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REVIEW

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State of the art of adjuvant immunotherapy in urothelial cancer: New developments and upcoming changes

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ABSTRACT

In recent years, several clinical trials focused on the potential role of immune-checkpoint inhibitors (ICIs) in the adjuvant treatment of muscle-invasive urothelial cancer (UC). Heretofore, only the anti-programmed death protein 1 (anti-PD1) nivolumab received European Medical Agency (EMA) approval for cisplatin-unfit patients. In our work, we deeply analyzed the results of the three pivotal studies in view of the rapidly evolving therapeutic advanced UC's scenario. Furthermore, there are several ongoing research to investigate ICIs and other emerging immune agents in this setting; results are awaited. Additionally, current efforts have been made to assess the role of these agents in earlier disease settings, particularly in high-risk non-muscle-invasive bladder cancer (NMIBC). In our review, we analyzed the potential role of predictive and/or prognostic biomarkers that may improve patient selection and treatment efficacy. To conclude, we highlighted the upcoming changes that could redefine the standard of care for patients with early-stage UC.

PLAIN LANGUAGE SUMMARY

This review discusses the latest advancements in post-surgery immune treatments given to prevent recurrences from urothelial cancer. Urothelial cancer is the most frequent type of tumor that affects the bladder and the upper parts of the urinary tract. Immunotherapy is a treatment that helps the body's immune system to fight cancer cells more effectively. Unlike other treatments – such as chemotherapy – that directly kills tumor cells, immunotherapy boosts the natural defenses of the body to attack the cancer. Our article provides a summary of the current state of adjuvant immunotherapy, with the most recent progress and the ongoing clinical trials based on immune checkpoint inhibitors (ICIs). These agents help the immune defenses to recognize and attack cancer cells by blocking specific proteins that prevent the immune system from working properly. Additionally, the review analyzes the innovative drugs that are being developed for earlier stages of the disease, especially for non-muscle-invasive bladder cancer (NMIBC), a type of tumor that has not spread into the muscle layer of the bladder wall. Furthermore, our work also examines potential biological indicators, known as biomarkers that can help clinicians to identify which patients are most likely to benefit from specific treatments, making therapies more effective and personalized. Finally, the article looks at how ongoing research and recent approval of new treatments could lead to significant changes in clinical practice, potentially setting up new standards for treating patients with early-stage urothelial cancer.

Introduction

Urothelial cancer (UC) is the 10th most common type of neoplasia worldwide, with more than 600.000 estimated new cases of bladder cancer (BC) in 2022.[1] In the last few years, astonishing innovations rapidly changed the therapeutic scenario, including the approval of several immune-checkpoint inhibitors (ICIs) and antibody-drug conjugated (ADCs) among different disease settings.[2,3] Despite these great strides, UC has been responsible for more than 200.000 deaths for BC in 2022 globally.[1]

Focusing on localized disease, non-muscle invasive bladder cancer (NMIBC) is one of the most common malignancies, accounting for about 75% of all BCs.[4] NMIBC is generally managed with endoscopic resection (with proven better outcomes for en-bloc transurethral resection of bladder tumor) and intravesical treatments; for patients with disease recurrence or refractory to prior interventions, radical cystectomy (RC) still represents the standard of care (SoC), even though several ongoing trials are trying to modify this assumption.[5–9] Tumors invading the detrusor muscle are named muscle-invasive bladder cancer (MIBC) and represent approximately 25% of all UCs

cases.[4] MIBC has an intrinsic propensity to become metastatic and it is generally associated with a poorer prognosis compared to NMIBC, especially in the case of lymph nodes involvement.[10] The current SoC for patients with MIBC involves the use of cisplatin-based neoadjuvant chemotherapy (NAC), followed by RC with lymph nodes dissection.[11–13] The benefits of pre-surgical medical treatment account for a 35–40% rate of pathologic complete response (pCR) and a 5–8% absolute gain in terms of overall survival (OS) at five.[14,15] Regarding the best combination strategy in the neoadjuvant setting, the GETUG/AFU V05 VESPER trial showed increased outcomes with ddMVAC regimen (dose-dense methotrexate, vinblastine, adriamycin and cisplatin) compared to gemcitabine-cisplatin, which, nonetheless, still remains a valid option considering the more manageable toxicity profile.[16,17] Despite this solid evidence, NAC remains underutilized in clinical practice, particularly in elderly patients.[18] One of the critical issues in the management of early-stage MIBC is to determine which patient may benefit the most from NAC, due to the lack of selection criteria and validated predictive factors of response, that may avoid unnecessary toxicity in potentially non-responders patients.[19] As widely known, comorbidities – such as renal impairment – do not allow the use of cisplatin-based regimen in about 50% of patients, leading them to undergo upfront RC.[20] Moreover, in this scenario, several trials already proved the activity of ICIs in the neoadjuvant setting, showing encouraging pCR, and many are ongoing to assess their role in the future clinical practice, both alone or in combination with ADCs.[21–23]

On the other hand, the role of adjuvant chemotherapy following upfront RC is still controversial, due to the lack of solid-randomized prospective data. In fact, no randomized clinical trial (RCT) has shown a clinically significant survival difference for patients with BC.[24–26] Nevertheless, a recently updated meta-analysis based on 10 RCTs demonstrated an improvement in terms of absolute improvement in survival of 6% at 5 years.[27] The only glimmer of light came from the randomized phase III POUT trial in UTUC patients, that showed a significant improvement in terms of disease free survival (DFS) for gemcitabine-platinum combination compared to observation alone (62% vs 45%, hazard ratio – HR 0.55, 95% confidence interval – CI 0.38–0.80). [28]. Furthermore, a statistically significant superior 5-year OS was seen in univariable analysis (66% vs 57%, HR = 0.68, 95% CI 0.46–1.00, p = .049), although the multivariate model did not show the same statistically significant benefit but only a positive trend in favor of adjuvant chemotherapy.[28] In view of these contrasting results, international guidelines suggest the use of platinum adjuvant therapy with limited evidence for BCs (and currently not recommended after NAC) and with a larger consensus for UTUCs.[11] Undiscussed is the role of ICIs in the current treatment scenario of advanced UCs, both as a second-line strategy or as a maintenance therapy.[29,30] Moreover, immunotherapy now represents also the cornerstone of first-line treatment, according to the amazing results of the EV-302 trial, showing increased outcomes with the combination of pembrolizumab plus enfortumab vedotin (EV) compared to platinum-based chemotherapy, and CheckMate-901 study, investigating cisplatin-based chemotherapy in combination with nivolumab.[31,32] With this background, it is immediately clear how difficult the therapeutic choices will be in view of this rapidly evolving therapeutic scenario. With these premises, the aim of our review of the literature is to focus on the controversial results of the RCTs exploring adjuvant ICIs, with particular emphasis on the therapeutic impact in view of the first-line upcoming changings. Indeed, we deeply analyzed the role of potential predictive biomarkers of immunotherapy response, which represent an urgent clinical need in this disease setting. Furthermore, we will give an overview of novel possible immunological adjuvant strategies in MIBC, with a brief focus on the promising novel immune compounds also explored in the earlier stages of disease (high-risk BCG-unresponsive NMIBC).

