

Optimizing enfortumab vedotin plus pembrolizumab therapy

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ABSTRACT

Often associated with a poor prognosis, advanced urothelial carcinoma (aUC) has progressed to muscle-invasive or metastatic stages. Traditionally, chemotherapy has been the primary treatment for aUC, though its effectiveness in advanced stages remains limited. Recent developments have introduced promising therapies, notably the combination of enfortumab vedotin with pembrolizumab, which is now recommended as the first-line therapy following the EV-302 trial results. This combination has demonstrated significant improvements in survival rates. This review aims to explore the evolution of treatment strategies for aUC, emphasizing the shift towards immunotherapy and targeted therapies, and discusses the potential for optimized treatment algorithms to improve patient outcomes.

INTRODUCTION

Bladder cancer (BC) is the ninth most common cancer type worldwide, with its incidence approximately four times higher in men than in women [1] with 614298 new cases and 220596 deaths in 2022 [2]. BC arises from the urothelium and accounts for approximately 90% of all urothelial cancers [3]. It progresses through distinct biological processes. Non-muscle invasive bladder cancer (NMIBC), which represents about 75% of BC cases, remains confined to the mucosa and submucosa, often recurring but rarely progressing. In contrast, muscle-invasive bladder cancer (MIBC), comprising about 25% of cases, penetrates the detrusor muscle, increasing the risk of metastasis through lymphatic and vascular invasion. Many key pathways and genetic alterations contribute to its pathogenesis: FGFR3 mutations promote tumor proliferation in NMIBC [4], TP53 and RB1 loss drive genomic instability in MIBC [5]; KDM6A mutations disrupt chromatin remodeling, aiding tumor progression [6]; PI3K-AKT-mTOR activation supports survival and growth [7]; PD-L1 overexpression through PD1 enables immune evasion [8]; Nectin-4 and Trop-2

overexpression enhance tumor adhesion, invasion, and progression, serving as targets for Enfortumab Vedotin and Sacituzumab Govitecan, respectively [9, 10] (Figure 1). Cystoscopy is the gold standard method in detecting BC where suspicious areas can be biopsied. It can be preceded in some cases by urine cytology. MRI, US and CT-Scan can also help in BC staging. Tumor stage (TNM), grade, histological subtype and metastasis are usually the main prognostic factors of urothelial cancer, Metastasis is present in ten to fifteen percent of cases of muscle-invasive BC (MIBC) at time of diagnosis [11]. Moreover, the prognosis for such patients is poor despite recent advancements in treatment regimens. The 5-year survival rate is about 8% at the metastatic stage and about 39% when the tumor extends beyond the bladder and invades adjacent tissue or reaches nearby lymph nodes [12]. The latest trials for advanced-stage urothelial carcinoma (aUC) that introduced new drugs such as antibody-drug conjugates (ADCs) and immune checkpoint inhibitors (ICIs) showed significant improvement in these survival rates. Nevertheless, there is still a major necessity for establishing treatment algorithms that could effectively implement these novel

drug combinations. In this article, we will present the major shift in treatment sequences for advanced bladder cancer by starting with the historical standard of care then moving on to the influential impact of enfortumab vedotin plus pembrolizumab and ending with a discussion of other potential treatment strategies.

Molecular subtypes

Molecular subtypes exhibit distinct clinical outcomes and differ in the expression of cell-cycle genes, cytokeratins, cell adhesion genes, and mutation frequencies. These subtypes transcend pathological classification, with gene signatures consistently expressed across stages and grades, suggesting they are intrinsic tumor properties.

Furthermore, drug susceptibility is more strongly associated with molecular subtypes than pathological classification, highlighting the potential for molecular stratification to guide targeted therapies and personalized treatment approaches.

aUC can be classified into three intrinsic molecular subtypes groups (Table 1): basal, luminal, and neuro-endocrine. The molecular markers identified in these subtypes also exhibit different clinicopathologic characteristics, as well as responses to different therapeutic modalities [13–15].

Histological subtypes

The histological subtypes (Table 2) of urothelial carcinoma are classified as follows: infiltrating urothelial carcinoma with divergent differentiation; nested, microcystic; micropapillary; lymphoepithelioma-like; plasmacytoid/signet ring cell/diffuse; sarcomatoid; giant cell; poorly differentiated; lipid-rich; and clear cell. Each subtype carries unique prognostic and therapeutic implications, making accurate classification essential for effective management and treatment [16].

This table summarizes the most common histological subtypes of urothelial carcinoma, detailing their specific genetic alterations, clinical implications, and prognosis. It highlights the distinct features of each subtype, emphasizing the importance of accurate classification in guiding treatment decisions and predicting patient outcomes [17–19].

Predictive biomarkers

Recent biomarkers have been identified to predict treatment response and prognosis in aUC. They play a key role in personalized treatment, emphasizing the need for further research. Table 3 summarizes the most significant biomarkers, while others, including PARP, HER2, HER1, ERCC1, and ERCC2, show potential for guiding future therapies or serving as prognostic indicators in aUC [20, 21].

