Recent Advances in the Development of Antibody-Drug Conjugates in Urothelial Cancer

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Abstract: Antibody-drug conjugates (ADCs) have joined the armamentarium against urothelial cancer (UC) as an effective therapy option. Since 2019, the US Food and Drug Administration has approved 2 ADCs for advanced previously treated UC: enfortumab vedotin, which targets nectin-4 and sacituzumab govitecan, which targets trophoblast cell-surface antigen 2. These ADCs are now being tested in earlier disease settings and in previously untreated patients. Furthermore, novel ADCs (e.g., anti–HER-2) are being tested in the clinic and show promising clinical benefit. The next frontier is to understand the mechanisms of resistance and response, gaining experience with ADC-related adverse events and learning the best strategy to sequence and combine these agents with existing therapies. Here, we highlight the recent advances in the development of ADCs for treating localized and metastatic UC.

Key Words: Antibody-drug conjugates, HER-2, nectin-4, Trop-2, tumor-associated antigens, urothelial carcinoma

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Bladder cancer is one of the 10 most common forms of cancer with more than half a million cases diagnosed each year across the globe.[1] In the United States and Europe, urothelial carcinoma (UC) is the most common histology of bladder cancer.[2,3] Platinum-based chemotherapy has been the cornerstone of frontline treatment of patients with UC.[4] Antibody-drug conjugates (ADCs) have emerged as a new therapeutic option for platinum-refractory and checkpoint inhibitor (CPI)–refractory disease. Urothelial carcinoma represents a suitable candidate for ADCs, as it expresses unique surface tumor-associated antigens (TAAs) that allow for specific targeting, delivering cytotoxic drugs directly to tumor cells, and limiting systemic adverse effects.[5] Enfortumab vedotin (EV) was among the first ADCs to attract attention for the treatment of UC.[6] In December 2019, the US Food and Drug Administration (FDA) granted accelerated approval of EV for the treatment of metastatic UC.[7] Five months later, in April 2021, the US FDA granted another accelerated approval to sacituzumab govitecan (SG) for the treatment of a similar patient population.[8] In this review, we highlight the success and challenges in the clinical development of ADCs in the treatment of localized and metastatic UC.

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STRUCTURE OF ADCS

Antibody-drug conjugates are often composed of a target-specific antibody, a linker, and a payload (Table 1).

Choice of Targets and Antibodies

The target TAA ideally should have a preferential expression,[9] surface location,[10] and the ability to internalize and transport the ADC into the cell.[11] However, internalization might not be necessary in some ADCs as they may be cleaved extracellularly or via other mechanisms.[12] Regarding the antibody moiety, it is preferred to have high specificity,[13] high affinity and low immunogenicity.[14] The development of antibodies in ADCs has gone through 3 generations starting with murine antibodies and then humanized/chimeric antibodies, and finally reaching fully human antibodies.[15]

Choice of the Linker

The linker is a key component of ADCs that plays an essential role in their activity and tolerance.[16] When antibodies circulate in the body, the linker's main role is to prevent cytotoxic drug release in off-target tissue, keeping the ADC in a nontoxic state while circulating, but at the same time should have the property of unleashing the cytotoxic drug upon internalization. There are 2 types of linkers that ensure the aforementioned conditions: cleavable and noncleavable linkers.

Cleavable linkers are designed to be cleaved by responding to an environmental difference between the extracellular and intracellular environments (pH, redox potential, etc.) or by specific lysosomal enzymes. In most cases, the linkers in this class are designed to release the payload after bond cleavage. A cleavable linker can be a hydrazone linker that releases the payload after interring acidic endosomes or lysosomes. Cathepsin B–responsive linker responds to lysosomal proteases that are overexpressed in various cancer cells and involved in numerous oncogenic processes in humans. Disulfide linkers are glutathione-sensitive linker relying on the higher concentration of reducing molecules such as glutathione in the cytoplasm compared with the extracellular environment and at the same time resists reductive cleavage in circulation. Pyrophosphate diester linkers, which are novel cleavable linkers with an anionic structure, have greater aqueous solubility than traditional linkers, with excellent circulatory stability. Furthermore, upon internalization, the pyrophosphate diester is promptly

cleaved through the endosomal-lysosomal pathway to liberate unmodified payloads.[17]

Noncleavable linkers consist of more stable bonds that are resistant to proteolytic degradation, relying on complete degradation of the antibody component of ADC by proteases, which eventually liberates the payload.