The dawn of immunotherapy in bladder cancer

Intravesical therapy with Bacillus of Calmette-Guerin (BCG) was first introduced in 1976, long before modern ICIs, as a pioneer immunotherapy for BC.[33] BCG is a live attenuated strain of *Mycobacterium Bovi*s, mainly used as a vaccine against tuberculosis. Its antitumor activity in bladder cancer consists of different mechanisms, still largely unexplored, such as a unique interaction with urothelial cells and activation of both the innate and the adaptive immune response.[34] Very interesting is the role of the adaptive immune system: T lymphocytes, key players with respect to B cells, seem to react both to BCG- and tumor antigens, eliciting a T-cell dependent tumor-specific immunity.[35] Nowadays, BCG has become a cornerstone of treatment for patients with intermediate and high-risk disease.[6] BCG treatment includes an induction phase and, subsequently, a maintenance period, with a duration of 1 and 3 years, respectively, to reach a consistent benefit in terms of DFS and recurrence rate.[36] According to a recent meta-analysis, BCG resulted to be superior in terms of reduction of recurrence rate and time to recurrence with respect to chemotherapy with mitomycin-C.[37] Furthermore, when compared with intravesical epirubicin, BCG reduced rates of metastatic disease and demonstrated better OS and DFS.[38] On the other hand, BGC is not free of adverse events (AEs), although rare and temporary; the most frequent are dysuria, urinary frequency, and urgency. Much less often, systemic AEs occur, such as fever or skin rash. Compared to an induction course alone, BCG maintenance is not associated with a higher rate of AEs.[39] Nowadays, several upcoming trials are exploring the potential role of new immune-combinations to take BCG's undoubted results a step forward, as discussed later on.

Immune-checkpoint inhibitors in miBC: joys and sorrows

IMvigor010 trial: atezolizumab

The potential role of atezolizumab as an adjuvant treatment in high-risk muscle-invasive urothelial cancers has been investigated in IMvigor010 trial, published in 2021 by J. Bellmunt *et al* (Table 1).[40] This phase III multicentric study enrolled 809 patients affected predominantly by BCs (93% in the atezolizumab group and 94% in the observation group, respectively)

Table 1. Principal characteristics of the three pivotal trials of adjuvant immune-checkpoint inhibitors in urothelial cancers.

	ImVigor 010 [40, 41]	CheckMate 274 [43, 44]	Ambassador Alliance A031501 [46]
Phase	III	III	III
Publication	Lancet Oncology, 2021, J. Bellmunt et al	NEJM, 2021, D.F. Bajorin et al	ASCO Genitourinary Cancer Symposium, 2024, A. Apolo et al
Primary endpoints	DFS in ITT	DFS in ITT and DFS in PD-L1 CPS ≥ 1	DFS and OS
Investigated drug	atezolizumab	nivolumab	pembrolizumab
Target	PD-L1	PD-1	PD-1
Maximum time of treatment	1 year or up to 16 cycles	1 year	1 year or up to 18 cycles
Comparator arm	Observation	Placebo	Observation
Number of Patients enrolled (total)	809	709	702
Bladder and upper tract cancers (experimental vs comparator arm, %)	93% vs 94% 7% vs 6%	79% vs 78.9% 21% vs 21.1%	75.4% vs 75.9% 22.9% vs 20.7%
Prior neoadjuvant therapy (experimental vs comparator arm, %)	48% vs 47%	43.3% vs 43.5%	65.3% vs 62.6%
PD-L1 positivity status (experimental vs comparator arm, %)	48% vs 49% (PD-L1 IHC assay VENTANA, SP142, IC2/3)	39.7% vs 39.9% (PD-L1 IHC 28–8 pharmDx assay Dako, 22C3)	57.1% vs 57.8% (IHC assay Dako 22C3, CPS ≥ 10)
Positive nodal status, N+ (experimental vs comparator arm, %)	52% vs 52%	47.3% vs 47.2%	50.9% vs 48.8%
Median Follow-up (months)	21.9	36.1	22.3 for DFS 36.9 for OS
DFS (months), HR [CI 95%]	ITT population: 19.4 vs 16.6 0.89 [0.74–1.08] PD-L1 IC2/3 24.8 vs 41. 4 1.01 [0.76–1.35]	ITT population: 22.0 vs 10.9 0.71 [0.58–0.86] PD-L1 ≥ 1% population: 52.6 vs 8.4 0.52 [0.37–0.72]	DFS: 29.0 vs 14.0 0.69 [0.55–0.87]
OS (months), HR [CI 95%]	61.4 vs 59.0 HR 0.91 [0.73–1.13])		OS (i.a.): 50.9 vs 55.8 0.98 [0.76–1.26]
G3/4 adverse events (experimental vs comparator arm, %, according to CTCAE version 4.0)	37% (16% drug related) vs 20%	42.7% (17.9% drug related) vs 36.8%	48.4% vs 31.8%%

NEJM = New England Journal of Medicine, ASCO = American Society of Clinical Oncology, PD-1 = programmed death 1, PD-L1 = programmed death ligand 1, IC2/3 = tumor-infiltrating immune cell, DFS = disease free survival, OS = overall survival, ITT = intention to treat population, CPS = combined positive score, HR = hazard ratio, G = grade, CTCAE = Common Terminology Criteria for Adverse Events, i.a. = interim analysis, IHC = immunohistochemical, IC2/3 = defined as PD-L1- expressing tumor-infiltrating immune cells covering ≥ 5% of the tumor area.

and, to a lesser extent, by UTUCs (7% in the experimental arm and 6% in the comparator arm, respectively).[40] Patients were recruited after NAC in case of an unsatisfactory pathologic response (ypT2–4(a) or ypN+) or following upfront surgery, if pT3–4(a) or N+ and ineligible for or refused adjuvant platinum therapy.[40] Patients have been randomized 1:1 to receive the anti PD-L1 agent atezolizumab (1200 mg intravenously every 3 weeks up to 12 months or 16 cycles) or to undergo observation.[40] The primary endpoint was DFS in the intention-to-treat population (ITT); OS was a secondary endpoint.[40] Furthermore, programmed death ligand 1 (PD-L1) expression on tumor-infiltrating immune cells was one of the prespecified stratification factors (using the VENTANA SP142 immunohistochemical – IHC assay). Crossover between the two arms was not permitted.[40] At a median follow-up of 21.9 months, the DFS in the ITT population was 19.4 months in the atezolizumab group and 16.6 months in the observation group, with an HR of 0.89 (95% CI 0.74–1.08). According to the unsatisfactory result of its primary endpoint, the trial was considered negative. Furthermore, no prespecified subgroup showed DFS benefit with the anti-PD-L1 agent. At the time of the primary analysis, median OS in the ITT population was not reached[40]; moreover, at the updated data cutoff, median OS was not statistically improved with atezolizumab (61.4 months) versus observation only (59.0 months) (HR 0.91, 95% CI 0.73–1.13).[41] Interestingly, as if we will further

investigate in the following chapter, a subsequent analysis on 581 patients evaluable for circulating tumor DNA (ctDNA) proved that ctDNA positive patients at the start of the therapy had improved DFS and OS with atezolizumab.[42] The incidence of grade 3–4 AEs was 37% in the atezolizumab group compared to 20% in the observation group, according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.[40] Severe treatment-related adverse events (TRAEs) were 11% in the experimental group (the most frequent pyrexia).[40] There was one G5 event in the atezolizumab group due to acute respiratory distress. Common TRAEs of any grade in the experimental group included pruritus (24%), fatigue (24%), diarrhea (21%) and urinary tract infections (21%).[40] G3 or more immune-mediated AEs were 10% (3% of which required steroids therapy to recover) in the atezolizumab group and 4% in the observation group (1% of which required system corticosteroids).[40]