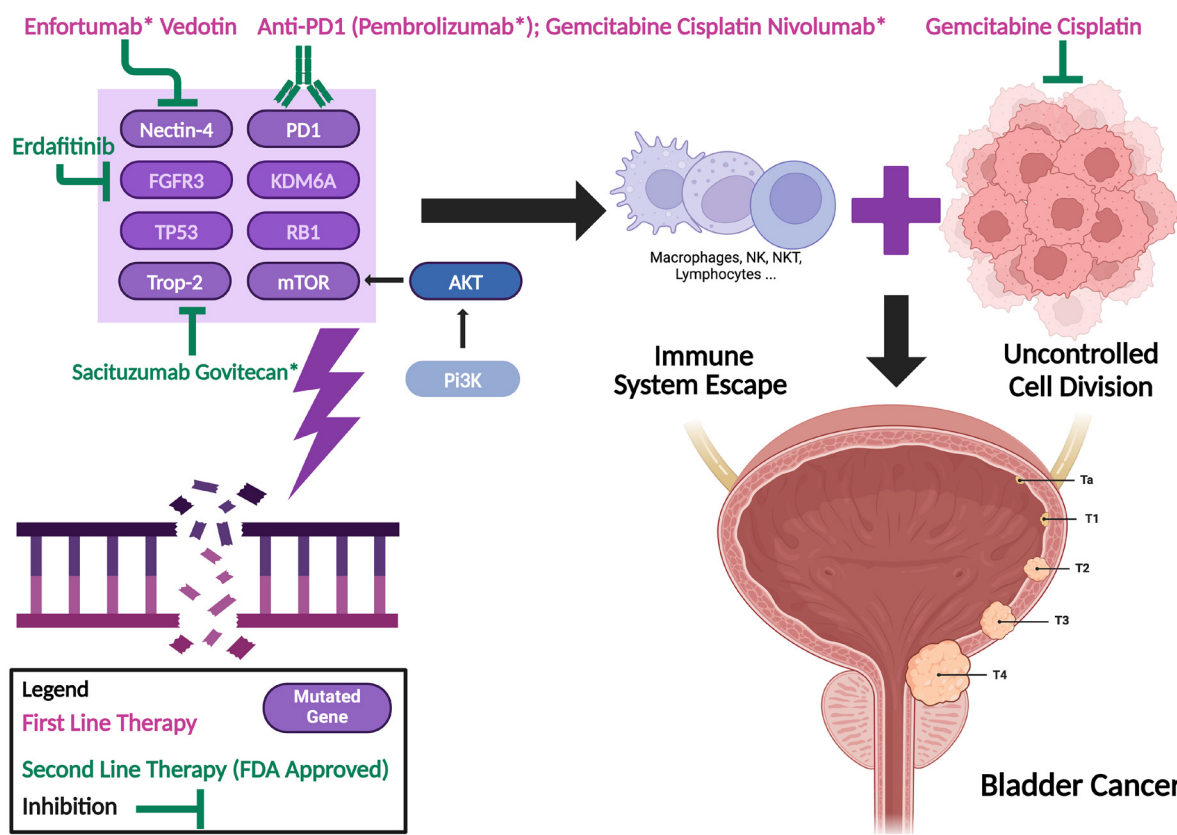


Figure 1: Bladder cancer progression. Genetic alterations, signaling pathways, and targeted therapies; Created in BioRender. Prince, G. (2025) <https://BioRender.com/r59h797>.

Table 1: Molecular subtypes for aUC

Molecular subtype	Subtype	Expression	Clinical implications	Frequency	Treatment	Genetic mutations	
Luminal	Luminal papillary	SHH+	Patients <60 years old	35%	FGFR3 inhibitors	FGFR3 mutation, fusion, amplification	
	Luminal non-specified	GATA3, uroplakins, CK18, CK20, and, FOXA1, and PPARG), KRT20+	EMT markers (TWIST1, ZEB1) miR-200 family, Medium CD274 (PD-L1), CTLA-4 Myofibroblast markers	Patients >80 years old	19%	Anti-PD-L1, PD-1, CTLA-4	Wild type p53
	Luminal infiltrated	Immune checkpoint markers (PD-L1, PD-1, and CTLA-4)			6%	Immune checkpoint therapy and radiation therapy	UPKs KRT20 SNX31.
Basal		KRT5/6 and KRT14 CK5/6, CK14, and p63 High CD274 (PD-L1), CTLA4 Immune infiltrates	Advanced or metastatic disease More common in woman	19%	Anti-PD-L1, PD-1, CTLA-4 Cisplatin-based chemotherapy EGFR targeted therapy		
Neuro-endocrine			Most aggressive	5%	Etoposide Cisplatin-based chemotherapy	SOX2 DLX6 MS11 PLEKHG4B E2F3/SOX4 amplification High cell cycle	

