Combined radiotherapy and immunotherapy in urothelial bladder cancer: harnessing the full potential of the anti-tumor immune response

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Abstract
Purpose Radiotherapy (RT), as part of trimodal therapy, is an attractive alternative treatment in patients with urothelial muscle-invasive bladder cancer (MIBC). There is accumulating evidence suggesting the immunomodulatory effects of RT and its potential synergy when combined with immunotherapy. The aim of this review was to report on the most recent advances on this combination, including the mechanisms of RT immunomodulation, practical approach to combining RT and immunotherapy, and ongoing clinical trials in bladder cancer.

Methods Using the PubMed database, we identified articles published between March 2004 and April 2020 on the combination of RT with immunotherapy in localized or metastatic MIBC. A search of the Clinicaltrials.gov and Clinicaltrialsregister.eu retrieved ongoing clinical trials on the topic as well.

Results Combination of RT with immunotherapy leads to immunogenic cell death and an increase in immune markers thus leading to improved tumor control. For localized MIBC, there are safety concerns related to the use of concurrent immunotherapy with hypofractionated RT, thus neoadjuvant or adjuvant immunotherapy is preferred. In the metastatic setting, the combination of multi-site RT with SBRT-like doses (≥ 6 Gy per fraction) and concurrent immunotherapy seems most efficacious at harnessing the abscopal effect. At least 25 clinical trials combining immunotherapy and RT in MIBC are currently ongoing and will answer pending questions on safety, efficacy, and practical considerations on RT scheduling, fractionation, and targets volumes.

Conclusion RT has the potential to synergize with immunotherapy to improve oncological outcomes in patient with localized or metastatic MIBC. Clinical trials results are eagerly awaited.

Keywords Radiotherapy · Radiation therapy · Immunotherapy · Immune checkpoint inhibitors · Urothelial carcinoma · Bladder cancer
Introduction

Trimodal therapy (TMT) is an attractive alternative treatment in patients with urothelial muscle-invasive bladder cancer (MIBC). TMT involves transurethral resection of the bladder tumor (TURBT), followed by radiotherapy (RT) and concurrent chemotherapy [1]. Its efficacy is comparable to that of the surgery in an appropriately selected population [2, 3]. Unfortunately, despite advances in these strategies, the 5-year overall survival (OS) for patients with non-metastatic T2–T4a disease remains around 50% [4] while patients with metastatic disease have a 5-year OS of 13% [5]. Moreover, local control rates with TMT range from 60 to 80% depending on disease stage and patient characteristics [3, 4]. Thus, therapeutic innovations are urgently needed in the treatment of MIBC.

Immunotherapy has shown many promises in the past decade for the treatment of locally advanced and metastatic MIBC [6, 7]. Since 2016, five immune checkpoint inhibitors (ICIs) targeting the programmed-cell-death-1 (PD-1) and programmed-cell-death-ligand-1 (PD-L1) pathway have been approved by the FDA as second-line agents in the treatment of metastatic MIBC patients who have progressed on Cisplatin-based chemotherapy [8–12]. These agents showed a benefit in overall response rate (ORR) [8, 10–12] and in the case of Pembrolizumab, an OS benefit compared to chemotherapy [9]. Atezolizumab and Pembrolizumab are also approved as first line treatment in patients who are cisplatin-ineligible and whose tumors/infiltrating immune cells express PD-L1 (≥ 5%) [13]. Despite these promising results, only about 20% of patients will respond to ICIs, although the majority of responders have a durable response [8, 14, 15].

There is an accumulating body of evidence showing the immunomodulatory role of RT and its increased efficacy when combined with immunotherapy [16–19]. Thus, combining ICIs with RT could enhance both local and occult distant disease control in MIBC. The aim of this literature review is to report on the most recent advances on the topic, including the mechanisms of RT immunomodulation, practical approach to combine RT and ICIs and perspectives on ongoing clinical trials in metastatic and localized MIBC.

Methods for evidence acquisition

A literature search was performed in the PubMed database for articles on immunotherapy and RT in localized or metastatic MIBC. The following keywords were used in various algorithms: “radiotherapy,” “radiation therapy,” “immunotherapy,” “immune checkpoint inhibitors,” “urothelial carcinoma,” “urothelial cancer,” “bladder cancer.” All sources published from March 2004 to April 2020 were included in the search. Original or review papers reporting on radiotherapy, immunotherapy, or the combination of, in localized or metastatic MIBC were included. Articles on upper urinary tract urothelial carcinomas and in language other than English or French were excluded. The articles were screened and further references relevant to the subject used. A search query was also done in Clinicaltrials.gov and Clinicaltrialregister.eu to retrieve ongoing clinical trials on combined immunotherapy and RT in localized and/or metastatic MIBC.

Radiotherapy and the immune system

RT induces cell death by causing DNA damage, either directly through charged particles producing double strand breaks in DNA or indirectly by generating hydroxyl free radicals that will cause DNA damage, both leading to apoptotic cell death [20]. Apoptotic cell death has long been thought to be non-immunogenic; however, several pre-clinical studies have now shown that RT has both immunostimulatory and immunosuppressive properties through modulation of the tumor microenvironment (TME) (Table 1).