CheckMate 274 trial: nivolumab

The CheckMate 274 trial evaluated the efficacy of anti-PD-1 agent nivolumab in patients with high-risk MIBC after radical surgery (Figure 1, Table 1).[43] This phase III, double-blind, multicentric RCT involved 709 patients, randomly assigned 1:1 to receive either nivolumab (240 mg flat dose intravenously
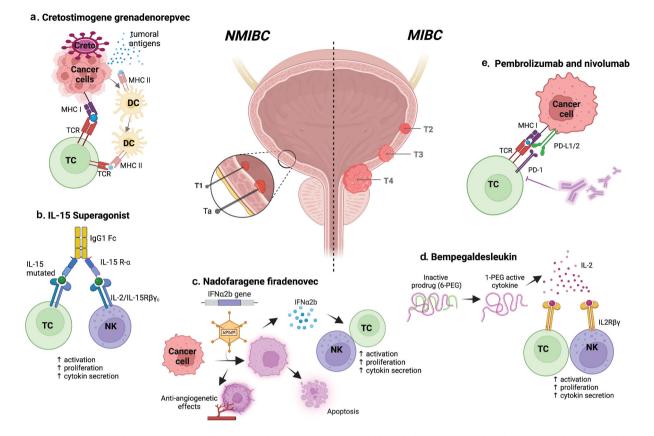
Figure 1. Immune agents tested in MIBC and NMIBC that proved positive evidence in published phase II and III clinical trials created with Biorender.com. (a) Cretostimogene grenadenorepvec. Cretostimogene is a modified oncolytic virus designed to target and destroy bladder cancer cells. Upon direct injection into the bladder, the adenovirus preferentially infects tumor cells with RB pathway alterations and replicates inside them, causing tumor lysis. This process releases new viral particles that can infect neighboring tumor cells. Additionally, the virus is engineered to stimulate an immune response against the cancer: the releasing of tumor antigen presented by DCs leads to the activation and recruitment of T cells that recognize and selectively destroy tumor cells. The combination of direct viral action and indirect immune system activation potentiates its global effect. (b) IL-15 Superagonist. Nogapendekin alfa-inbakicept (*N*-803) is a fusion complex of a dimeric human IL-15 Rα sushi domain/human IgG1 Fc. This protein has a IL-15 N72D mutation, specifically designed to enhance binding to IL-2/IL-15 Rβγc, leading to activation and proliferation of NK cells and T cells, without concomitant expansion of immunosuppressive T-reg cells. c. Nadofaragene firadenovec. Nadofaragene firadenovec works by internalization of an adenoviral vector into urothelial cancer cells. Inside the nucleus, the gene is transcribed into mRNA and subsequently translated into the IFNα2b protein, which is produced continuously. IFNα2b protein has both direct and indirect antitumor effects within the tumor environment. Direct effects consist of endoplasmic reticulum stress in tumor cells and increase expression of proteins that trigger cell death. Nadofaragene also has antiangiogenetic effects, causing tumor hypoxia and necrosis. Furthermore, it enhances the immune response by increasing the presentation of tumor antigens on the cell surface, making the cancer cells more recognizable to the immune system. Indirectly, IFNα2b stimulates the immune system by promoting the activation of cytotoxic CD8+ T cells and NK cells, able to target and kill cancer cells, particularly those with reduced MHC-I expression. d. Bempegaldesleukin PEGylated IL-2 is a modified variant of IL-2, administered as inactive prodrug (6-PEG), turned in vivo to the active cytokine form. The modification involves attaching PEG molecules to IL-2. This PEGylation increases the molecule's size, stability, and half-life in the bloodstream, while reducing its toxicity compared to unmodified IL-2. This molecule activates the IL-2 receptor pathway and selectively enhances expansion and proliferation of T cells and NK cells, minimizing the role of immunosuppressive T-reg cells. E. Pembrolizumab and nivolumab. Pembrolizumab and nivolumab are monoclonal antibodies that target PD-1 receptors on T cells. Normally, PD-1 binds to its ligands, PD-L1 or PD-L2, which are often overexpressed on tumor cells within the tumor microenvironment. This interaction inhibits T cell activity, allowing cancer cells to evade immune detection and destruction. Pembrolizumab and nivolumab block the binding between PD-1 and its ligands, allowing T cells to recognize and attack tumor cells more effectively. Pembrolizumab proved interesting results both in MIBC and NIMBC. Abbreviations: Creto = cretostimogene grenadenorepvec, MHC-I = major histocompatibility complex I, MHC-II = major histocompatibility complex II, TC = T-cell, RB= retinoblastoma, NK = natural killer, DC = dendritic cell, mRNA = messenger ribonucleic acid, IL-15 = interleukin 15, IL-15 Rα = interleukin 15 receptor alpha, IgG1 Fc = immunoglobulin gamma class 1 fragment crystallizable, IL-15 Rβγ = interleukin 15 receptor beta-gamma c chains, IL-2 = interleukin-2, T-reg = regulatory T cells, PEG = polyethylene glycol, programmed cell death protein 1 (PD1), MIBC = muscle invasive bladder cancer, NIMBC = non-muscle invasive bladder cancer, PD-1 = programmed death cell-1, PD-L1 = programmed cell death-ligand 1.

every 2 weeks up to 12 months) or placebo (every 2 weeks for up to 12 months).[43]

Most patients were affected by BCs (79% in the experimental arm and 78.9% in the comparator one) and a lower percentage by UTUCs (21% vs 21.1% for the experimental group and the placebo group, respectively).[43] In this study, patients were required to undergo surgery with negative margins (R0) and could have previously received NAC (43.3% in the experimental arm vs 43.5% in the placebo arm).[43] In the subgroup not exposed to prior NAC, patients were included if they had a pathological stage pT3-T4a or pN+ and if not eligible for or declined adjuvant platinum-based combination chemotherapy.[43] On the other hand, patients who received NAC were eligible if their pathological stage was ypT2-T4 or ypN+.[43] The co-primary endpoints were DFS in both the ITT population and in patients with tumor PD-L1 expression of ≥ 1% (on tumor cells with the use of Dako IHC 28–8 pharmDx assay).[43] Furthermore, OS and non – urothelial tract recurrence-free survival (NUTRFS) in both populations were secondary endpoints.[43] At the primary analysis, results showed that nivolumab significantly improved DFS compared to placebo in both groups.[43] The extended follow-up (31.6 months) data confirmed the benefit of adjuvant nivolumab: median DFS in the ITT population was 22.0 months in the nivolumab group versus

10.9 months in the placebo group (HR 0.71, 95% CI 0.58–0.86).[44] Among patients with PD-L1 expression 1% or more, the median DFS was 52.6 months with nivolumab, compared to 8.4 months with placebo (HR 0.52, 95% CI 0.37–0.72).[44] Interim OS results presented by Dr Matthew Galsky at the American Society of Clinical Oncology Genitourinary (ASCO GU) Meeting in 2024 showed a benefit for nivolumab group versus placebo in the ITT analysis (HR 0.76, 95% CI 0.61–0.96) and in PD-L1 > 1% populations (HR 0.56, 95% CI 0.36–0.86).[45] A continued follow-up of OS is ongoing. The consistency of DFS improvement was maintained across different subgroups including age, sex, performance status, nodal involvement and prior cisplatin-based treatment.[44] In August 2021 these excellent results led to Food and Drug Administration (FDA) approval of adjuvant nivolumab for UCs at high risk of recurrence after radical cystectomy. In connection with this, the European Medical Agency (EMA) restricted the indication for patients with PD-L1 expression ≥1% and pT3/4 and/or pN+ disease not eligible for, or who declined, adjuvant cisplatin-based chemotherapy (weak recommendation).[11] The incidence of grade 3–4 AEs was 18.2% in the nivolumab group compared to 7.2% in the placebo one, according to the CTCAE version 4.0.[44] Common TRAEs of any grade in the nivolumab group included pruritus (23.1%), fatigue (17.4%), diarrhea (16.8%), and rash (15.1%).[43] Three treatment-related deaths were reported in the nivolumab arm, two occurred due to pneumonitis and one due to bowel perforation.[44]