Table 2: Histological subtypes for aUC

Histological subtype	Corresponding molecular subtype	Genetic alterations	Marker expression	Clinical implications	Prognosis
Plasmacytoid Urothelial Carcinoma	Luminal and Basal	TP53, RB1, KMT2D, ARID1A mutations, CDH1 loss-of-function mutations, CDH1 promoter hypermethylation, loss of E-cadherin, abnormal expression of p120		<ul style="list-style-type: none"> Advanced stage at presentation High relapse rates Peritoneal carcinomatosis 	<ul style="list-style-type: none"> Poor survival, High cancer-specific mortality High risk of recurrence and metastasis Local recurrences
Micropapillary Urothelial Carcinoma	Luminal	ERBB2 Amplifications, PPARG enrichment and suppression of p63 target genes	Downregulation of miR-296 and activation of chromatin-remodeling complex RUVBL1	<ul style="list-style-type: none"> High-grade tumor cells Intra-tumoral heterogeneity Vascular invasion and nodal metastasis 	<ul style="list-style-type: none"> Poor survival Intratumoral heterogeneity complicates prognosis
Small-Cell/ Neuroendocrine Carcinoma	Luminal and Basal	TP53, RB1 mutations; TERT promoter mutations, chromatin-remodeling gene mutations (CREBBP, EP300, ARID1A, KMT2D, APOBEC) APOBEC mutation signature, high level of chromosomal instability and genomic doubling	Neuroendocrine markers CD56, synaptophysin, chromogranin, and INSM1, NEUROD1, ASCL1, POU2F3, YAP1, and DLL3	<ul style="list-style-type: none"> De novo neuroendocrine differentiation Association with paraneoplastic syndromes Aggressive course 	<ul style="list-style-type: none"> Poor response to treatment Frequent disseminated metastasis
Sarcomatoid Urothelial Carcinoma	Basal	TP53, RB1, PIK3CA mutations; dysregulation of epithelial-mesenchymal transition pathway	High molecular keratins CK 34βE12 or CK5/6; and or (3) GATA3 expression in the sarcomatous areas.	<ul style="list-style-type: none"> Aggressive Bi-phasal 	<ul style="list-style-type: none"> Extremely poor prognosis due to high invasiveness and aggressive behavior

Squamous cell carcinoma	Basal	TERT promoter mutations	Basal and stem-like markers (CD44, CK5, CK6, and CK14), epidermal growth factor receptor (EGFR) and desmogleins (DSC1-3) and desmogleins (DSG1-4), TGM1 (transglutaminase 1), and PI3 (elafin)	<ul style="list-style-type: none"> • Unfavorable prognosis • High-grade urothelial carcinoma • Poor response to chemotherapy and radiation
Nested Urothelial Carcinoma		TERT promoter mutations, <i>TP53</i> , <i>JAK3</i> , <i>CTNNB1</i> , <i>FGFR3</i>	FOXA1, GATA3, and CK20, PAX8 expression	<ul style="list-style-type: none"> • Aggressive clinical course • Variable prognosis • Aggressive behavior • High frequency of metastasis
Urothelial carcinoma with glandular differentiation		<i>TERT</i> promoter, chromatin-modifying genes, and DNA damage response (DDR) genes.	MUC5AC and CDX2	<ul style="list-style-type: none"> • High stage at presentation
Adenocarcinoma		TP53, KRAS, SMAD4 (similar to colorectal adenocarcinoma), EGFR and ERBB2 amplification	CK20 and CDX2	<ul style="list-style-type: none"> • Pure glandular morphology • Resembles colorectal adenocarcinomas • Intestinal metaplasia, bladder exstrophy, chronic irritation, and obstruction due to nonfunctioning bladder or endemic schistosomiasis. • Poor prognosis • High-grade urothelial carcinoma • Poor response to chemotherapy

Table 3: Predictive and prognostic biomarkers in aUC

Biomarker	Mechanism of action	Role	Drug	Drug mechanism of action
FGFR	Promote angiogenesis and the regeneration of tissue in cellular proliferation, differentiation and steroid synthesis.	Predictive of response to FGFR inhibitors	Erdafitinib	Pan-FGFR tyrosine kinase inhibitor [22]
TMB		Predictive of response to immunotherapy (PD-1 inhibition) Prognostic factor	Pembrolizumab	Block immune-suppressing ligands (PD-L1 and PD-L2), from interacting with PD-1 to help restore T-cell response and immune response [23]
Nectin-4	Cell-cell adhesion, proliferation, angiogenesis, epithelial to mesenchymal transition, metastasis, DNA repair, tumor relapse	Potential predictive biomarker for response to ADC	Enfortumab Vedotin	Binds to cells expressing Nectin-4, leading to internalization of the ADC-Nectin-4 complex. The MMAE is then released through proteolytic cleavage, where it induces cell cycle arrest and apoptotic cell death [24]
Trop-2	Cell proliferation, survival and invasion	Potential predictive factor for Sacituzumab govitecan response Prognostic factor	Sacituzumab govitecan	The delivery of SN-38 to the tumor cell results in inhibition of topoisomerase I and the accumulation of lethal DNA double strand break [25]

THE STANDARD OF CARE BEFORE EV-302

First line therapy

For the past decades, platinum-based chemotherapy has served as the standard frontline therapy for patients with aUC, with cisplatin preferred over carboplatin in the first-line setting. In fact, treatment algorithm selection depends on the patient's tolerance to platinum-based cytotoxic drugs, more specifically to cisplatin. Numerous first-line combinations for cisplatin-eligible patients have been investigated in historical clinical trials during the last thirty years. The MVAC regimen, comprising methotrexate, vinblastine, doxorubicin, and cisplatin, administered every 28 days for six cycles, demonstrated significant improvements in objective response rate (ORR), PFS, and OS compared to single-agent cisplatin in aUC [26]. MVAC therapy is associated with significant toxicity, including myelosuppression, neutropenic fever, sepsis, mucositis, and nausea and vomiting. These considerable adverse effects led to establishing different combinations, such as dose-dense MVAC and gemcitabine plus cisplatin, to circumvent these marked side effects.