Immune-stimulating effects of RT

Immunogenic cell death and modulation of the tumor microenvironment

RT can induce a process known as immunogenic cell death by causing tumor cell stress and apoptosis, thus releasing tumor antigens in the TME [17, 21]. RT has been shown to induce the expression and release of damage-associated molecular patterns (DAMPs) such as calreticulin, HSP70 and HMGB1 that are hallmarks of immunogenic cell death [16]. This process turns apoptotic cells into in-situ vaccines by releasing tumor antigens that are then presented to primed T-cells in the TME and draining lymph nodes [22]. Moreover, RT increases the expression of MHCI, pro-inflammatory cytokines as well as immune co-stimulatory molecules and adhesion molecules, thus facilitating CD8+ T-cell infiltration into the TME and priming [23]. Finally, RT can modulate the innate immune system by upregulating the complement pathway the co-stimulatory receptor NKG2D type II integral membrane protein leading to activation of NK cell-mediated responses [24].
The abscopal effect is the phenomenon by which systemic anti-tumor responses are observed outside of the primary site of local irradiation [25]. It has been described in a number of different malignancies, including metastatic renal cell carcinoma, melanoma and hepatocellular carcinoma among others [25]. The exact mechanisms of this phenomenon are not well known but are thought to be mediated by a systemic anti-tumor immune response [26]. Ionizing radiation is thought to increase tumor antigen presentation, subsequent activation of cytotoxic T-cells and increased production of a pro-inflamatory response [26]. Thus, combining RT with immunotherapy could provide an opportunity to boost abscopal response rates. In a mouse model of MIBC the combination of RT and anti-PD-L1 treatment resulted in significantly slower growth rate compared with RT alone in the irradiated xenograft tumors but also in the contralateral non-irradiated tumors, resulting in improved survival [27]. This abscopal effect has also been described when RT is combined with ICIs in several types of malignancies [28]. In a proof-of-principle clinical trial, Formenti et al. [19] showed an objective abscopal response in 9/34 patients (27%) with solid metastatic cancers that received GM-CSF and irradiation to one metastatic lesion. In a randomized phase 1 trial, Sundahl et al. compared Pembrolizumab with sequential versus concomitant stereotactic body radiotherapy (SBRT) to the largest metastatic lesion in MIBC patients. There was a 44% ORR in non-irradiated metastatic sites when SBRT was given concomitantly vs. 0% when given sequentially, correlating with a median OS of 12.1 and 4.5 months, respectively [29]. Table 2 lists ongoing trials combining immunotherapy and RT in the metastatic setting, and the phase 2 trial NCT03601455 specifically studies the abscopal effect as a secondary objective.

Radiation-induced abscopal effect

The abscopal effect is the phenomenon by which systemic anti-tumor responses are observed outside of the primary site of local irradiation [25]. It has been described in a number of different malignancies, including metastatic renal cell carcinoma, melanoma and hepatocellular carcinoma among others [25]. The exact mechanisms of this phenomenon are not well known but are thought to be mediated by a systemic anti-tumor immune response [26]. Ionizing radiation is thought to increase tumor antigen presentation, subsequent activation of cytotoxic T-cells and increased production of a pro-inflamatory response [26]. Thus, combining RT with immunotherapy could provide an opportunity to boost abscopal response rates. In a mouse model of MIBC the combination of RT and anti-PD-L1 treatment resulted in significantly slower growth rate compared with RT alone in the irradiated xenograft tumors but also in the contralateral non-irradiated tumors, resulting in improved survival [27]. This abscopal effect has also been described when RT is combined with ICIs in several types of malignancies [28]. In a proof-of-principle clinical trial, Formenti et al. [19] showed an objective abscopal response in 9/34 patients (27%) with solid metastatic cancers that received GM-CSF and irradiation to one metastatic lesion. In a randomized phase 1 trial, Sundahl et al. compared Pembrolizumab with sequential versus concomitant stereotactic body radiotherapy (SBRT) to the largest metastatic lesion in MIBC patients. There was a 44% ORR in non-irradiated metastatic sites when SBRT was given concomitantly vs. 0% when given sequentially, correlating with a median OS of 12.1 and 4.5 months, respectively [29]. Table 2 lists ongoing trials combining immunotherapy and RT in the metastatic setting, and the phase 2 trial NCT03601455 specifically studies the abscopal effect as a secondary objective.