Ambassador (alliance A031501) trial: pembrolizumab

Ambassador study is an open-label phase III RCT analyzing the potential role of the anti-PD-1 pembrolizumab versus observations as an adjuvant 1-year treatment after radical surgery in miBC (Figure 1, Table 1).[46] Recruited patients were stage pT2 and/or node involvement or positive margins at surgery following NAC or, if NAC naive, they should be cisplatin-ineligible or refusing chemotherapy.[46] The 702 total patients enrolled were randomized 1:1 to receive either pembrolizumab (200 mg flat dose every 3 weeks for 12 months, up to 18 cycles) or observation.[46] It is important to underline that this RCT was closed early due to the FDA approval of adjuvant nivolumab for MIBC, as said before.[43] The double primary endpoints were DFS and OS in the ITT.[46] The population was stratified according to pathologic stage (nodes positive 50.9% in the experimental arm versus 48.8 in the observation group), PD-L1 status assessed as combined positive score with Dako IHC 22C3 pharmDx assay (57.1% vs 57.8%, respectively) and prior NAC (65.3% vs 62.6%, respectively).[46] Moreover, 75.4% of patients in the experimental group had BCs versus 75.9% in the observation group, while 24.6% vs 24.1% had UTUCs, respectively.[46] Dr Andrea B. Apolo presented the results of the interim analysis at the American Society of Clinical Oncology Genitourinary (ASCO GU) Meeting in January 2024: median DFS was 29.0 months with pembrolizumab versus 14.0 months with observation (HR 0.69, 95% CI 0.55–0.87), consistent across the subgroup analysis except for UTUCs

(HR 1.05, 95% CI 0.61–1.82).[46] On the other hand, median OS was 50.9 months with the anti-PD-1 and 55.8 months with observation (HR 0.98, 95% CI 0.76–1.26), still not yet demonstrating a benefit for adjuvant pembrolizumab.[46] Additional events are needed for the final analysis. Grade 3 AEs were observed in 48% and 32% of patients in the pembrolizumab and observation arms, respectively.[46] The most common TRAEs of any grade were fatigue (47%), pruritus (22%), diarrhea (21%), and hypothyroidism (20%).[46] Furthermore, PD-L1 status was a prognostic biomarker both for DFS and OS; unfortunately, it showed no predictive value for OS or DFS response.[46]

Upcoming news in MIBC adjuvant setting

Several ongoing phase III clinical trials are assessing the role of perioperative or adjuvant ICIs, alone or in combination with chemotherapy, ADCs or novel immunotherapy compounds, as we summarized in Table 2.

In particular, NIAGARA (NCT03732677) and Keynote-886 (NCT03924856) are evaluating the addition of durvalumab and pembrolizumab to chemotherapy as perioperative treatment; a recent press release of the NIAGARA study showed positive event-free survival (EFS) and OS for the combination treatment, suggesting future promising results (Table 2).[47]

KeynoteB15/EV304 (NCT04700124) and Keynote905/EV303 (NCT03924895) are investigating the perioperative combination strategy of pembrolizumab and the anti-nectina 4 ACD enfortumab-vedotin (EV), in a population of cisplatin-eligible and ineligible patients, respectively (Table 2). Analogously, VOLGA trial (NCT04960709) is assessing the potential role of durvalumab, either alone or with the addition of the anti-CTLA-4 tremelimumab, in combination with EV in a population of cisplatin unfit patients. Results of the three studies are currently awaited (Table 2).

CA045–009 is a phase 3 RCT analyzing the role of perioperative nivolumab plus bempegaldesleukin (arm A), versus nivolumab alone (arm B) versus radical surgery alone (arm C) in patients with MIBC who are cisplatin ineligible (NCT04209114). Bempegaldesleukin (also known as BEMPEG/NKTR-214) is an immunostimulatory IL-2 prodrug engineered to selectively bind the dimeric IL-2 receptor predominantly expressed on natural killer and CD8+ T cells (Figure 1, Table 2). This led to a selectively expansion and activation of these two cell types, without an undesidered proliferation of regulatory T cells (T regs) within the tumor microenvironment (TME).[49] The same combination explored in CA045009 showed promising results in a phase I/II study for cisplatin-ineligible patients affected by locally advanced/mUC.[50] Primary endpoints of trial CA045–009 were pCR and EFS among arm A and C, resulting in 10.8% vs 2.5% and 22.11 months vs 15.18 months, respectively (NCT04209114). Completely results are still awaited.

As we further analyze in Chapter 5, IMvigor011 (NCT04660344) and MODERN (NCT05987241) are biomarkers driven ongoing trials designed to assess the role of a restriction of the adjuvant ICI therapy to ctDNA positive patients (Table 2).

Table 2. Ongoing phase three clinical trials evaluating the role of adjuvant immune agents in high-risk muscle invasive urothelial cancers.