Choice of the Payload

Another important component of ADCs is the cytotoxic payload, which is typically a small cytotoxic molecule that gets released inside the cytoplasm of tumor cells with a subnanomolar half-maximal inhibitory concentration value for cancer cell lines and sufficient solubility in the aqueous environment of antibody. The 2 main classes of payloads are microtubule-disrupting agents (e.g., auristatin in monomethyl auristatin E [MMAE] and mertansine in drug maytansinoid 1) and DNA-damaging agents (e.g., calicheamicin and deruxtecan).[18]

EXISTING AND EMERGING TARGETS IN UC

Nectin-4

Enfortumab Vedotin

The differential expression of nectin-4 in UC led to the development of EV as a novel ADC. Enfortumab vedotin is a fully human antibody against nectin-4 linked via a cleavable drug linker to a payload microtubule-disrupting chemotherapy agent: MMAE.[6]

EV as a Single Agent in Metastatic Disease

In the phase I dose escalation trial (EV-101, NCT02091999), 112 patients with metastatic UC were treated with 1.25 mg/kg of EV on days 1, 8, and 15 of 28-day cycles.[19] Of the enrolled patients, 81% had received prior platinum therapy and 75% had received prior CPIs. Confirmed overall response rate (ORR) was 42%, median duration of response (DoR) was 7.7 months, and median overall survival (OS) was 12.5 months.[19] The encouraging EV-101 data in heavily pretreated patients with metastatic UC held true in the phase II trial (EV-201, NCT03219333) which confirmed a response rate of 42%.[20] Treatment-related adverse events (TRAEs) included rash, peripheral neuropathy, and hyperglycemia.[20] Based on these results, EV was granted an accelerated approval by the US FDA in December 2019.[7] An open-label randomized phase III multicenter trial (EV-301, NCT03474107) confirmed the survival advantage of EV over standard-of-care chemotherapy with median OS of 12.9 versus 9.0 months, respectively (hazard ratio, 0.70; 95% confidence interval, 0.56–0.89)[21] (Table 1). Based on the level 1 evidence, EV was granted regular approval from the FDA in July 2021 for patients with metastatic UC who have previously received prior CPI and platinum-containing chemotherapy or are ineligible for cisplatin-containing chemotherapy and have previously received 1 or more prior lines of therapy.[8] Of note, a boxed warning for serious skin reactions,[22] including Stevens-Johnson syndrome and toxic epidermal necrolysis,[23] and a warning for pneumonitis were added to EV's drug label.

EV in Combinations for Metastatic Disease

The EV-103 trial (NCT03288545) is a multicohort study assessing various combinations of EV in patients with metastatic and localized UC. The trial has cohorts for first- or second-line setting as well as cohorts for cisplatin-eligible and -ineligible patients: cohorts A through G as expansion cohorts and cohort K as a randomized cohort. Cohort A is assessing EV combined with pembrolizumab (P) for cisplatin-ineligible patients and has shown

TABLE 1. Comparing Different ADCs and BTC Currently Studied in Urothelial Cancer

Drug Name	Target	Function of Target	Antibody/Peptide	Linker	Spacer	Payload	Payload's Mechanism of Action
EV	Nectin-4	Nectin-4/nectin-4 interaction for cell adhesion Nectin-4/TIGIT interaction inhibitory immune signal	Fully human antibody	Protease-cleavable maleimidocaproyl valine-citrulline	N/A	MMAE	Microtubule-disrupting chemotherapy
BT8009	Nectin-4	Same as above	Bicyclic peptide	Cleavable	Sarcosine chain	MMAE	Microtubule-disrupting chemotherapy
SG	Trop-2	Has several ligands (e.g., claudin-1, claudin-7, cyclin D1, and insulinlike growth factor 1) and is a calcium signal transducer	Humanized monoclonal antibody	pH sensitive cleavable/ hydrolyzable	N/A	SN-38	Topoisomerase I inhibitor leading to DNA damage
DS-1062a or Dato-DXd	Trop-2	Same as above	Humanized monoclonal antibody	Protease-cleavable tetrapeptide-based	N/A	DXd	Topoisomerase I inhibitor leading to DNA damage
RC48 or disitamab vedotin	HER-2	A membrane tyrosine kinase, epidermal growth factor receptor 2	Humanized monoclonal antibody	Protease-cleavable maleimidocaproyl valine-citrulline	N/A	MMAE	Microtubule-disrupting chemotherapy
DS8201a or T-DXd	HER-2	Same as above	Humanized monoclonal antibody	Protease-cleavable tetrapeptide-based	N/A	DXd	Topoisomerase I inhibitor leading to DNA damage

BTC indicates Bicycle toxin conjugate; N/A, not applicable.