While dose-dense MVAC did not significantly improve OS compared to classic MVAC, it demonstrated benefits in terms of PFS and toxicity reduction. Compared to classic MVAC, dose-dense MVAC showed a more favorable toxicity profile, with lower rates of grade ≥ 3 leukopenia, mucositis, and neutropenic fever [27–29]. In a phase III trial comparing gemcitabine plus cisplatin (GC) with classic MVAC [29, 30], GC regimen has shown comparable efficacy and reduced toxicity compared to the MVAC regimen, making it a promising alternative. GC demonstrated similar ORR and OS outcomes. GC was associated with less grade ≥ 3 toxicity than MVAC, including lower rates of neutropenia, neutropenic sepsis, and mucositis.

These multiple first-line cisplatin-based combinations were recommended for aUC as a standard of care for multiple years although GC remained the most commonly used regimen. However, nearly half of patients are ineligible for cisplatin use due to frequent medical comorbidities and/or reduced renal function [31]. Thus, the addition of carboplatin to gemcitabine was recommended as the first-line therapy for these cases. It is particularly suitable for those who cannot tolerate more complex carboplatin-based combination regimens like MCAVI (methotrexate, carboplatin, and vinblastine).

The evidence for this indication comes from the randomized phase II/III EORTC 30986 trial [32] that involved chemotherapy-naïve patients with advanced or metastatic UC and impaired kidney function or poor performance status. The gemcitabine plus carboplatin combination was compared with MCAVI. The study found that treatment with carboplatin plus gemcitabine resulted

in similar OS and PFS compared to MCAVI. Although the ORR was slightly higher with gemcitabine plus carboplatin, the difference was not statistically significant. Gemcitabine plus carboplatin was associated with lower rates of grade 3 to 4 toxicity compared to MCAVI, particularly in terms of neutropenia and febrile neutropenia. However, it was linked to a higher incidence of serious thrombocytopenia.

For patients unfit for both cisplatin and carboplatin, immunotherapy for PD-L1-positive cases such as pembrolizumab is recommended [33]. In the phase II KEYNOTE-052 [34] study, pembrolizumab demonstrated significant efficacy as initial therapy in patients with aUC who were ineligible for a cisplatin-based regimen. The ORR for the entire cohort was 29%, with complete and partial response rates of 9% and 20%, respectively. Importantly, the median duration of response was 33 months, indicating durable responses. Response rates remained consistent across various subgroups, with higher ORR observed in patients with a combined positive score (CPS) >10 compared to CPS ≤ 10 . The median OS was 11 months, with a four-year OS rate of 19%. Finally, combination therapy involving nivolumab alongside gemcitabine–cisplatin demonstrated superior outcomes compared to gemcitabine–cisplatin alone in the CheckMate 901 trial [35]. The positive overall survival findings of this study led to a recent FDA approval of this combination as a first-line treatment for unresectable or metastatic urothelial carcinoma [36].

Maintenance therapy

Although platinum-based cytotoxicity was cemented as the optimal first-line approach for increasing patient survival, the poor durability of response highlighted the need for first-line maintenance therapy in cases without disease progression. As a result of the JAVELIN Bladder 100 phase III trial, avelumab first-line maintenance therapy has been integrated into international guidelines as a standard of care for patients with aUC who do not experience progression following first-line platinum-based chemotherapy. In the avelumab group, the median overall survival (OS) was 21.4 months, compared to 14.3 months in the control group (BSC). Additionally, the median progression-free survival (PFS) was 3.7 months in the avelumab group and 2.0 months in the control group [37, 38]. While there have been notable improvements observed with avelumab maintenance treatment, it is evident that survival rates remain relatively low.

Second-line therapy

Several innovative treatments such as FGFR inhibitors, ADCs, and ICIs [39–41] were implemented as second-line therapies for aUC patients with disease progression after receiving standard-of-care first-line drugs.

Erdafitinib, a FGFR inhibitor, demonstrates promising efficacy as a second-line drug in patients with advanced or metastatic UC harboring a FGFR3 genetic alteration. Initial efficacy, supported by early phase II clinical trials, included an ORR of 40% and a median OS of 11 months [39]. Furthermore, in the phase III THOR trial [22], erdafitinib showed significant improvements in OS and PFS compared to chemotherapy, with benefits observed across all clinically relevant subgroups. Erdafitinib is generally well tolerated in long-term treatment, maintaining patient quality of life. Mutations in FGFR3, AKT1, and TP53, detected in cfDNA may contribute to acquired resistance to erdafitinib [42].

Enfortumab vedotin, an ADC targeting Nectin-4, demonstrated significant improvements in OS and PFS compared to chemotherapy in the randomized phase III EV-301 trial. In this study involving 608 patients with locally advanced unresectable or metastatic UC previously treated with platinum-based chemotherapy and PD-1/PD-L1 inhibitor, enfortumab vedotin exhibited superior OS (median 13 vs. 9 months) and PFS (median 6 vs. 4 months) compared to chemotherapy [43, 44]. Additionally, overall response rates were higher with enfortumab vedotin than with chemotherapy (41% vs. 19%). Sacituzumab govitecan, an antibody-drug conjugate targeting Trop-2, demonstrated promising efficacy in a phase II trial (TROPHY-U-01) involving 113 patients with advanced UC previously treated with platinum-based chemotherapy or immunotherapy. The study reported objective and complete response rates of 27% and 5%, respectively, with median OS and median PFS of 5 and 11 months, respectively [45].