Study name	Study ID	Status	Sample size	Immune Therapeutic Agents	Arms	Primary Endpoints	Resulted posted
NIAGARA	NCT03732677	Active, not recruiting	1063 actual (1050 estimated)	Anti PD-L1 durvalumab	*Experimental:* cisplatin + gemcitabine + durvalumab (neoadjuvant) → adjuvant durvalumab *Control:* cisplatin + gemcitabine (neoadjuvant) → no adjuvant treatment	pCR EFS	Press release positive for EFS and OS (25 June 2024)[47]
Keynote886	NCT03924856	Active, not recruiting	907 actual	Anti PD-1 pembrolizumab	*Experimental:* 4 cycles Pembrolizumab + Gemcitabine + Cisplatin (neoadjuvant) → 13 cycles adjuvant pembrolizumab *Control:* 4 cycles Placebo + Gemcitabine + Cisplatin(neoadjuvant) → 13 cycles adjuvant placebo	EFS	None
KeynoteB15/ EV304	NCT04700124	Active, not recruiting	784 estimated	Anti PD-1 pembrolizumab	*Experimental ARM A:* 4 cycles Pembrolizumab + Enfortumab Vedotin (EV) (neoadjuvant) → 5 cycles adjuvant EV + 13 cycles adjuvant pembrolizumab *Control ARM B:* 4 cycles cisplatin + gemcitabine (neoadjuvant) → no adjuvant treatment	EFS	None
Keynote905/ EV303	NCT03924895	Active, not recruiting	857 estimated	Anti PD-1 pembrolizumab (cisplatin ineligible/ refused pts)	*Experimental ARM A:* 3 cycles Pembrolizumab (neoadjuvant) → 14 cycles adjuvant pembrolizumab *Experimental ARM C:* 3 cycles Pembrolizumab + EV (neoadjuvant) → 6 cycles adjuvant EV + pembrolizumab + 8 cycles pembrolizumab alone *Control ARM B:* surgery alone	EFS (arm C and B)	None
IMvigor011	NCT04660344	Active, recruiting	800 estimated	Anti PD-L1 atezolizumab (cisplatin ineligible/ refused pts)	*Experimental ARM A:* 12 cycles adjuvant atezolizumab (ctDNA - → + within 21 mo post cystectomy) *Control ARM B:* adjuvant placebo	DFS investigator-assessed	Analysis of ctDNA negative persistent: 17/171 events in DFS (mFUP 16.3 mo)[48]
MODERN	NCT05987241	Active, recruiting	1190 estimated	Anti PD-1 nivolumab Anti LAG-3 relatlimab (cisplatin ineligible/ refused pts)	*Cohort A:* ARM I: 12 cycles adjuvant nivolumab ARM II: 12 cycles of adjuvant nivolumab and relatlimab *Cohort B* ARM III: 12 cycles adjuvant nivolumab ARM IV: surveillance (ctDNA - → +) 12 cycles adjuvant nivolumab	*Cohort A phase II:* proportion of pts ctDNA- phase III: OS *Cohort B:* DFS	None
VOLGA	NCT04960709	Active, recruiting	677 estimated	Anti PD-L1 durvalumab Anti CTLA-4 tremelimumab (cisplatin ineligible/ refused pts)	*Experimental (ARM I):* 3 cycles Durvalumab + Tremelimumab + EV (neoadjuvant) → 1 cycle adjuvant tremelimumab and 9 cycles adjuvant durvalumab *Experimental (ARM II):* 3 cycles durvalumab + EV → 9 cycles adjuvant durvalumab *Control (ARM III):* adjuvant treatment according to approval (nivolumab or surgery alone)	EFS (ARM I vs ARM III and ARM II vs ARM III) Safety run-in part	None
CA045009/ 18-214-13	NCT04209114	Completed	114 actual	PEGylated IL-2 prodrug bempegaldesleukin (BEMPEG, NKTR-214) Anti PD-1 nivolumab (cisplatin ineligible pts)	*Experimental Arm A:* 3 cycles nivolumab + bempegaldesleukin (neoadjuvant) → 12 cycles adjuvant nivolumab + bempegaldesleukin *Experimental ARM B:* 3 cycles nivolumab (neoadjuvant) → 12 cycles adjuvant nivolumab *Control ARM C:* surgery alone	pCR EFS (ARM A vs ARM C)	10.8% vs 2.5% 22.11 mo vs 15.18 mo (*no publications available*)

pCR = pathologic complete response, EFS = event free survival, NCT = number of clinical trial, ID= identification, OS = overall survival, DFS = disease free survival, ctDNA = circulating tumor DNA, mFUP = median follow up, mo = months, pts = patients, LAG 3 = anti-Lymphocyte Activation gene 3, CTLA-4= Cytotoxic T-Lymphocyte Antigen 4, IL-2 = interleukin-2.

How to interpret the results of adjuvant ICIs trials in the current treatment scenario of MIBC

A hot topic of interest is to analyze the divergent results of the three pivotal and similar trials of adjuvant treatment (IMVigor010, CheckMate274, Ambassador) in the wider context of the actual therapeutic scenario of MIBC. In fact, as we previously stated, several studies showed promising insights with the use of ICIs in neoadjuvant disease setting.[21–23] Furthermore, in the previous paragraph, we gave an overview of the ongoing phase III trials evaluating the potential role of perioperative strategies (Table 2). Moreover, it should be considered also the upcoming role of ICIs in first-line therapy in light of the results of the EV-302 and CheckMate 901 trials, making the choice and the proper timing of immunotherapy use even more complex. To unravel this tangle, it may be useful to focus on the differences between the three pivotal adjuvant trials, although making cross-study comparisons is not formally correct. In particular, differently from the other two, the IMvigor010 study did not reach its primary endpoint.[40] The reasons why atezolizumab did not perform as well as the other two investigated ICIs is still a matter of debate. Nevertheless, it may be plausible that the high proportion of individuals with UTUCs, the type of compound used (anti PD-L1 vs anti-PD-1), the choice of the comparator arm (observation vs placebo) and the amount of nodes positive and NAC-pretreated patients may have enhanced its negative results.[51] Furthermore, an interesting meta-analysis of IMvigor010 and CheckMate-274 trial showed no significant difference in DFS in the experimental arms, but a significantly different outcome in the control arms, with more recurrences and shorter DFS in the placebo-treated patients.[52] Moreover, 40 patients discontinued treatment from IMvigor010 compared with only seven in CheckMate274.[40,43]

The lack of consistent DFS benefits and OS final outcomes, despite FDA and EMA nivolumab approval, make adjuvant ICIs not widespreadly used in clinical practice. Otherwise, a recent meta-analysis of the three RCTs highlighted a 23% overall benefit of adjuvant ICIs in reducing disease recurrence rates.[53] In particular, this DFS benefit resulted stronger among individuals exposed to prior NAC (HR 0.69, 95% CI 0.53–0.90) and with pathologic nodal involvement (HR 0.75, 95% CI 0.59–0.95).[53] On the other hand, no benefit in terms of DFS in patients with UTUCs has been found (HR 1.19, 95% CI 0.86–1.64), although nivolumab received approval despite disease location.[53] This latter evidence may support the use of cisplatin-based combination in UTUCs in eligible patients.[28] Although not statistically significant, the pooled analysis of OS showed a 13% survival benefit in favor of ICIs.[53]

On the basis of these assumptions, there is an urgent need for predictive biomarkers – as we further examine later – to better customize adjuvant immunotherapy according to patient's characteristics, in order to offer the best treatment strategy to the correct individual.

A look into the future: the role of novel immune therapies in NMIBC

Despite these promising results in the adjuvant setting, researchers are exploring several immunological strategies also in an earlier setting. Many studies are evaluating immune agents, either alone or in combination, in NMIBC. Some of these compounds have received FDA approval, as we will further examine, while other studies are ongoing with very promising insights. These recent breakthroughs represent a significant advancement, especially for patients with BCG-unresponsive NMIBC, who historically had limited therapeutic options beyond radical cystectomy.[54]

Nadofaragene firadenovec-vncg (Adstiladrin, also known as rAd-IFN/Syn3) is a replication-deficient recombinant adenovirus delivering human interferon alfa-2b cDNA into urothelial cells, which had been recently approved by FDA for adults with high-risk BCG-unresponsive NMIBC with carcinoma in situ (CIS), with or without papillary tumors (Figure 1).[7] The efficacy and safety of this novel intravesical agent were previously assessed in a multicentric, randomized, open-label, parallel arm phase II trial, comparing two different dose regimens (1×10^{11} viral particles (vp)/mL or 3×10^{11} vp/mL).[55] The approval of rAd-IFN/Syn3 is based on data from the phase III, multicentre, open-label repeat-dose trial (CS-003), in which patients received a single intravesical 75 ml dose of nadofaragene firadenovec every 3 months until disease recurrence.[7] The primary endpoint was complete response (CR) in individuals with CIS with or without concomitant Ta or T1 papillary disease; CR was 53.4% (95% CI 43.3%–63.3%) all noted at 3 months, with a median duration of response of 9.69 months.[7] According to the recently presented 5-year follow-up data, nadofaragene firadenovec-vncg allowed bladder preservation in nearly half of the patients at 60 months, with a cystectomy-free survival rate of 49% (40.0%–57.1%).[56] Safety profile was confirmed at the long-term follow-up with any G4 or G5 AEs registered.[56] G3 toxicity occurred in 3.8% of patients, most commonly micturition urgency (1.3%).[56] Common G1 and G2 AEs included instillation site discharge (25%), fatigue (20%), and bladder spasms (16%).[56]