Immune-suppressing effects of radiotherapy

Radiation-induced lymphopenia (RIL):
- Preferential depletion of CD4+ T cells and B cells after RT
- Effects on infiltrating immune cells:
  - ↑ CD4+ T-reg cells
  - ↑ MDSCs
- Effects on immune cell surface markers:
  - ↑ PDL1 expression
  - ↑ CTLA4 expression on T-reg cells

Effects on tumor infiltrating immune cells:
- ↑ tumor antigens → ↑ APCs → ↑ pro-inflammatory cytokines → ↑ CD8+ T cells

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**Table 1 The effects of radiotherapy on the immune system**

| Immune-stimulating effects of radiotherapy | Immune-suppressing effects of radiotherapy |
|------------------------------------------|------------------------------------------|
| **Induces immunogenic cell death:** | Radiation-induced lymphopenia (RIL): |
| Release of tumor antigens and DAMPs (calreticulin, HSP70, HMGB1) | Preferential depletion of CD4+ T cells and B cells after RT |
| Increased MHCI expression and APCs maturation | |
| Increased CD8+ T-cell infiltration and tumor cell death | |
| Increases: | Effects on infiltrating immune cells: |
| Pro-inflammatory cytokines: interferon gamma, tumor necrosis factor-α, type I interferons | ↑ CD4+ T-reg cells |
| Cos-stimulatory molecules | ↑ MDSCs |
| Adhesion molecules | |
| Activates the innate immune system: | Effects on immune cell surface markers: |
| Upregulation of NKG2D type II | ↑ PDL1 expression |
| NK-cell activation | ↑ CTLA4 expression on T-reg cells |
| Abscopal effect: | |
| ↑ tumor antigens → ↑ APCs → ↑ pro-inflammatory cytokines → ↑ CD8+ T cells | |

DAMPs: damage-associated molecular patterns, MHCI: major histocompatibility complex class I, APC: antigen presenting cell, T-reg: T regulatory cells, MDSCs: myeloid-derived suppressive cells, PDL1: programmed-cell death-ligand-1, CTLA4: cytotoxic T-lymphocyte-associated protein 4
| Study          | Phase | Status/estimate Enrollment | Eligibility                                      | Intervention                                      | Details                                                                 | Outcomes measured                                      |
|---------------|-------|----------------------------|--------------------------------------------------|--------------------------------------------------|-------------------------------------------------------------------------|--------------------------------------------------------|
| NCT03601455  | II    | Open 74 patients           | Unresectable locally advanced or metastatic MIBC | Durvalumab + RT vs durvalumab, tremelimumab + RT | EBRT for 5 fractions beginning on day 8 of cycle 1 of durvalumab ± tremelimumab given q4 weeks | Primary: AEs, PFS  Secondary: LC, pCR, ORR and duration, abscopal response, DSS, OS, late AEs |
| NCT03115801  | II    | Active, not recruiting 112 patients | M + RCC or MIBC with 2+ sites of metastases | IO (nivolumab/atezolizumab/pembrolizumab) versus IO + RT | RT: 30Gy×3 every other day by 3DCRT or IGRT/IMRT IO to start on day 1 of RT: nivolumab q2 weeks for RCC and atezolizumab/pembrolizumab q3 weeks for BC | Primary: best ORR  Secondary: PFS, AEs, OS |
| NCT03486197  | II    | Recruiting 20 patients     | M + MIBC with 2+ sites of measurable disease     | Pembrolizumab + neutron based RT                  | Pembrolizumab on days 1 and 22. On day 23, neutron RT×3–5 fractions over 2 weeks (days 23–42)  Pembrolizumab maintenance until progression or unacceptable toxicity | Primary: ORR  Secondary: OS, PFS |
| NCT03915678  | II    | Not yet recruiting 247 patients | M + adult solid tumors (pancreatic, Virus-assoc. tumors, MIBC, NSCLC, TNBC melanoma) | Combination atezolizumab + intratumoral G100 + RT | Atezolizumab on day 1, q3 weeks G100 intratumoral injection 1 week before atezolizumab, qweek×6–12 weeks RT 1–2 weeks before atezolizumab: 2 Gy×2 on injected metastasis OR SBRT 27-60 Gy in 2–5 fractions on non-injected metastasis | Primary: CR, PR  Secondary: OR, PFR, PFS (1–2 years), OS (1–2 years), AEs, immune markers |
| NCT03693014  | II    | Recruiting 60 patients     | M + cancer of any histology with limited progression on ICI | SBRT | Image guided SBRT: 27 Gy/3 fractions to 1–3 lesions ICI continues as previously scheduled until progression or unacceptable toxicity | Primary: ORR |
| NCT02560636  (PLUMMB) | I      | Active, not recruiting 34 patients | T2–4, N0–3, M0–1 MIBC | Pembrolizumab + RT | Pembrolizumab 2 weeks prior to RT then weekly adaptive bladder RT (24 Gy/6 vs 24 Gy/4 vs 30 Gy/5 fractions) followed by Pembrolizumab q3 weeks | Primary: MTD, AE rates  Secondary: late grade 2+/3+ AEs, response, PFS, OS |
caused an increase in the proportion of CD4+ T regulatory cells (T-reg) infiltrating the tumors [38]. This would a priori be detrimental to the anti-tumor response; however, the increase in infiltrating T-reg cells was abrogated by addition of anti-PD-1 blockade, resulting in improved local control [38]. In their abscopal model on MIBC, Rompre-Brodeur et al. [27] showed that, compared to RT alone, mice treated with combined RT and ICIs had increased infiltration of cytotoxic T-cells, downregulation of immunosuppressive genes, and upregulation of T-cell activation markers. RT has also been shown to increase the infiltration and activation of myeloid-derived suppressive cells (MDSCs), which are known mediators of immunosuppression [39]. In another study of patients with oligometastatic solid tumors, treatment with concurrent SBRT and Sunitinib (but not SBRT alone) decreased the numbers of MDSC and T-reg cells, correlating with improved PFS and cause-specific survival [40].