Pembrolizumab was also a potential therapeutic alternative for relapsed aUC as shown in the KEYNOTE-045 trial. This ICI improved median OS compared to paclitaxel, docetaxel, or vinflunine (10.3 vs. 7.4 months, HR 0.73, 95% CI 0.59–0.91) in recurrent aUC cases. The durability of response was also demonstrated in a three-year follow-up update [46, 47].

THE CHANGING TREATMENT LANDSCAPE AFTER EV-302 AND CHECKMATE 901

After years of employing platinum-based established protocols, the EV-302 trial served as a pivotal milestone that introduced ADCs into the first-line armamentarium against aUC. Enfortumab vedotin plus pembrolizumab is now approved as a first-line treatment regardless of cisplatin-eligibility [48]. The CheckMate-901 trial also prompted a significant shift in the treatment algorithm by proving an increased survival with the use of nivolumab with the standard gemcitabine-cisplatin combination in the first-line [35, 49], this could be useful for patients who cannot receive enfortumab vedotin plus pembrolizumab. In this section, we will discuss the intricacies of these

clinical trials and the implications behind the major change in the treatment sequences.

The favored regimen of enfortumab vedotin plus pembrolizumab

For patients diagnosed with aUC, it is recommended starting treatment with enfortumab vedotin in conjunction with pembrolizumab over platinum-based [35, 50].

Enfortumab vedotin is a fully human ADC consisting of a human IgG1 antibody targeting Nectin-4, linked to monomethyl auristatin E (MMAE), a microtubule-disrupting agent. Its anticancer effect occurs when the ADC binds to cells expressing Nectin-4, leading to internalization of the ADC-Nectin-4 complex. The MMAE is then released through proteolytic cleavage, where it induces cell cycle arrest and apoptotic cell death [24]. Nectin 4 is expressed in more than 90% of urothelial carcinoma [51]. The luminal subtype of urothelial carcinoma is the one who expresses Nectin 4 the most. Resistance to this treatment includes down regulation or knockdown of NECTIN4 [52].

The most common adverse events associated with EV therapy include peripheral sensory neuropathy, pruritus, fatigue, reduced appetite, diarrhea, dysgeusia, and nausea [53].

Pembrolizumab is a programmed death 1 (PD-1) inhibitor that binds to the PD-1 receptor, blocking immune-suppressing ligands (PD-L1 and PD-L2), from interacting with PD-1 to help restore T-cell response and immune response.

PD-L1 and PD-L2 bind to PD-1, a receptor expressed on activated and exhausted T cells, as well as on antigen-presenting cells like macrophages, dendritic cells, and B cells. When PD-L1 interacts with PD-1, it triggers phosphorylation of the immunoreceptor tyrosine-based switch motif within the intracellular domain of PD-1, leading to the recruitment of SHP-1 and SHP-2 phosphatases. These phosphatases modulate kinases associated with the T-cell antigen receptor, thereby reducing cytokine production, T-cell activation, and target cell lysis [23].

Various biomarkers defining the tumor microenvironment may aid in predicting the response to pembrolizumab monotherapy in aUC. For example, TMB and Tcell_{int}GEP show a correlation with better outcomes [54].

The most frequent long term adverse events to a treatment with Pembrolizumab include Fatigue, Pruritus, Rash, Decreased appetite, Hypothyroidism, Diarrhea and Nausea [55].

The phase III EV-302 trial [56] involving 886 patients with previously untreated, locally advanced or metastatic UC demonstrated superior OS at a median follow-up duration of 17.2 months (31.5 months vs. 16.1 months, HR 0.47, 95% CI 0.38–0.58), PFS (12.5 months vs. 6.3

months, HR 0.45, 95% CI 0.38–0.54), and response rates with enfortumab vedotin plus pembrolizumab compared to chemotherapy [56, 57].

This efficacy extended to cisplatin-eligible and cisplatin-ineligible patients, with significant improvements in OS and PFS across various clinically relevant subgroups. Grade ≥ 3 toxicities were lower with enfortumab vedotin plus pembrolizumab compared to platinum-based chemotherapy, with manageable profiles. However, it's important to note that while these toxicities were reported less, the resulting profiles from these complications differ significantly. Therefore, long-term studies and patient-reported outcomes are crucial in determining the long-term management and prognosis. The findings of this trial led to an FDA approval in December 2023 for first-line aUC indication of enfortumab vedotin plus pembrolizumab irrespective of cisplatin-eligibility [48].

In the case of disease progression after enfortumab vedotin plus pembrolizumab, an optimal approach to second-line therapy remains uncertain. For patients eligible for cisplatin, it is advised to opt for cisplatin-based chemotherapy, while using the treatment options previously discussed, although data on this context are limited. If patients are ineligible for cisplatin, gemcitabine plus carboplatin represents a suitable alternative, although data on this context are limited. Moreover, further research is needed to determine the efficacy of avelumab maintenance treatment following platinum-based therapy after pembrolizumab plus enfortumab vedotin. The ESMO Clinical Practice Guideline 2024 interim update states that second-line platinum-based combinations should be administered without avelumab in FGFR negative cases. For example: Sacituzumab Govitecan can be administered or Enfortumab Vedotin if not already administered. Whereas if the patient has FGFR mutations, Erdafitinib can be administered. In addition, single ICI rechallenge is also not advised without upcoming evidence [58]. For individuals ineligible for platinum-based therapy, the investigation of FGFR2/3 mutations remains crucial in the pursuit of suitable treatment options such as erdafitinib. Ultimately, defining second-line therapy after this novel combination remains challenging; it is still unclear whether this sequence could provide better survival benefits in comparison to the previous GC-avelumab followed by enfortumab vedotin sequence.