The potential role of pembrolizumab monotherapy was assessed in the KEYNOTE-057 trial in a population of BCG-unresponsive high-risk NMIBC patients, not eligible for or refusing RC (Figure 1).[8,9] This phase II, multicentre, single-arm study included: cohort A, composed of individuals with CIS (with or without papillary tumor) and cohort B, involving patients with papillary tumors without CIS. In cohort A, the primary endpoint was clinical CR achieved in 41% (95% CI 30.7%–51.1%) of patients after 3 months.[8] Notably, 46% of responders patients maintained CR at 12 months.[8] These findings led to FDA approval of pembrolizumab for the treatment of patients with BCG-resistant high-risk NMIBC with CIS (with or without papillary tumors) who are either ineligible for or have elected not to undergo RC. In cohort B, DFS at 12 months was 43.5% (95% CI 34.9%–51.9%) in high-risk NMIBC without CIS.[9] Notably, patients with a PD-L1 combined positive score (CPS) of 10 or more had a 12-month DFS rate of 54.1%, compared to 39.4% in those with CPS < 10.[9] In cohort A, 13% of patients experienced G3 or G4 TRAEs, and no treatment-related death was reported.[8] In cohort B, G3 and G4 TRAEs were described in 14% of patients, without any G5 events.[9] Generally, the safety profile of pembrolizumab was similar to what referred in the literature.

The phase II CORE-001 trial investigated the potential synergism between intravesical administration of the

adenovirus cretostimogene grenadenorepvec and systemic pembrolizumab in patients with BCG-unresponsive high-risk NMIBC.[57] Cretostimogene grenadenorepvec is a serotype-5 oncolytic virus engineered to selectively target and destroy tumor cells with an altered retinoblastoma pathway (Figure 1).[58] Tumor lysis leads to the release of antigens and the consequent immune system stimulation.[58] Furthermore, this molecule carries a transgene encoding granulocyte-macrophage colony-stimulating factor (GM-CSF), a potent immune-stimulating protein.[57] This trial met its primary endpoint with a significant proportion of patients achieving a CR at 12 months (57.1% in the ITT population, 95% CI 40.7%–73.5%).[57] The most common TRAEs attributed to cretostimogene were low-grade and limited to urinary symptoms; no G3 TRAE was reported.[57] G3 TRAEs related to pembrolizumab occurred in 14.3% of patients.[57] No G4-G5 TRAEs associated with either cretostimogene or pembrolizumab were observed, without evidence of synergic or overlapping toxicities between the two agents.[57] The combination of the two agents received FDA Breakthrough Therapy Designation in May 2023.

The role of the anti-PD-L1 agent durvalumab in treating BCG-unresponsive NMIBC with CIS of the bladder has been explored in a phase II trial.[59] The enrolled patients received durvalumab (1500 mg intravenously every 4 weeks) for up to 12 months and the primary endpoint was CR rate after 6 months.[59] Unfortunately, this trial showed limited efficacy of the anti-PD-L1 agent, with CR of only 12% after 6 months, not achieving the predetermined threshold.[59] The only severe TRAE described was a G3 elevation of lipase, no G4 or G5 were reported.[59] The reasons why this trial had negative results may be found in molecular analysis, showing elevated complement activation genes post-ICI.[59]

Similarly, the phase II trial SWOG S1605 - investigating the role of the anti-PD-L1 atezolizumab in patients with BCG-unresponsive high-risk NMIBC not eligible for or unwilling to undergo RC – showed negative data.[60] In fact, its primary endpoint was pCR at 6 months for patients with CIS (with or without Ta or T1 disease), which had been observed only in 27% of them (95% CI 17%–38%), not meeting the prespecified threshold.[60] Regarding safety, G3 and G4 TRAEs were registered in 14% of patients and three deaths occurred, respectively, due to a sepsis, a myositis and a respiratory failure secondary to immune-related myasthenia gravis.[60]

Nogapendekin alfa-inbakicept (NAI) or N-803 is an interleukin 15 (IL-15) superagonist and its potential synergism with BCG was preliminarily explored in a completed phase I study, showing promising antitumor activity and limited toxicity (Figure 1).[61] This novel agent is now being investigated in the QUILT-3.032 (NCT03022825), a phase II/III ongoing registrational, pivotal, multicentre, open-label, single-arm trial. The protocol is designed to evaluate the efficacy and safety of intravesical NAI in combination with BCG (cohort A) or NAI alone (cohort C) in patients with BCG-unresponsive high-grade NMIBC with CIS, with or without high-grade papillary tumors. So far, CR rate in cohort A was 62% (95% CI: 51, 73), and this data led to NAI's FDA approval in combination with BCG for patients affected by BCG-unresponsive NMIBC with CIS, with or without papillary tumors.[62,63]

Several phase III trials are ongoing with promising agents that may potentially provide interesting insights for this patient population (Table 3).

In particular, KEYNOTE-676 is an ongoing phase III clinical trial evaluating the potential efficacy of BCG combined with pembrolizumab in BCG persistent/recurrent high-risk NMIBC patients after BCG induction therapy (Table 3).[65]

Intravesical cretostimogene grenadenorepvec monotherapy is under evaluation in BOND-003, a single-arm open-label trial for BCG-unresponsive high-risk NMIBC (NCT04452591) (Table 3). The primary endpoint in patients with CIS, with or without Ta or T1 papillary tumor, is CR at any time. DOR, PFS and recurrence free survival (RFS) were secondary endpoints. Recently, preliminary findings have shown a CR rate of 75.2% (95% CI 65%–83%) in this population (cohort C).[64]

The potential role of the anti-PD-1 subcutaneous agent sasanlimab in BCG-unresponsive NMIBC is currently being explored by the CREST trial (NCT04165317) (Table 3). This ongoing randomized open-label phase III trial is evaluating Sasanlimab as a single agent in patients with BCG unresponsive CIS or papillary tumors in cohort B (to date, closed to enrollment).

Cohort B of the previously cited QUILT 3.032 trial includes patients with BCG unresponsive high-grade Ta and T1 papillary tumor without CIS (Table 3).[63] Recent findings have shown a DFS rate of 55.4% (95% CI 42.0–66.8%) at 12 months in this population.[63]

Potential biomarkers of treatment response: beyond ctDNA

Several studies sought to develop predictive biomarkers in both NMIBC and MIBC.

In NMIBC, although BCG instillations may be considered a curative treatment, from 40% to 60% of patients will experience a tumor recurrence within 2 years.[54] Considering this, several efforts have been made to understand the potential underlying molecular explanations and to find biomarkers of BCG response. A retrospective study on 22 BCG-resistant patients showed that the treatment significantly enhanced PD-L1 levels on tumor tissues.[66] Even more interestingly, in a cohort of 63 patients with NMIBC, a higher pretreatment PD-L1 expression was found in BCG non-responders with respect to responders.[67] In fact, this study proved evidence of a different pretreatment adaptive immune response within the tumor microenvironment (TME): among non-responders, one patient out of three showed a pre-BCG colocalization of PD-L1 positive cells in areas of high density of CD8^{+} cells and a lack of CD4^{+} T cells. In contrast, PD-L1 expression was scarce among BCG responders, whose TME was enriched with CD8^{+} and CD4^{+} T cells.[67] Despite these findings, it is still a matter of study whether PD-L1 alone may be used as a predictive or prognostic biomarker in NMIBC treated with BCG.[68,69]

A more modern approach may suggest to analyze PD-L1's role in the dynamic and more complex context of TME, together with other active players such as CD8^{+} and CD4^{+} T cells.[70] In fact, TME seems to influence the therapeutic

Table 3. Ongoing phase three clinical trials evaluating the role of adjuvant immune agents in high-risk non-muscle invasive urothelial cancers that failed prior BCG-therapy.