TABLE 2. Phase III ADC Trials in Metastatic Urothelial Cancer

Trial (NCT Identifier)	Intervention	Comparator	Phase	Sample Size	Primary Outcome	Results
EV-301 (NCT03474107)	EV (arm A)	Treatment of physician choice (TPC) (paclitaxel, docetaxel, or vinflunine) (arm B)	3	608	OS	Median OS was 12.9 mo in arm A vs. 9.0 mo in arm B (HR, 0.70; 95% CI, 0.56–0.89; $P = 0.001$)
EV-302 (NCT04223856)	EV + pembrolizumab (arm A)*	Platinum-based chemotherapy (arm B)	3	990	PFS, OS	NR
TROPiCS-04 (NCT04527991)	SG (arm A)	TPC (paclitaxel, docetaxel, or vinflunine) (arm B)	3	696	OS	NR

Enfortumab vedotin + pembrolizumab + platinum-based chemotherapy (arm C) was present in the original study and removed after a modification.[25]
CI indicates confidence interval; HR, hazard ratio; NR, not reported.

promising results in 45 patients[24] with a confirmed ORR of 73.3% including 15.6% complete responders. Responses seemed durable with a median DoR of 25.6 months. However, grade ≥3 TRAEs occurred in 64.4%. Cohort K randomized similar previously untreated cisplatin-ineligible patients to EV as monotherapy or in combination with P. One hundred forty-nine patients were treated, and results were recently reported at the European Society of Medical Oncology Congress 2022 showing a confirmed ORR for EV + P of 64.5% (median DOR was not reached) and confirmed ORR for EV of 45.2% (median DOR was 13.2 months). Although not compared with the currently approved P single agent, these results are promising and may change the treatment landscape for cisplatin-ineligible patients. Another ongoing phase Ib trial (NCT04963153) is assessing EV in combination with erdafitinib in previously treated patients harboring somatic FGFR2/3 genomic alterations. On the other hand, NCT04878029 and NCT03606174 are studying the safety and efficacy of EV in combination with other tyrosine kinase inhibitors (cabozantinib and sitravatinib, respectively) regardless of genomic profile. Enfortumab vedotin-302 (NCT04223856) began as a global 3-arm, open-label, randomized phase III study evaluating the efficacy and safety of EV + P (arm A) against standard platinum (arm B) in patients with previously untreated metastatic UC.[25] Enfortumab vedotin + pembrolizumab + platinum-based chemotherapy (arm C) was present in the original study and removed after a modification[25] (Table 2).

EV in Localized Disease

Given its success in the metastatic setting, EV is being tested across all stages of UC. In patients with non–muscle-invasive bladder cancer, NCT05014139 is testing the safety of EV via the novel intravesical administration. In muscle-invasive bladder cancer (MIBC), there are 4 trials worth mentioning. First is VOLGA (NCT04960709), which is a global phase III, multicenter, randomized trial to determine the efficacy and safety of durvalumab + tremelimumab + EV or durvalumab + EV for the perioperative treatment in cisplatin-ineligible patients undergoing radical cystectomy. The goal of the study is to explore the triplet combination in terms of efficacy and safety compared with the current standard of care. Second, EV-304/KEYNOTE-B15 (NCT04700124) is a randomized phase III study of perioperative EV + P versus chemotherapy in cisplatin-eligible patients with MIBC. Third, EV-303/KEYNOTE-905 (NCT03924895) is a randomized phase III study evaluating perioperative P or EV + P in cisplatin-ineligible patients with MIBC with the primary outcomes of pathological complete response (pCR) and event-free survival. Lastly, study EV-103 cohort H has reported the promising antitumor activity of neoadjuvant treatment with EV monotherapy in patients with MIBC who are cisplatin ineligible.[26] In the locally advanced or node-positive disease, NCT05239624 is assessing EV + P as first-line therapy with the primary outcome of pCR (Table 3).