Treatment sequences for platinum-based regimens

The recommendation for initial therapy in aUC favors enfortumab vedotin plus pembrolizumab due to its superior efficacy and manageable toxicity profile observed in clinical trials. However, patients with the following conditions are ineligible for treatment with pembrolizumab plus enfortumab vedotin [56]:

uncontrolled diabetes mellitus, severe dermatologic conditions, grade ≥ 2 neuropathy, creatinine clearance ≤ 30 mL/minute or failure of immunotherapy in the adjuvant setting. Moreover, the economic considerations [57] surrounding the utilization of enfortumab vedotin and pembrolizumab cannot be overlooked. A recent analysis [59] revealed that the estimated annual cost of treatment with these agents was 3.8 times higher compared to platinum-based chemotherapy followed by avelumab maintenance (\$455,630 vs. \$120,253). Considering that certain patients may not be suitable candidates, decline treatment, or lack access to the initial therapy, alternative options must be explored.

Patients who cannot receive enfortumab vedotin plus pembrolizumab will benefit from the previous platinum-based standard of care with the addition of a novel combination evaluated in the CheckMate 901 trial. After the negative findings of Keynote 362 and IMvigor 130, this study was the first to demonstrate overall survival benefits in the case of a first-line GC-ICI combination. In the CheckMate 901 phase III trial, 608 patients were randomized (1:1) into receiving either gemcitabine-cisplatin plus nivolumab every 3 weeks for a maximum of 6 cycles followed by up to 24 months of nivolumab monotherapy (every 4 weeks) or gemcitabine-cisplatin alone for a maximum of 6 cycles. After a median follow-up duration of 33.6 months, adding nivolumab to gemcitabine-cisplatin (GCN) significantly improved survival compared to GC alone. Notably, OS was prolonged with GCN therapy, with a median OS of 21.7 months compared to 18.9 months with GC alone. PFS was also extended with nivolumab-combination therapy, with a median PFS of 7.9 months versus 7.6 months. It should be noted that these positive survival results were present irrespective of PD-L1 expression of the tumor. This trial demonstrated that the GCN combination was also responsible for an early and durable response as seen in the ORR rates (58% for the GCN arm vs. 43% for the GC arm). The GCN group showed a higher complete response (CR) rate than the GC group (22% vs. 12%), this response was also more durable (median duration of CR: 37.1 months vs. 13.2 months). However, it's important to note that grade ≥ 3 toxicity was more prevalent in the GCN group, primarily involving neutropenia and thrombocytopenia [35, 50]. Patients who received GCN as a first-line treatment for aUC can benefit from maintenance nivolumab therapy [29].

In the case of lack of access to CGN or ineligibility, it is recommended that patients receive the previously established standard of care that consisted of gemcitabine with the addition of either cisplatin or carboplatin (based on cisplatin eligibility). Avelumab maintenance therapy is advised in the absence of disease progression.

If the patient experiences disease recurrence, several second-line options can be proposed. Pembrolizumab has shown favorable results in recurrent aUC after platinum-

based chemotherapy in the Keynote-045 trial and could be recommended as a second-line treatment in ICI-naïve cases. It is preferred that patients who have received ICI (such as nivolumab) with platinum-based regimens in the first line and experience disease recurrence benefit from erdafitinib depending on FGFR DNA fusions and mutations or enfortumab vedotin [58]. Sacituzumab govitecan has been also proven to be effective in cases with disease progression after receiving platinum chemotherapy with PD-1/PD-L1 inhibitors as shown in the TROPHY-U-01 trial [45].

Recommendations for platinum-ineligible patients

Immune checkpoint inhibitor monotherapy can be recommended in the first-line if patients are unfit or lack access to enfortumab vedotin plus pembrolizumab or platinum-based regimens. Pembrolizumab monotherapy in this case is indicated based on the KEYNOTE-052 phase II study which has proven positive response rates as previously mentioned in the first section [34]. After experiencing disease relapse post-ICI monotherapy, patients can benefit from enfortumab vedotin based on the findings of the EV-201 phase II trial. In this study comprising 91 such patients, enfortumab vedotin yielded an ORR of 52%, with complete and partial response rates of 20% and 31% respectively [60].

Further research is needed to determine the optimal second-line treatment following the first-line combination

of Pembrolizumab and Enfortumab Vedotin. In addition to efficacy, factors such as cost differences and toxicity profiles must be considered when selecting subsequent therapies. Figure 2 presents a proposed algorithm outlining potential treatment sequences for advanced urothelial carcinoma (aUC).

UNCHARTED WATERS: NAVIGATING OBSTACLES AFTER EV-302

With the introduction of novel combinations into the treatment arsenal against advanced-stage urothelial carcinoma, several dilemmas arise, and questions remain unanswered. For instance, is there a specific population more likely to respond to pembrolizumab + enfortumab vedotin as a first-line treatment? Subgroup analyses are necessary to correctly stratify patients and ensure an effective personalized treatment plan. Moreover, there is still uncertainty around the efficacy of these new combinations in subgroups that have already received adjuvant immunotherapy.