Study name	Study ID	Immune Therapeutic Agents	Arms	Cohorts	Status	Sample size	Primary Endpoints	Resulted posted
Keynote-676	NCT03711032	Anti PD-1 pembrolizumab	*Arm A-1:* pembrolizumab + BCG *Arm A-2:* BCG monotherapy	Cohort A: patients BCG persistent/ recurrent)	Active, recruiting	430 estimated	pCR between participants with CIS	None
BOND-003	NCT04452591	Oncolytic adenovirus cretostimogene grenadenorepvec	*Single Arm Experimental cohort C and cohort P:* cretostimogene grenadenorpvec monotherapy:	Cohort C: CIS ± hG Ta or HG T1 papillary	Closed	112 actual	CR at any time	75.2% [95% CI 65%-83%][64]
				Cohort P: HG Ta/T1 papillary without CIS.	Open to enrollement	75 estimated	HG EFS	None
CREST	NCT04165317	Anti PD-1 sasanlimab	*Experimental:* sasanlimab	Cohort B B1: CIS ± hG Ta or HG T1 papillary	Discontinuation of enrollment (not for safety reasons)	110	CR	None
				B2: HG Ta/T1 papillary without CIS		50	EFS	None
QUILT 3.032	NCT03022825	*IL-15 superagonist NAI*	*NAI + BCG*	Cohort A: CIS ± hG Ta or HG T1 papillary	closed	190 actual (200 estimated)	CR	62% (95% CI = 51, 73)[62]
			NAI + BCG	Cohort B: HG Ta/T1 papillary without CIS	closed		DFS rate	55.4% (95% CI 42.0–66.8%)[63]
			NAI	Cohort C: CIS ± hG Ta or HG T1 papillary	discontinued for futility		CR	None

PD-1 = programmed cell death protein 1, BCG = Bacillus Calmette-Guérin, pCR = pathologic complete response, CIS= carcinoma in situ, CI = confidence interval, CR = complete response, HG = high-grade, EFS = event-free survival, NAI = nogapendekin alfa inbakicept, NCT = number of clinical trial, ID = identification, DFS = disease free survival, FDA = Food and Drug Administration, BCG = Bacillus of Calmette-Guerin.

response to BCG as it has been proved by the results of a retrospective study analyzing the local immune cell subset; in particular, a significant association was found among a low density of $CD4^+$ and GATA-binding-protein-3$^+$ (GATA3) T-cells, and increased expression of forkhead box P3$^+$(FOXP3), T regs and tumor-associated macrophage (TAMs), and treatment failure.[71] Subsequently, to find more objective and comparable parameters, several urinary cytokines levels have been studied to predict treatment success. In particular, interleukin-2 (IL-2), normally secreted by $CD4^+$ cells (reflecting a T-helper 1 predominant activity), has been detected in larger quantities in the urine of BCG-responders than from non-responders.[72] A combination of nine urinary cytokines, namely IL-2, IL-6, IL-8, IL-12, IL-18, IL-1ra, tumor necrosis factor-α (TNF–α), TNF-related apoptosis-inducing ligand (TRAIL), interferon-γ (INF-γ), accurately predicted the likelihood of response in a prospective trial including 130 patients.[73] An intriguing retrospective study on 243 patients affected by NMIBC with variant histologies (predominantly, squamous or glandular) pointed out the potential prognostic role of an high preoperative neutrophil-to-lymphocyte ratio to identify individuals with poorer outcomes.[74]

Although their use in clinical practice may help to identify individuals at higher risk of recurrence during BCG treatment and to guide modification of therapy's dose/duration, still no validated predictive biomarker is available to help clinicians.[6]

Recently, few steps are moving in the direction of finding some biomarkers of novel immunotherapies' response. For example, a subsequent analysis of the trial investigating nadofaragene firadenovec-vncg showed that a combination of post-therapy serum levels of anti-human adenovirus type-5 antibody and fold change from baseline can predict the durability of therapeutic response.[75] Despite these interesting findings, several efforts must be made in the next future to match the best therapy and the most correct patient.

Focusing on MIBC, lots of strain have been made to highlight the potential role of ctDNA as a biomarker for molecular residual disease (MRD) and tumor recurrence following radical surgery. As previously pointed out, Powles et al. conducted a study on a biomarker-evaluable population of 581 individuals among the 809 total amount of patients enrolled in the ImVigor010 RCT.[40,42] In this evaluation, the ctDNA-positive status at the start of adjuvant therapy identified patients at a higher risk of disease recurrence than those with a ctDNA-negative status.[42] Furthermore, individuals who were positive for ctDNA had improved DFS with adjuvant atezolizumab compared with patients undergoing observation (HR 0.58, 95% CI 0.43–0.79) and, interestingly, clearance of ctDNA with the anti-PD-L1 occurred in 18% of patients and was associated with better outcomes.[42] On the other hand, no difference in DFS was found between the two arms for negative-ctDNA individuals.[42] To find out the underlying

molecular mechanisms, an exploratory transcriptional analysis from sample tumors was performed. ctDNA-positive patients were enriched in cell-cycle and keratin genes compared with those who were negative for ctDNA, which might correlate to a more aggressive behavior; furthermore, in this sub-group of individuals, tumor-mutational burden (TMB) and PD-L1 positivity have been related to improved clinical outcomes with atezolizumab.[42] These findings were confirmed at the extended follow-up analysis, where ctDNA-positive patients continued to show shorter OS and greater benefit if treated with atezolizumab versus observation (HR 0.59, 95% CI 0.42–0.83).[41] Interestingly, in the atezolizumab arm, non-relapse in ctDNA-positive patients was associated to a higher expression of interferon-inducible genes, while recurrence was associated with angiogenesis and tumor-growth factor (TGF)β signaling.[42] On the other hand, patients negative for ctDNA and who had relapsed showed increased expression of extra-cellular matrix, stromal and TGFβ-inducible genes, underlying the previously discussed central role of TME; the ones that did not relapse were enriched in interferon-inducible genes, suggesting a preexisting tumor immunity.[42,70] To conclude, these analyses have highlighted a different way to relapse: locally, for patients who were negative for ctDNA, and at a distance for ctDNA-positive patients.[42] Recently, an interesting systematic review confirmed the prognostic role of ctDNA measured right after cystectomy and a promising predictive value when used to monitor disease recurrence, anticipating radiological evidence of disease relapse.[76] Interesting insights came from a prospective study on 112 patients, where detectable ctDNA before radical cystectomy was associated with poor outcomes (nodal involvement, locally advanced disease and risk of recurrence) regardless of clinical stage or prior NAC.[77] Furthermore, analysis of longitudinal ctDNA status showed that patients with persistently undetectable ctDNA before and after surgery had better prognosis than the ones with detectable ctDNA at any time.[77] Based on this wide background, monitoring patients for ctDNA is a minimally invasive approach that results appealing for selecting individuals eligible for adjuvant treatment with ICIs. IMvigor-011 (NCT04660344) is an ongoing phase III trial evaluating the role of adjuvant atezolizumab in a selected high-risk (i.e. (y)pT2-T4a N0 M0 or (y)pT0-T4a N$^+$ M0) population following cystectomy (Table 2). Prior neoadjuvant chemotherapy is allowed but not required, and patients do not need to be eligible for adjuvant chemotherapy. Patients who are ctDNA positive at any point are 2:1 randomly assigned to receive 1 year of atezolizumab or placebo. On the other hand, ctDNA-negative patients are being included in the surveillance group. Investigator-assessed DFS is the study's primary end point, and OS is a key secondary end point. At the 2024 European Association of Urology Annual Meeting, Prof. T. Powles presented an analysis of the outcomes of patients with persistent ctDNA negativity enrolled in the surveillance cohort of ImVigor-011, showing that only 9.9% (17/171) of them experienced a DFS event at a median follow-up 16.3 months, enhancing the role of a future ctDNA tailored therapy.[48] A similar biomarker-integrated phase II/III RCT (MODERN, NCT05987241) is trying to assess the role of nivolumab alone or in combination with the anti-Lymphocyte Activation gene 3 (CD223 or LAG3) relatlimab in patients with urothelial cancer after radical surgery based on their ctDNA status (Table 2). Similarly, Dr. Anderson and colleagues are evaluating the role of atezolizumab administered at the time of biochemical recurrence (monitored by ctDNA periodical assessment) in patients undergoing radical cystectomy within the phase II clinical trial TOMBOLA (NCT04138628). These three ongoing trials have the ambitious plan to personalize and de-escalate treatment according to patient's recurrence risk, avoiding futile adverse events to low-risk individuals. Results of these RCTs are eagerly awaited.