Effects on immune cell surface marker expression

RT has also been shown to upregulate PD-L1 expression in several cancer types, notably in MIBC [41, 42]. RT upregulated the expression of PD-L1 in the human HT1197 and the murine MB49 MIBC cells and PD-L1 blockade in an orthotopic MB49 model was associated with tumor growth delay following irradiation [41]. Interestingly, when specimens from MIBC patients treated with chemoradiation were analyzed, high PD-L1 expression correlated with higher clinical stage, lower complete response rate and reduced disease-free survival. There was also a positive correlation between PD-L1 overexpression and lymph nodes metastases or loco-regional failure [41]. RT has also been shown to upregulate CTLA4 expression in T-reg cells [43].

Practical considerations of combining RT with ICIs in MIBC

Although there is a large body of evidence supporting the synergistic effect of immunotherapy and RT, many questions remain on how to optimally combine these two modalities. Studies emphasize the importance of the sequencing, total dose, fractionation, and target volumes in harnessing this synergy.

Sequencing

Pre-clinical studies have explored the optimal sequencing of RT and immunotherapy in eliciting a synergistic immune response. In a colorectal cancer mouse model, Young et al. [44] showed that anti-CTLA4 was most effective when given 7 days prior to RT versus one day or one week after. Interestingly, anti-OXO was most effective
when delivered one day post RT, highlighting the nuances in optimally combining RT with different immunotherapy regimens [44]. While some pre-clinical data show an increase PD-L1 expression and improved survival when RT was given concurrently with ICIs [41, 44], we were unable to show any difference in tumor growth rate inhibition when ICI was given either neoadjuvantly, concomitantly or adjuvantly with TMT (Tholomier et al. [45], in press). In contrast, in Sundahl et al. phase I trial of metastatic MIBC, ORR was 44.4% in the concomitant Pembrolizumab-SBRT vs. 0% in the sequential arm [29]. Ongoing clinical trials are evaluating combined immunotherapy with TMT in MIBC with various administration schedules: SWOG 1806 (NCT03775265) and KEYNOTE-992 (NCT04241185) are assessing concurrent chemoradiotherapy with atezolizumab or pembrolizumab, the CCTG BL13 study (NCT03768570) is evaluating adjuvant durvalumab after TMT, whereas the soon to open UK trial will examine neoadjuvant durvalumab followed by TMT.

**Doses and fractionation**

Different RT fractionation schemes and doses have been shown to have various immunomodulatory effects, either favoring immunostimulation or immunosuppression [46]. Suppressor T-cells are particularly radiosensitive whereas macrophages and regulatory T-cells are more radioresistant [23, 46]. This poses a challenge in normalizing response to treatment as RT doses and techniques can vary, ranging from delivering a single fraction to a metastatic deposit to a more protracted course of several weeks of conventionally or hypofractionated RT [47].

Pre-clinical studies have shown that dose per fraction greater than 6–8 Gy are required to produce an effective immunogenic response [22, 46, 48]. Furthermore, most of the studies describing an abscopal effect used SBRT or SBRT-like dose regimens (doses per fraction of ≥ 6 Gy) [49]. The abscopal effect also seems to be related to the fractionation used. In many tumor types, a multi-fractionated regimen was superior to single dose regimens in decreasing tumor growth at non-irradiated sites [18, 48]. In a mouse model of breast and colon cancer, while all fractionations were effective at controlling the primary irradiated tumor, only the multi-fractionated regimens (8 Gy x 3 fractions or 6 Gy x 5 fractions), but not the single dose regimen (20 Gy/1 fraction), synergized with anti-CTLA4 to decrease distant tumor growth [48]. Specifically in bladder cancer mouse models, ICIs were more effective when combined with a 10 Gy x 2 [27] or 6.25 Gy x 2 [45] RT regimens than with a 10 Gy x 1 regimen. In the clinical setting, establishing the ideal RT dose and fractionation when combined with immunotherapy remains a challenge requiring further evaluation.

**RT volume and sites of disease**

RT could be delivered to the whole pelvis, to the bladder only, bone metastases or visceral metastases. In the context of TMT, it would be intuitive to treat the gross tumor disease ± whole bladder. However, it remains unanswered whether pelvic elective nodal irradiation (ENI) could directly or indirectly affect the immune response. Preclinical data suggest that ENI can decrease the synergy between RT and ICIs likely by inhibiting the antigen-presentation process within the TME and in nearby draining lymph nodes. In a mouse model of colorectal or melanoma tumors treated with ICIs and 12 Gy in one fraction to the tumor ± draining lymph nodes, ENI attenuated immune cell infiltration, chemokine expression and intratumoral antigen-specific CD8+ T-cells, thus decreasing the synergistic effect between RT and ICIs [50]. ENI also adversely affected survival when combined with ICIs [50]. Other studies have shown a strong correlation between the RT volume and RT-induced lymphopenia [30, 33]. Thus, to enhance the synergistic effect between RT and ICIs, target volumes not involving the pelvic lymph nodes may be preferable when combining RT with immunotherapy in the localized MIBC setting since indirect irradiation of bone marrow structures during ENI could induce lymphopenia. To our knowledge, there are no clinical trials currently addressing this question in MIBC.