EV Pharmacokinetics Studies

NCT04995419 (EV-203) and NCT03070990 are 2 trials assessing the safety and pharmacokinetic of EV in Chinese and Japanese patients, respectively. In NCT03070990, patients were randomized 1:1 to receive 1.0 mg/kg (arm A) or 1.25 mg/kg (arm B) of EV on days 1, 8, and 15 of each 28-day cycle. Pharmacokinetic data suggest a dose-dependent increase in EV maximum concentration and area under the concentration-time curve at day 7. Enfortumab vedotin was well tolerated across both doses.[27]

TABLE 3. ADC Trials in Localized Bladder Cancer

Trial (NCT Identifier)	Intervention (Followed by Cystectomy)	Comparator	Patient Population	Phase	Sample Size	Primary Outcome	Results
VOLGA (NCT04960709)	Durvalumab + tremelimumab + EV (arm 1) or durvalumab + EV (arm 2)	Cystectomy with or without approved adjuvant therapy (arm 3)	Cisplatin-ineligible	3	830	pCR, EFS	NR
EV-303/KEYNOTE-905 (NCT03924895)	Pembrolizumab (arm 1) or EV plus pembrolizumab (arm 2)	Cystectomy (arm 3)	Cisplatin-ineligible	3	857	pCR, EFS	NR
EV-304/KEYNOTE-B15 (NCT04700124)	EV plus pembrolizumab	Gemcitabine plus cisplatin	Cisplatin-eligible	3	784	pCR, EFS	NR

EFS indicates event-free survival; NR, not reported.

Other Agents Targeting Nectin-4

BT8009 is a Bicycle toxin conjugate (BicycleTx, Cambridge, United Kingdom) in which a nectin-4 binding Bicycle (bicyclic peptide) is conjugated through an inert sarcosine spacer chain and a cleavable linker to MMAE. NCT04561362 is a phase I/II, multicenter, first-in-human, open-label dose-escalation study of BT8009 given either as a single agent once weekly or in combination with nivolumab.[28]

Trophoblast Cell-Surface Antigen 2

Sacituzumab Govitecan

Trophoblast cell-surface antigen 2 (Trop-2) is overexpressed in UC.[29–31] Sacituzumab govitecan is a humanized antibody against Trop-2 linked to the cytotoxic payload SN-38 (active metabolite of irinotecan).[32,33]

SG as a Single Agent in Metastatic Disease

NCT03547973 (TROPHY-U-01) is a multicohort, open-label, phase II, registrational study. In cohort 1, 113 patients with metastatic UC who progressed after platinum and CPI were treated with SG 10 mg/kg on days 1 and 8 of 21-day cycles. Overall response rate was 27%, and median DoR was 7.2 months, with median progression-free survival (PFS) and OS of 5.4 and 10.9 months, respectively. Key grade ≥3 TRAEs included neutropenia (35%), anemia (14%), diarrhea (10%), and febrile neutropenia (10%).[34] Based on these results, on April 13, 2021, the US FDA granted accelerated approval to SG after progression on platinum and CPI.[35] Aiming to confirm TROPHY's cohort 1 results, TROPiCS-04 (NCT04527991) is a global, multicenter, open-label trial randomizing SG against standard-of-care chemotherapy (Table 2).

SG in Combinations for Metastatic Disease

At 2022 American Society of Clinical Oncology Genitourinary Cancers Symposium, Grivas et al.[36] presented the interim efficacy and safety results of combining SG with P as second-line therapy in CPI-naive patients with metastatic UC who progressed after platinum-based chemotherapy (cohort 3 of TROPHY-U-01). Overall response rate was 34%, and grade ≥3 TRAEs occurred in 59% of patients.[36] A single-center, open-label, nonrandomized phase I trial (NCT04724018) is testing the safety and efficacy of SG combined with EV in metastatic UC. Another ongoing phase I/II study (NCT04863885) is combining SG with ipilimumab and nivolumab as first-line treatment for cisplatin-ineligible patients with metastatic UC.

SG in Localized Disease

SURE (NCT05226117) is a trial assessing whether SG as a single-agent (SURE-01) or combined with P (SURE-02) results in pCR in cisplatin-ineligible patients with MIBC undergoing radical cystectomy.

Other Agents Targeting Trop-2

Datopotamab deruxtecan (DS-1062a or Dato-DXd) is ADC conjugated, via an enzymatically cleavable tetrapeptide-based linker, to the cytotoxic DNA topoisomerase I inhibitor DX-8951 (deruxtecan). NCT03401385 (TROPION-PanTumor01) is a first-in-human study of Dato-DXd for advanced solid tumors including patients with metastatic UC. Furthermore, SKB264 (Kelun Pharmaceuticals, Sichuan Chengdu, China) and Bio-106 (BiOneCure Therapeutics, Germantown, MD) are novel Trop-2 ADCs that are being tested in a range of solid tumors.[37]