Focusing on other potential biomarkers, PD-L1 positivity status – according to CheckMate 274 study – seems to highlight patients with a greater benefit from adjuvant nivolumab (PD-L1 expression according to tumor-cell score) (HR 0.55, 98.72% CI 0.35–0.85) although a DFS improvement has been underlined in the ITT population.[43] Actually, a not-prespecified post-hoc analysis by Prof. M.D. Galsky et al. showed that the DFS benefit with nivolumab compared with placebo was observed in the CPS >or = 1, TC >or = 1%, and TC < 1% sub-populations.[78] In the latter sub-group, median DFS with nivolumab for patients with both TC < 1% and CPS >or = 1 was nearly double that with placebo, suggesting a benefit of immunotherapy also in these patients.[78] According to these results, we might be cautious about pointing PD-L1 out as a potential driver for clinicians treatment's choice, also considering the differences between CPS and TC as measures of PD-L1 expression among different RCTs. Analogously, PD-L1 status could not identify patients with a greater benefit from adjuvant atezolizumab according to the subsequent analyses of ImVigor010.[42] Interestingly, among ctDNA-positive patients, PD-L1 positivity status has been related to improved clinical outcomes with atezolizumab, with respect to ctDNA-negative individuals.[42] When focusing on PD-L1 as a potential predictive biomarker of response to ICIs, it is mandatory to underline its intrinsic dynamic nature, with a marked heterogeneity of expression within the TME and among primary versus secondary neoplastic lesions.[79] About microenvironment's cross-talking, interesting seems the role of epidermal growth factor receptor (EGFR) and signal transducer and activator of transcription 3 (STAT3), which both enhance PD-L1 expression on tumor cells by proliferative oncogenic boost, underling the complex interaction between innate and adaptive immune system.[80]

To conclude, other potential biomarkers already known from the metastatic setting, such as TMB or gene expression profiles (i.e. tGE3 signature – made of CD274, IFNγ and C-X-C Motif Chemokine Ligand 9 or CXCL9 - related to greater atezolizumab responce or pan-fibroblast TGFβ response (F-TBRS) signature, related to atezolizumab worse response) have been analyzed in the biomarker-evaluable population of IMvigor010.[42] Anyway, none of them are still used in clinical practice to drive patient's selection. Interesting insights may – in a near future – come from the distinct UC's subtypes identified through transcriptomic analyses using clustering analysis (i.e. luminal papillary, luminal non-specified, luminal unstable, stroma-rich, basal/squamous, and neuroendocrine).[81] In previous studies analyzing samples from patients undergoing NAC, these subgroups showed different molecular alterations, immunological phenotypes and prognosis.[19] Furthermore, several exploratory analyses focused on their potential role in

patients receiving immune-checkpoint inhibitors, but in a neoadjuvant or advanced setting, underlying a different response to ICIs.[82] To the best of our knowledge, there is actually no evidence for the use of these molecular classifications to guide the adjuvant immunotherapy for UC.

Conclusions

Exactly in the field where the first immunotherapy grew up, several efforts have recently been made to enhance the use of immune agents in early-stage urothelial cancer.

The success of nivolumab in the CheckMate 274 study has underscored its potential as an adjuvant therapy, offering a significant disease-free survival (DFS) benefit, particularly in patients with PD-L1 expression ≥ 1%, as we previously extensively analyzed. However, the contrasting results from other pivotal trials, such as IMvigor010 and Ambassador, highlight the complexity of integrating ICIs into routine practice. The observed divergence may stem from differences in trial designs, patient populations, biomarker integration, and treatment comparators, underscoring the urgent need for tailored therapeutic strategies.

Emerging data show the promising role of ctDNA as a dynamic biomarker for molecular residual disease, enabling a more personalized approach to adjuvant treatment. Efforts such as IMvigor011 and MODERN trials are paving the way for ctDNA-driven therapeutic algorithms, aiming to enhance treatment specificity and minimizing unnecessary exposure to adverse effects.

Additionally, the exploration of ICIs in earlier disease stages, including high-risk non-muscle-invasive bladder cancer (NMIBC), has already yielded promising results. The approval of pembrolizumab and nadofaragene firadenovec for BCG-unresponsive NMIBC marks a significant cornerstone, providing viable alternatives for patients unsuitable for or declining radical cystectomy.

Despite these advances, several challenges remain. Integrating these therapies into the rapidly evolving treatment landscape of metastatic urothelial carcinoma raises critical questions about optimal timing and treatments sequencing. Moving forward, the focus must be on refining patient stratification through predictive biomarkers, enhancing the understanding of tumor microenvironment dynamics. By addressing these gaps, we may establish a more precise and effective therapeutic paradigm, improving outcomes for patients with urothelial cancer.

Short biographical note

Since 2017, Prof Francesco Massari has held the National Scientific Qualification in Hematology, Oncology, and Rheumatology as an Assistant Professor (II Level). He currently serves as the head of the genitourinary oncology area within the Medical Oncology Unit at the IRCCS Azienda Ospedaliero-Universitaria di Bologna, University Hospital of Bologna. His clinical and research activities focus on neoplasms of the male and female urogenital system, with particular emphasis on studying mechanisms of treatment resistance in prostate and kidney cancers, as well as evaluating new therapeutic molecules for these malignancies. His scientific work also involves significant participation as a principal investigator in approximately 30 clinical trials (phase I-II-III studies, prospective, observational, and retrospective studies), all conducted in compliance with good clinical practice guidelines, primarily concerning kidney, prostate and bladder cancers. He is the author of approximately more than 400 publications in international journals.

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This work does not involve human participants and it was not necessary an ethical approval by the ethic committee.

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