In the metastatic setting, a relevant question is which metastatic site to irradiate if several are present. Most reported cases of the abscopal effect involved RT to visceral metastases [25], suggesting that visceral sites may be more immunogenic than osseous sites; although direct comparative studies are lacking. In a recent review, Brooks et al. [51] proposed the provocative idea that the single-site irradiation abscopal approach should be abandoned to the benefit of comprehensive multi-sites irradiation when combining RT and ICIs. They formulated the hypothesis that irradiating multiple sites of disease reduces tumor burden while also increasing the likelihood of exposure and priming to the desired tumor-associated antigens. This would circumvent the inhibitory effects of the TME within each individual tissue bed, thus increasing the probability of activation of the anti-tumor immune process. Recent clinical trials studying ICIs in combination with multi-site irradiation support this hypothesis [52, 53]. Randomized trials comparing single to multi-site irradiation and stratifying patients with limited and extensive metastatic burden are needed.
Toxicities

The adverse effects (AEs) associated with ICIs use (irAEs) and their management are well documented [54]. RT-related AEs are thought to be in part related to the immune system response, mostly through its effects on pro-inflammatory and fibrogenic cytokines [55]. There are concerns that the combination of RT and immunotherapy could lead to a cumulative toxicity profile. The safety considerations related to the combination of RT with ICIs in solid cancers have been reviewed elsewhere, with grade ≥ 3 irAEs ranging from 7–31% across studies [56].

In the treatment of localized MIBC, acute AEs are mostly related to the combination of pelvic irradiation and concomitant chemotherapy. These, most commonly, include gastrointestinal (GI) and genito-urinary (GU) AEs. Acute GU AEs range from 4 to 21% across studies, whereas acute GI AEs range from 2 to 21% [1]. Late grade 3 pelvic toxicities occur in 2–7% of patients [47, 57]. The use of hypofractionated RT can also lead to more GI toxicity in the TMT setting [47]. Recently, a phase I trial evaluated the safety of concomitant intravenous Atezolizumab (anti-PDL-1) in combination with hypofractionated TMT in patients with T2–T4aN0M0 MIBC (NCT03620435, Table 3). The study closed prematurely due to unacceptable grade 3 GI toxicity in 50% of the patients (Table 4) [58]. In addition, Tree et al. also reported unacceptable toxicity when using pembrolizumab and weekly hypofractionated RT for metastatic or locally advanced MIBC in the phase 1 PLUMMB trial (NCT02560636) [59]. The trial was stopped for amendment after two out of five patients developed grade 3 GU AEs and one experienced grade 4 rectal perforation (Table 4) [59]. Thus, caution should be taken when ICIs are given concurrently with hypofractionated RT. Since the sequencing of TMT and immunotherapy does not appear to affect efficacy in MIBC [45], and in light of acute toxicity concerns presented herein, currently we favor neoadjuvant or adjuvant immunotherapy.

Finally, it is important to note that the toxicity of combined ICIs and RT could be enhanced when chemotherapy is used in the context of TMT. In metastatic MIBC, RT delivered to visceral metastases, such as the lungs or liver could also yield different irAEs, including pneumonitis, hepatitis or hematologic toxicities [54, 56]. Of course, the relative sensitivity of the irradiated organ and the technique/dose used will also impact on the toxicity profile.

Perspectives

Through its immunomodulatory capability, RT is being studied as a targeted therapy modality that can enhance systemic tumor control. A search of the ClinicalTrials.gov database as of March 31st, 2020 showed 615 ongoing clinical trials combining immunotherapy and RT, of which 24 are in MIBC patients. Several trials are looking into combined immunotherapy and RT in the locally advanced or metastatic setting (Table 2). Other studies are investigating combined ICIs and RT either in the neoadjuvant setting or concurrently with TMT as a bladder-preserving approach (Table 3). The use of ICIs as maintenance treatment after TMT is also being studied in patients that cannot undergo salvage radical cystectomy. These trials may improve outcomes in MIBC and broaden treatment options for patients, particularly for the non-negligible proportion who are too frail to either undergo chemotherapy or surgery.

