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First-in-Human, Phase I/II Dose Escalation and Expansion Study of Zelenectide Pevedotin in Patients With Advanced Solid Tumors: Results From Monotherapy Dose Escalation

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ABSTRACT

PURPOSE Zelenectide pevedotin (BT8009) is a Bicycle Drug Conjugate comprising a highly selective Nectin-4–targeting Bicycle peptide, linked to monomethyl auristatin E. We report monotherapy dose-escalation results from Duravelo-1 (Phase I/II; ClinicalTrials.gov identifier: NCT04561362).

METHODS Adults with advanced/metastatic solid tumors associated with Nectin-4 expression received zelenectide pevedotin intravenously at 2.5, 5.0, or 7.5 mg/m² once weekly on a 28-day cycle; or 7.5 mg/m² on days 1 and 8 of a 21-day cycle; or 7.5 or 10.0 mg/m² once every 2 weeks on a 28-day cycle. Primary objectives were to evaluate safety and tolerability; antitumor activity and pharmacokinetic characterization were secondary objectives.

RESULTS Forty-nine patients, most with urothelial carcinoma (UC; 25 of 49), received three previous lines of therapy (median). Common treatment-related adverse events (TRAEs) included nausea (49% [grade 3/4 2%]), likely because of a lack of prophylactic antiemetics during the dose-limiting toxicity period, and fatigue (39% [grade 3/4 6%]). The most common TRAEs of clinical interest were peripheral neuropathy (33% [grade 3/4 2%]), neutropenia (22% [grade 3/4 16%]), and skin reactions (22% [grade 3/4 2%]). The maximum tolerated dose was 7.5 mg/m² once every 2 weeks; the recommended phase 2 doses were 5.0 mg/m² once weekly and 7.5 mg/m² on days 1 and 8 of a 21-day cycle. Across doses (efficacy-evaluable; all tumor types), the objective response rate (ORR) was 24% and the clinical benefit rate (CBR) was 48% (n = 10 of 42; 95% CI, 12.1 to 39.5); the ORR was 38% and the CBR was 57% for patients with UC (n = 8 of 21; 95% CI, 18.1 to 61.6). The median duration of response and the median follow-up for all patients were 11.1 and 7.4 months, respectively.

CONCLUSION Zelenectide pevedotin monotherapy demonstrated a generally well-tolerated safety profile and preliminary efficacy, particularly in UC, supporting investigation of UC and non-UC populations in the expansion phase.

ACCOMPANYING CONTENT

Appendix

Data Sharing Statement

Protocol

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INTRODUCTION

Bicycle molecules are an innovative therapeutic class of synthetic, highly constrained bicyclic peptides that offer the manufacturing and pharmacokinetic (PK) properties of a small molecule with the high binding specificity of a biologic. Bicycle molecules are ideally suited for the targeted delivery of a range of payloads such as cytotoxins to solid tumors.[1-7] Bicycle Drug Conjugates (BDCs) have a short systemic exposure and are largely eliminated by renal excretion. They also have a molecular weight approximately 40 times smaller than antibody-drug conjugates (ADCs).[1-7] BDCs can penetrate tumor tissue and bind their targets with high selectivity. This potentially provides toxin delivery directly to the tumor and reduces toxicity in nontarget tissues.[1,4]

Nectin-4 is a cell adhesion molecule that is expressed in multiple cancers, including bladder (urothelial),[8,9] lung,[10] esophageal,[11] ovarian,[12] breast,[8] pancreatic,[13] and head and neck cancers.[14] Expression of Nectin-4 in tumor tissue may alter cellular functions, promote angiogenesis, and is a marker for poor prognosis.[11,13,15,16] Clinical data for a

CONTEXT

Key Objective
To evaluate the tolerability and efficacy of the Nectin-4–targeting Bicycle Drug Conjugate, zelenectide pevedotin, in patients with Nectin-4–associated advanced solid tumors.

Knowledge Generated
The recommended Phase II doses of zelenectide pevedotin were generally well tolerated, with the incidence of monomethyl auristatin E (MMAE)–associated adverse events generally lower than has been observed with antibody drug conjugates with an MMAE payload. Preliminary efficacy was demonstrated, particularly in urothelial carcinoma. Zelenectide pevedotin represents a promising treatment option in the therapeutic pathway for this patient population and is a potentially well-suited partner for combination therapy in earlier lines of treatment.

Relevance (R.G. Maki)
Pharmacokinetics of novel agents remain an important issue in drug development. The shorter half-life of this agent compared to others agents using an MMAE payload may make zelenectide easier to combine with other therapy.*

*Relevance section written by *JCO* Associate Editor Robert G. Maki, MD, PhD, FACP, FASCO.