HER-2

Targeting HER-2 in metastatic UC has yielded inconsistent results in the past. RC48-ADC (disitamab vedotin) is a novel humanized anti–HER-2 ADC that has shown efficacy and safety in patients with HER-2–positive metastatic UC. RC48-C005 (NCT03507166) and RC48-C009 (NCT03809013) are 2 trials that evaluated RC48-ADC in 107 patients with HER-2 positive (immunohistochemistry [IHC] 2+ or 3+) UC. In a combined analysis, confirmed ORR was 50.5%, median PFS was 5.9 months, and median OS was 14.2 months.[38] Grade ≥3 TRAEs were numbness (15.0%) and neutropenia (12.1%), with no dose-limiting toxicity reported.[38] Furthermore, RC48-ADC has shown favorable results in HER-2–negative (IHC – or 1+) patients with metastatic UC (NCT04073602). A total of 19 patients were enrolled with ORR of 26.3%. Median PFS was 5.5 months, and median OS was 16.4 months.[39] These promising results encouraged investigating the synergistic efficacy of the combination of RC48-ADC with CPIs. In an open-label, multicenter, phase Ib/II trial (NCT04264936), the safety and the efficacy of RC48-ADC were evaluated in combination with toripalimab (anti–programmed cell death 1 antibody) in patients with metastatic UC. Forty-one patients were enrolled, of which 61% were treatment-naive. HER-2 expression was positive in 59% patients, and programmed cell death 1 ligand was positive in 32%. Preliminary results showed ORR of 82.4% for previously untreated patients and 100% for patients with positive HER-2 and programmed cell death 1 ligand.[40] Grade ≥3 TRAEs were γ-glutamyl transferase increase (12.2%), alanine aminotransferase/aspartate aminotransferase increase (7.3%), asthenia (7.3%), hypertriglyceridemia (4.9%), and neutropenia (4.9%). Another combination trial testing the synergy between HER-2 targeting and CPI is the DS8201-A-U105 trial of trastuzumab deruxtecan (T-DXd) with nivolumab in previously treated HER-2–expressing (with high defined as IHC 2+ or 3+ and low defined as 1+) UC. Overall response rate was 36.7%, median PFS was 6.9 months, and median OS was 11.0 months.[41] Grade ≥3 treatment-emergent AEs occurred in 73.5% and led to drug discontinuation in 32.4% of all patients.

BIOMARKERS OF RESPONSE AND RESISTANCE TO ADC

Resistance to ADC can occur in different mechanisms such as downregulation of TAA (also known as antigen escape), which is demonstrated in tumors resistant to HER-2 targeting ADCs.[42] Other reported resistance mechanisms include decreased sensitivity to payload, decreased receptor internalization, and increased drug efflux pump.[42] Studies have reported that nectin-4 expression is heterogeneous across molecular subtypes of bladder cancer and significantly expressed in luminal subtypes.[43] Nectin-4 expression is thought to be required for EV-induced cell death, and downregulation or loss of nectin-4 may be an important mechanism of resistance and that, furthermore, inducing nectin-4 expression may increase EV sensitivity. The upregulation of nectin-4 expression in tumor cells showed marked increase in sensitivity to EV. Nectin-4 expression level in different UC subtypes showed correlation with EV sensitivity. Nectin-4 mRNA expression was found to be highest in luminal subtypes of bladder cancers, which demonstrated more sensitivity to EV-induced cell death.[44] Downregulation or the loss of nectin-4 in basal or nonluminal subtypes of UC showed more resistance to EV.[43]

ADCS AGAINST NONUROTHELIAL HISTOLOGIES

The activity of ADCs in nonurothelial bladder cancer histologies (e.g., urachal, small cell, etc.) is not clear. Sacituzumab govitecan was studied in patients with pretreated metastatic small cell lung carcinoma. A total of 53 patients were enrolled after receiving a median of 2 prior lines of chemotherapy. Sixty percent of patients showed tumor shrinkage from baseline CT scans. On an intention-to-treat basis (N = 50), the ORR was 14% with the

median DoR of 5.7 months. The study showed a suggested improvement in PFS with SG in second-line patients who were sensitive to first-line therapy. Nonetheless, the ability to extrapolate these data to small cell bladder cancer is limited at this time.[45]

CONCLUSION

Antibody-drug conjugates are active against UC and have provided patients with meaningful clinical benefits. Existing agents are being tested in earlier disease settings and might provide an alternative to the current standard-of-care platinum regimens. Novel agents are being developed using different peptide structures and payloads. The next frontier is to understand the mechanisms of response and resistance, gaining experience with ADC-related adverse effects, and learning the best strategy to sequence and combine these agents with existing therapies.

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