Conclusion

The accumulating pre-clinical and clinical body of evidence reviewed in this article supports the hypothesis that through its cytotoxic and immunotherapy effects, RT has the potential to synergize with ICIs to improve oncological outcomes in patients with localized or metastatic MIBC. Increased toxicity might be challenging especially when combining ICIs and hypofractionated RT regimens. The many ongoing clinical trials on the subject will help answer many practical questions related to RT scheduling, dose, fractionation, and targets for RT. Undoubtedly, well-designed randomized trials are warranted in this newly developing field with special attention given to how effectively and accurately measure treatment response.
| Study | Phase | Status/estimated Enrollment | Eligibility | Intervention | Details | Outcomes measured |
|-------|-------|-----------------------------|-------------|--------------|---------|-------------------|
| NCT03775265 (SWOG/ NRG-1806) | III Recruiting | 475 | T2–T4a MIBC | Randomizing patients to chemoRT vs chemoRT and concurrent atezolizumab | Daily 3DCRT or IMRT over 7 weeks with concurrent chemotherapy (gemcitabine, cisplatin or 5FU/mytomycin) at physician’s discretion. Atezolizumab on day 1 of chemo and q3 weeks x 6 months | Primary: BI-EFS Secondary: OS, modified BI-EFS, biopsy, CR duration, PFS, MFS, CSS, QoL |
| NCT04241185 (MK-3475–992/KEYNOTE-992) | III Recruiting | 636 patients | T2–T4 N0M0 MIBC | Randomizing patients to pembrolizumab with CRT or CRT alone | Pembrolizumab q6 weeks + CRT at investigator’s choice. RT: 64 Gy/32 to bladder only, 64 Gy/32 to bladder + pelvis or 55 Gy/25 fractions to bladder only. Chemo: cisplatin, gemcitabine, 5FU, mytomycin-C | Primary: BI-EFS Secondary: OS, MFS, time to cystectomy, time to occurrence of NMIBC, AEs rate, tolerability, QoL and function outcomes |
| NCT02621151 | II Recruiting | 54 patients | T2–T4a N0M0 MIBC | Pembrolizumab, gemcitabine, and hypofractionated RT | Lead-in single dose pembrolizumab then TURBT EBRT: 52 Gy/20 fractions + concurrent gemcitabine and pembrolizumab q3 weeks starting on day 1 of RT | Primary: 2 year BI-DFS Secondary: AEs, CR, OS, MFS |
| NCT03617913 | II Active, not recruiting | 27 patients | T2–T4a N0M0 MIBC | Avelumab, RT and mitomycin-C/5FU or cisplatin chemotherapy | Avelumab q2 weeks x 10 cycles maximum. ChemoRT to start 29 days after avelumab | Primary: CR Secondary: AES, patient reported outcomes, PFS, RFS |
| NCT02662062 (PCR-MIB) | II Recruiting | 30 patients | T2–T4a N0M0 MIBC | Pembrolizumab, cisplatin and RT | RT: 64 Gy/32 fractions over 6 weeks with cisplatin given concurrently weekly. Pembrolizumab given concurrently with RT q3 weeks | Primary: grade 3–4 AEs Secondary: best response, rate metastases, salvage cystectomy |
| Study                  | Phase | Status/estimated Enrollment | Eligibility                  | Intervention                                                                                                                                   | Details                                                                                                                                                                                                 | Outcomes measured                                                                 |
|-----------------------|-------|-----------------------------|------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| NCT03620435           | II    | Closed 25 patients          | T2–T4 N0M0 MIBC             | Concurrent atezolizumab with gemcitabine and RT after TURBT (TMT)                                                            | Atezolizumab 1200 mg IV q3 weeks concurrently with TMT and adjuvant for up to one year IMRT: 50 Gy/20 fractions over 4 weeks Gemcitabine: 100 mg/m² q week × 4 weeks | Primary: DLT in stage 1, safety (grade ≥ 3 iRAEs or TRAEs) Secondary: OS, CR, QoL |
| NCT03844256 (CRMI)    | I/I   | Recruiting 50 patients      | T2–T4a N0–1, M0 MIBC       | Nivolumab or nivolumab and ipilimumab with mitomycin-C/capecitabine concurrent chemoRT                                             | RT: 40 Gy in 20 fractions with mitomycin-C/capecitabine Nivolumab q4 weeks vs nivolumab + ipilimumab q3 weeks | Primary: AEs, DLT, DFS, DFS rate Secondary: OS, OS rate, RR |
| NCT04216290 (INSPIRE) | I     | Not yet recruiting 114 patients | Any T, N1–2, M0 MIBC     | ChemoRT vs chemoRT + durvalumab     | RT over 6–8 weeks Durvalumab and chemotherapy 4 days before or after RT start                                      | Primary: clinical CR Secondary: MFS, B1-EFS, CSS, OS, PFS, CR duration, salvage cystectomy rate, AEs |
| Combined immunotherapy and RT in the neoadjuvant setting |       |                              |                              | Neoadjuvant nivolumab with RT before radical cystectomy                                  | Nivolumab q2 weeks, starting one week before RT RT: 45 Gy/5 fractions with 5, 4 Gy/3 boost Radical cystectomy with PLND at week 11–15 | Primary: completion rate Secondary: AEs, DFS, OS, ORR, pCR, R0/R1/R2 rates |
| NCT03529890 (RACE-IT) | II    | Recruiting 33 patients      | cT3–4N0/+ MIBC             | Concurrent nivolumab and RT followed by nivolumab monotherapy                                      | Nivolumab q2 weeks for up to 6 months RT to start on day 3, RT over 32–35 fractions on weeks 1, 3, 5, 7 and 9 | Primary: PFS Secondary: AEs, ORR, MFS, OS, QoL, PD-1/PD-L1 expression, cytokines profile |
| NCT03421652 (NUTRA)  | II    | Recruiting 34 patients      | T2–T4b N0/+ M0 MIBC       | Concurrent avelumab and RT                                                             | Avelumab q2 weeks with 6 doses with concurrent RT fractionation regimen at discretion of radiation oncologist | Primary: clinical CRR Secondary: OS, PFS, MFS, LRR, QoL |
| NCT03747419           | II    | Recruiting 24 patients      | T2–T4, N0M0 MIBC          | Concurrent atezolizumab with RT                                                        | Durvalumab + tremelimumab with concurrent RT                                                                 | Primary: pathological response (≤ cT1) Secondary: bladder preservation rate, salvage cystectomy, B1-EFS, DFS, OS, AEs |
| NCT03702179 (IMMUNO PRESERVE) | II    | Recruiting 32 patients      | Patients with localized MIBC treated with bladder preservation intent | TURBT followed by durvalumab + tremelimumab q4 weeks for 3 cycles RT 2 weeks after initiation of IO and concurrently: 64–66 Gy to the bladder and 46 Gy to the pelvis | Durvalumab + tremelimumab with concurrent RT |
Table 3 (continued)