Predictive and prognostic biomarkers are also lacking in the aUC sphere, with an unmet need for markers such as ctDNA that could help modify the administered medication in terms of escalation or cessation. Liquid biopsy could in fact aid in monitoring disease progression in a non-invasive manner which could lead to a possible reduction of adverse effects. ctDNA is secreted into the bloodstream by apoptotic tumor cells. It is used in

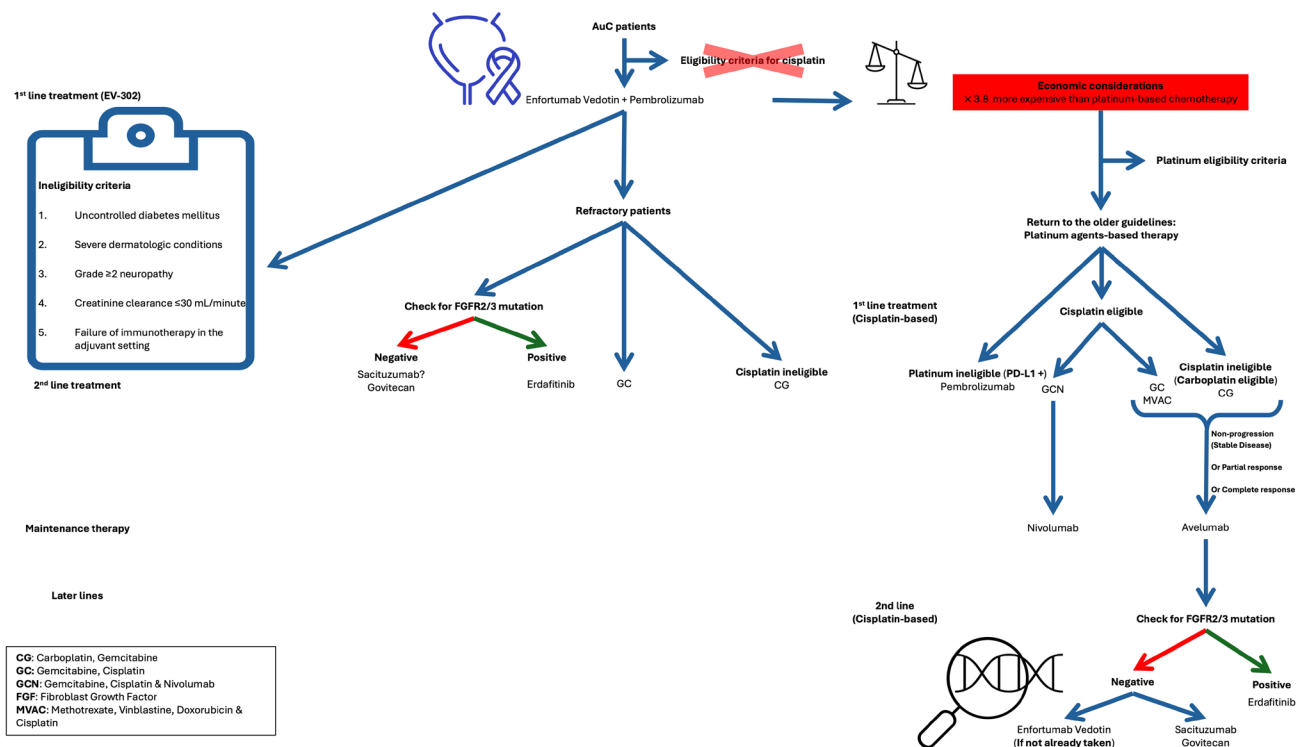


Figure 2: New sequence of treatment based on Phase III trial (EV-302). Bladder cancer Icon made by cube29 from <https://www.flaticon.com/>.

