdV-tk injection	2	Malignant Glioma
NCT01836432	NewLink Genetics Corporation	Algenpantucel-L (HAPa1, HAPa2)	50.4 Gy in 28 fractions	Radiation and immunotherapy on day 1.	3	Pancreatic Cancer
NCT01896271	University of Texas Southwestern Medical Center	High dose IL-2	Stereotactic ablative radiation therapy (SART) 8–20 Gy in 1–3 fractions	IL-2 administered immediately following radiation	2	Metastatic Renal Cancer
NCT01497808	Abramson Cancer Center of the University of Pennsylvania	Ipilimumab	Dose escalation for Stereotactic body radiation therapy (SBRT)	Not described	1/2	Metastatic Melanoma
NCT02303990	Abramson Cancer Center of the University of Pennsylvania	Pembrolizumab (anti-PD-1)	Hypofractionated radiation	Not described	1	Metastatic Cancers
NCT02086721	Maastricht Radiation Oncology	L19-IL-2	Patients receive a schedule of 1×30 Gy, 3×15–20 Gy; 5×12 Gy; 8×7.5 Gy	L19-IL-2 given one week after completion of radiation	1	Oligometastatic Solid Tumors
NCT01935921	National Cancer Institute	Ipilimumab	Standard of care	Ipilimumab started at beginning of week 4 of cetuximab course, given 3 courses total	1	Stage III-IVB Head and Neck Cancer
NCT02298946	National Cancer Institute	AMP-224 (PD-1 inhibitor)	Stereotactic body radiation therapy	Radiation day 0, AMP-224 on day 1 then q14 days	1	Metastatic Colorectal Cancer

Table 1
(*Continued*)

Clinical trials.gov identifier	Sponsor	Immunotherapy	Radiotherapy	Treatment timing	Phase	Condition
NCT02239900	M.D. Anderson Cancer Center	Ipilimumab	Depends on group- SBRT 50 Gy in 4 fractions (group 1)	Depends on group - Radiation days 1–4 of cycle 1 of ipilimumab (group 1)	1/2	Advanced Solid Tumors
NCT01689974	New York University School of Medicine	Ipilimumab	6 Gy × 5 IMRT	Ipilimumab first given day 4 after radiation	2	Metastatic Melanoma
NCT02221739	New York University School of Medicine	Ipilimumab	6 Gy × 5 IMRT	Ipilimumab first given within 24 hours of starting radiation	2	Metastatic Non-Small Cell Lung Cancer
NCT01276730	James Graham Brown Cancer Center	Interferon- α2b	40–45 Gy in conventional fx to whole pelvis	Interferon- α starts first day of radiation	2	Advanced Cervical Cancer
NCT01347034	H. Lee Moffit Cancer and Research Institute	Intratumoral injection of autologous dendritic cells	Conventional radiation with boost	Radiation begins prior to DC injection	2	Soft Tissue Sarcoma
NCT01449279	Stanford University	Ipilimumab	Palliative radiation	Ipilimumab given up to two days before radiation	1	Melanoma
NCT02254772	Stanford University	TLR-9 agonist (SD-101), ipilimumab	Not specified	SD-101 given intratumoral given starting day 1 and ipilimumab given on day 1, radiation on days 1,2	1/2	Low-Grade Recurrent B-Cell Lymphoma
NCT01565837	Wolfram Samlowski	Ipilimumab	Stereotactic ablative radiation	Radiation to 1–5 lesions after initial dose of ipilimumab	2	Melanoma
NCT01758458	Fred Hutchison Cancer Research Center	MCPyV TAg-specific polyclonal autologous CD8-positive T-cell vaccine	Unspecified	Radiation is given day 2 to 4 before treatment	1	Merkel Cell Carcinoma
NCT02303366	Peter MacCallum Cancer Center, Australia	MK-3475 (anti-PD-1)	Stereotactic ablative radiation	Radiation followed by 8 cycles of MK-3475	1	Oligometastatic Breast Neoplasia
NCT02318771	Thomas Jefferson University	MK-3475	8 Gy × 1	Radiation day 1, MK-3475 3–17 days later	1	Many Advanced Cancers
NCT01703507	Thomas Jefferson University	Ipilimumab	Whole brain or stereotactic radiation	Receive ipilimumab 4 × over 10 weeks. WBRT done for weeks 1-2.	1	Melanoma with Brain Metastases

to 30 Gy in 5 which is superior to 20 Gy in 1 fraction [107, 108]. Clinical data has demonstrated abscopal effects for a variety of different fractionation regimens including 8 Gy in one and 30 Gy in five fractions [107, 109, 110]. Although specific dosing information has yet to be fully established, data again from pre-clinical studies also suggests that increasing dose leads to increased interferon-γ production and improved antigen presentation, the caveat being that at higher doses there appears to be a concurrent rise in regulatory T-cells [111, 112]. Currently, when evaluating the data in totality, it is difficult to make a specific recommendation, but a dose of 8 Gy in one fraction up to 24 Gy in three fractions given with a concurrent checkpoint inhibitor appears to be the most well supported treatment regimen based on the above preclinical data and the encouraging results from NCT00861614 (ipilimumab with radiation for castration-resistant prostate cancer) which gave radiation within 2 days of initiating ipilimumab.

Melanoma has been at the forefront of the radio-immunotherapy clinical trials but it is now time to incorporate urinary bladder cancer. It was one the first malignancies in which an effective immunotherapy was utilized, and it behooves us to examine potential synergy with radiation. There are numerous contexts in which this combination therapy could be applied including BCG refractory NMIBC. A new phase of RTOG 0926 could include a checkpoint inhibitor and an immune activating dose of radiation (such as 8Gy x 1). Alternatively, further investigation may yield a novel hypofractionated regimen that in combination with immunotherapy optimizes radiation antigenicity. Immunotherapy could also be added as an upfront component of trimodality therapy for MIBC, or for use in salvage following trimodality therapy failure in MIBC or in non-muscle invasive disease. Finally, immunotherapy may even have an application in combination with radiation for metastatic disease to evaluate the likelihood of an abscopal response.

CONCLUSION

In conclusion, both radiation and immunotherapy are playing an increasingly important role in a number of malignancies including bladder cancer. It is evident that immunotherapy has great potential to improve survival for patients with both localized and advanced disease. This potential may be improved even further if these novel immunotherapeutic modalities are combined with radiation. Using melanoma as the model, there seems to be reason for great excitement for the future of bladder cancer therapy.

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