| Study                      | Phase | Status/estimated Enrollment | Eligibility                      | Intervention                                                                 | Details                                                                                                                                                                                                 | Outcomes measured                                      |
|----------------------------|-------|-----------------------------|----------------------------------|-------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|
| NCT02891161 (DUART)       | I/II  | Active, not recruiting      | 42 patients                      | Concurrent durvalumab and RT followed by durvalumab monotherapy               | Durvalumab ×2 doses q4 weeks concurrent with RT 64.8 Gy/36 fractions daily Adjuvant durvalumab to start 3–4 weeks post concurrent durvalumab and RT                                                                 | Primary: DLT, PFS, disease control rate Secondary: CR, OS, PD-L1 expression |
| NCT03697850 (GETUG-35     | II    | Suspended due to the Covid-19 pandemic | 77 patients                      | Adjuvant atezolizumab after TURBT and ChemoRT                                | Atezolizumab q3 weeks for 12 months beginning 30 days (± 5 days) after TURBT and chemoRT                                                                                                              | Primary: DFS Secondary: LC, DFS, OS, AEs, QoL          |
| BladderSpar)              |       |                              | pT2–T3, MIBC                     | Adjuvant nivolumab after TURBT and ChemoRT                                   | Nivolumab q4 weeks until disease recurrence or unacceptable toxicity for a maximum of 12 treatments                                                                                                       | Primary: 2-year FFS Secondary: FFSIB, AEs, QoL, LC, distant FFS, OS |
| NCT03171025 (NEXT)        | II    | Recruiting                  | 28 patients                      | Adjuvant immunotherapy after TMT in patients ineligible for or refusing cystectomy | Randomizing patients treated with TMT to adjuvant durvalumab or surveillance                                                                                                                        | Primary: DFS Secondary: RR, LRC, patters of recurrence, OS, BI-DFS, MFS, AEs, QoL, cost-effectiveness |
| (BL13)                    |       |                              | T2–T4a N0–2, M0 MIBC            |                                                                               | Bladder only: 64–66 Gy in 32–33 fractions; 50–55 Gy in 20 fractions using IMRT Pelvis and bladder: 45–46 Gy to pelvic nodes +17–20 Gy bladder boost in 33–35 fractions Durvalumab w4 weeks for 12 months                                                                 | Primary: DFS Secondary: RR, LRC, patters of recurrence, OS, BI-DFS, MFS, AEs, QoL, cost-effectiveness |

TMT trimodal therapy, **TURBT** transurethral resection of the bladder tumor, **RT** radiotherapy, **chemoRT** chemoradiotherapy, **IO** immunotherapy, **PLND** pelvic lymph node dissection, **MIBC** muscle-invasive bladder cancer, **RCC** renal cell carcinoma, **EBRT** external beam radiotherapy, **3DCRT** 3D conformal radiotherapy, **IGRT** image-guided radiotherapy, **IMRT** intensity modulated radiotherapy, **SBRT** stereotactic body radiotherapy, **MTD** maximum tolerated dose, **AEs** adverse events, **irAEs** immune related adverse events, **TRAEs** treatment-related adverse events, **ORR** overall response rate, **CRR** clinical response rate, **PFS** progression-free survival, **OS** overall survival, **DFS** disease-free survival, **RFS** recurrence-free survival, **MFS** metastasis-free survival, **RR** recurrence rate, **LRC** locoregional control, **BI-EFS** Bladder intact event-free survival, **QoL** quality of life
Table 4 Published studies on the safety of combined radiotherapy and immunotherapy in muscle-invasive bladder cancer