Table 4: Pivotal clinical trials in patients with aUC

Trial name	Agents	Type	Setting	Year	Trial	ORR/mPFS/ mOS	Toxicities
EV-302; NCT04223856	Enfortumab, Vedotin and Pembrolizumab	ADC	First line	2024	Phase III [56]	–/12.5 mo/ 31.5 mo”	Peripheral neuropathy, ocular, pulmonary, and cutaneous toxicities, hyperglycemia, and immune-related adverse events
–	MVAC	Cytotoxic and anti-angiogenic	First line, cisplatin-fit	1992	[26]	39%/10 mo/ 12.5 mo	Myelosuppression, neutropenic fever, sepsis, mucositis, and nausea and vomiting
Protocol No. 30924	Gemcitabine and cisplatin	Cytotoxic	First line, cisplatin-fit	2000 and 2005	Phase III [27]	49%/7.4 mo/ 13.8 mo and –/ 7.7 mo/14 mo	Lower rates of neutropenia, neutropenic sepsis, and mucositis
NCT03036098 ONO-4538-X41	Cisplatin, Gemcitabine and Nivolumab	Cytotoxic and ADC	First line, cisplatin-fit	2023	Phase III [35, 50]	57.6%/7.9 mo/ 21.7 mo	Neutropenia and thrombocytopenia
EORTC study 30986	Gemcitabine and Carboplatin	Cytotoxic	First line, cisplatin-unfit	2011	Phase II/III [32]	42.1%/5.8 mo/ 9.3 mo	lower rates of neutropenia and febrile neutropenia, but higher incidence of serious thrombocytopenia
KEYNOTE-052 NCT02335424	Pembrolizumab	Anti-PD-L1	First line, platinum-unfit	2017	Phase II [55]	29%/2 mo/ 11 mo	Incidence of severe or life-threatening toxicities is low
JAVELIN Bladder 100 trial NCT02603432	Avelumab	Anti-PD-L1	Maintenance therapy	2023	Phase III [38, 65]	–/5.5 mo/ 23.8 mo	Urinary tract infection, diarrhea, and arthralgias
BLC2001 study; NCT02365597 NCT03390504	Erdafitinib	FGFR inhibitor	Later lines, after platinum therapy and immunotherapy, FGFR2/3 positive patient	2022 and 2023	Phase II [40] and Phase II [22]	40%/–/11 mo and 45.6%/5.6 mo/ 12.1 mo	Palmar-plantar erythrodysesthesia, stomatitis, onycholysis, hyperphosphatemia, and diarrhea
EV-301	Enfortumab vedotin	FGFR inhibitor and Cytotoxic	Later lines, after platinum therapy and immunotherapy, FGFR2/3 negative patient	2022	Phase III [44, 53]	41%/6 mo/ 13 mo	Rash, peripheral neuropathy, and hyperglycemia, ocular toxicities, pneumonitis, and severe cutaneous adverse reactions
TROPHY-U-01; NCT03547973	Sacituzumab and govitecan	Antibody-drug conjugate targeting Trop-2	Later lines, after platinum therapy and immunotherapy, FGFR2/3 negative patient	2021	Phase II [45]	27%/11 mo/ 5 mo	Neutropenia, leukopenia, anemia, diarrhea, and febrile neutropenia
EV201; NCT03219333	Enfortumab and vedotin	FGFR inhibitor and Cytotoix	Later lines, cisplatin-unfit therapy after immunotherapy, FGFR2/3 negative patient, refractory aUC	2021	Phase II [60]	52%/5.8 mo/ 14.7 mo	–

Abbreviations: ADC: antibody-drug conjugate; mo: months; MVAC: Methotrexate, Vinblastine, Doxorubicin and Cisplatin; FGF: fibroblast growth factor; ORR: objective response rate; mPFS: median progression-free survival; mOS: median overall survival.

urothelial carcinoma for MRD assessment and follow-up after surgical treatment and chemotherapy [61, 62]. Further research needs to be done concerning the use of ctDNA monitoring in patients receiving the combination of pembrolizumab and enfortumab vedotin.

Ultimately, the increasing cost of such drugs and the potential resistance mechanisms that have not yet been elucidated are challenges that could arise with the new adoption of these combinations. New clinical trials are underway to validate the effectiveness of this combination, including EV-304, a phase 3 study evaluating the regimen of Pembrolizumab + Enfortumab Vedotin against the standard Neoadjuvant Gemcitabine and Cisplatin in Cisplatin-eligible participants with muscle-invasive bladder cancer. The primary endpoint is the Event-Free Survival [63]. Another ongoing clinical trial, EV-303, is a phase 3 study with three arms: Pembrolizumab + Surgery, Surgery alone, and Enfortumab Vedotin + Pembrolizumab + Surgery. It involves participants with muscle-invasive bladder cancer (MIBC) who are either ineligible for cisplatin or have declined it. The primary endpoint is the Event-Free Survival [64]. Other trials with published results are represented in Table 4.

In conclusion, the management of aUC has seen notable advancements, offering patients a variety of promising treatment options aimed at enhancing outcomes. Recent research has highlighted the effectiveness of pembrolizumab and enfortumab vedotin, solidifying their position as compelling choices for first-line therapy. Nevertheless, there is a pressing need for further investigation and clinical trials to better understand the implications of implementing these treatments as initial interventions. Continued efforts in research and collaboration are crucial to improve treatment strategies for individuals with aUC.

Abbreviations

ADCs: Antibody-drug conjugates; aUC: Advanced-stage urothelial carcinoma; BC: Bladder cancer; CPS: Combined positive score; CR: Complete Response; FGFR: Fibroblast Growth Factors; GC: Gemcitabine-cisplatin; GCN: Gemcitabine-cisplatin-nivolumab; ICIs: Immune checkpoint inhibitors; MCAVI: Methotrexate, carboplatin, and vinblastine; MIBC: Muscle-invasive bladder cancer; NMIBC: Non-muscle invasive bladder cancer; MVAC: Methotrexate, vinblastine, doxorubicin, and cisplatin; ORR: Objective response rate; OS: Overall survival; PFS: Progression-free survival; UC: Urothelial carcinoma.

AUTHOR CONTRIBUTIONS

Elias Karam: Conceptualization, primary drafting of the manuscript, critical review, and final editing. Céline Yaghi: Data collection, literature review, drafting of sections related to molecular subtypes and predictive

biomarkers. Gilles Prince: Preparation of figures and tables, assistance in literature search, and drafting of sections related to standard of care and treatment algorithms. Fouad Attieh: Contribution to the writing and analysis of immunotherapy and antibody-drug conjugate sections. Hampig Kourie: Critical revision of the manuscript for important intellectual content, and supervision of clinical recommendations. Joseph Kattan: Supervision, expert input in urothelial carcinoma management guidelines, critical appraisal of clinical trials discussed. Elie Nemer: Review and interpretation of clinical trial data, manuscript revision.

CONFLICTS OF INTEREST

Authors have no conflicts of interest to declare.

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