| Study                | Study characteristics                                                                 | Intervention                                                                 | Safety outcomes                                                                 | Type of toxicities including those that were not DLT (n)                        | References |
|----------------------|----------------------------------------------------------------------------------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------------|------------|
| NCT02560636 (PLUMMB trial) | Phase I trial involving 5 patients in first cohort with locally advanced or metastatic MIBC (T2–T4, N0–3, M0–1) | Pembrolizumab 2 weeks before weekly hypofractionated RT (24 Gy/6 vs 24 Gy/4 vs 30 Gy/5 fractions) | 2/5 patients met the predefined definition of dose-limiting toxicity Trial was stopped and RT doses reduced | G4 bowel perforation (1) G3 non-infective cystitis (1) G3 urinary tract/bladder infection (2) G3 hematuria (1) G3 urinary pain (1) G3 fatigue (1) G2 urinary urgency, incontinence (1) G2 pain (1) G2 anemia (1) | [59]       |
| NCT03620435          | Phase I trial TMT in first cohort of 8 patients with T2–T4a N0M0 MIBC                 | Concurrent atezolizumab with gemcitabine and hypofractionated RT (50 Gy/20 fractions) after TURBT (TMT) | Study stopped after 50% of patients experienced grade 3 GI toxicities despite atezolizumab dose reduction. No grade 4 toxicity | G3 colitis (3) G3 proctitis (1) G3 lymphopenia (1) G3 neutropenia (1) | [58]       |

DLT dose-limiting toxicity. MIBC muscle-invasive bladder cancer, RT radiotherapy, G grade, TURBT transurethral resection of the bladder tumor, TMT trimodal therapy

*Happened outside of the DLT window, i.e. 11 weeks post completion of radiotherapy, thus considered at least subacute. All other toxicities are considered acute unless otherwise stated.

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Compliance with ethical standards

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References

1. Ploussard G et al (2014) Critical analysis of bladder sparing with trimodality therapy and muscle-invasive bladder cancer: a systematic review. Eur Urol 66(1):120–137
2. Efstathiou JA et al (2012) Long-term outcomes of selective bladder preservation by combined-modality therapy for invasive bladder cancer: the MGH experience. Eur Urol 61(4):705–711
3. James ND et al (2012) Radiotherapy with or without chemotherapy in muscle-invasive bladder cancer. N Engl J Med 366(16):1477–1488
4. Giacalone NJ et al (2017) Long-term outcomes after bladder-preserving tri-modality therapy for patients with muscle-invasive bladder cancer: an updated analysis of the Massachusetts general hospital experience. Eur Urol 71(6):952–960
5. von der Maase H et al (2005) Long-term survival results of a randomized trial comparing gemcitabine plus cisplatin, with methotrexate, vinblastine, doxorubicin, plus cisplatin in patients with bladder cancer. J Clin Oncol 23(21):4602–4608
6. Lenfant L et al (2020) Current status and future directions of the use of novel immunotherapeutic agents in bladder cancer. Curr Opin Urol 30(3):428–440
7. Vera-Badillo FE, Tannock IF, Booth CM (2019) Immunotherapy for urothelial cancer: where are the randomized trials? J Clin Oncol 37(29):2587–2591
8. Rosenberg JE et al (2016) Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 387(10031):1909–1920
9. Bellmunt J et al (2017) Pembrolizumab as second-line therapy for advanced urothelial carcinoma. N Engl J Med 376(1):1590–1598
10. Sharma P et al (2016) Nivolumab monotherapy in recurrent metastatic urothelial carcinoma (CheckMate 032): a multicentre, open-label, two-stage, multi-arm, phase 1/2 trial. Lancet Oncol 17(11):1590–1598
11. Powles T et al (2017) Efficacy and safety of durvalumab in locally advanced or metastatic urothelial carcinoma: updated results from a phase 1/2 open-label study. JAMA Oncol 3(9):e172411
12. Patel MR et al (2018) Avelumab in metastatic urothelial carcinoma after platinum failure (JAVELIN solid tumor): pooled results from two expansion cohorts of an open-label, phase 1 trial. Lancet Oncol 19(1):51–64
13. Suzman DL et al (2019) FDA approval summary: atezolizumab or pembrolizumab for the treatment of patients with advanced urothelial carcinoma ineligible for cisplatin-containing chemotherapy. Oncologist 24(4):563–569
56. Hwang WL et al (2018) Safety of combining radiotherapy with immune-checkpoint inhibition. Nat Rev Clin Oncol 15(8):477
57. Efstathiou JA et al (2009) Late pelvic toxicity after bladder-sparing therapy in patients with invasive bladder cancer: RTOG 89-03, 95-06, 97-06, 99-06. J Clin Oncol 27(25):4055
58. Marcq G et al (2020) MP61-08 A phase I/II trial of transurethral surgery followed by a combination of atezolizumab an ANTI-PDL-1 (MPDL3280A) with trimodal therapy in patients with muscle-invasive bladder cancer. J Urol 203(Supplement 4):e938–e938
59. Tree AC et al (2018) Dose-limiting urinary toxicity with pembrolizumab combined with weekly hypofractionated radiation therapy in bladder cancer. Int J Radiat Oncol Biol Phys 101(5):1168–1171

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