Nectin-4–directed ADC have shown significantly improved outcomes versus chemotherapy in patients with locally advanced/metastatic urothelial carcinoma (UC).[17,18]

Zelenectide pevedotin is a first-in-class BDC (Appendix Fig A1, online only), comprising a highly selective Nectin-4–targeting bicyclic peptide conjugated to the cyto-toxin monomethyl auristatin E (MMAE) via a cleavable linker.[2,4] Zelenectide pevedotin binds to Nectin-4–expressing tumor cells, initiating protease cleavage of the linker and release of the MMAE toxin into the tumor microenvironment. Cell membrane–permeable MMAE then acts to disrupt intracellular microtubule dynamics, bringing about arrested mitosis, subsequent apoptosis, and cell death. Unlike with many ADCs, this process does not necessarily require internalization, which may avoid the development of resistance pathways that could emerge via altered endocytic/lysosomal processes or lysosomal toxin release.[2,19]

Preclinical studies of zelenectide pevedotin demonstrated antitumor activity in multiple Nectin-4–expressing solid tumor xenograft models, with rapid systemic clearance predominantly via renal excretion of zelenectide pevedotin, but high penetration and persistence of MMAE in the tumor.[2] These findings supported the initiation of a phase I/II first-in-human clinical trial (Duravelo-1/BT8009-100; ClinicalTrials.gov identifier: NCT04561362). Here, we report on the monotherapy dose-escalation part of Duravelo-1.

METHODS

Study Design

This is a phase I/II open-label, multicenter study of zelenectide pevedotin monotherapy or zelenectide pevedotin in combination with pembrolizumab in patients with advanced solid tumors associated with Nectin-4 expression. The study consists of four parts: monotherapy dose escalation (A-1) and dose de-escalation in combination with pembrolizumab (A-2); dose expansion; renal insufficiency; and supplementary PK. Herein, we report data from Part A-1; additional cohorts are ongoing. The first patient in Part A1 received Cycle (C) 1 Day (D) 1 dosing on September 9, 2020, and the last patient received C1D1 dosing on August 4, 2022.

The trial was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. The protocol was approved by the independent ethics committees and institutional review boards of the participating centers, and all patients provided written informed consent.

Patient Population

Full eligibility criteria are presented in the Study Protocol. Briefly, patients 18 years and older from the United States and Europe with advanced/metastatic measurable disease per RECIST v1.1, and who had exhausted all standard treatment options, were eligible for Part A-1. Eligible tumor types were UC with pure or predominant transitional cell histology, pancreatic, breast, lung, gastric, esophageal, head and neck, or ovarian cancer. After the 5.0 mg/m² once weekly dose level was found to be tolerable, backfill patients were added to enrich the population for patients with UC. Patients with grade ≥2 peripheral neuropathy or uncontrolled brain metastases at baseline were excluded.

Nectin-4 Expression

All patients were required to provide formalin-fixed paraffin-embedded tumor tissue at baseline (fresh or archival) for

central laboratory evaluation of Nectin-4 expression. A validated cutoff of H-score ≥100 (medium-high Nectin-4 expression) in either the tumor membrane or tumor cytoplasm was used to determine eligibility during prospective testing.[20] Additional details are provided in Appendix 1.

Dose Escalation

All patients received a 60-minute intravenous infusion of zelenectide pevedotin either:

- once weekly at 2.5, 5.0, or 7.5 mg/m^2 (days 1, 8, 15, and 22 of a 28-day cycle),
- once every 2 weeks at 7.5 or 10.0 mg/m^2 (days 1 and 15 of a 28-day cycle), or
- on days 1 and 8 of a 21-day cycle at 7.5 mg/m^2.

Treatment was continued until disease progression, intolerability of zelenectide pevedotin, investigator decision, or patient withdrawal.

Dose escalation proceeded with an initial single patient, followed by a 3 + 3 escalation design, with a once weekly dosing schedule and alternative dosing schedules assessed[21] (Fig 1). Additional details are provided in Appendix 1.

End Points

The primary objectives were the assessment of safety and tolerability and definition of the maximum tolerated dose (MTD) and/or recommended phase 2 dose(s) (RP2D), as determined by rates of treatment-emergent adverse events (AEs) and dose-limiting toxicities (DLTs). AEs were graded per the National Cancer Institute Common Terminology

Criteria for Adverse Events v5.0 and reported using MedDRA system organ class (SOC) and preferred terms or via Standardized MedDRA Queries (SMQs). AEs of clinical interest in this study were peripheral neuropathy (SMQs [broad]), hyperglycemia/new-onset diabetes mellitus (SMQs), skin reactions (Severe Cutaneous Adverse Reactions SMQs and AEs within Skin and Subcutaneous Tissue Disorders SOC, excluding alopecia), pneumonitis (preferred term), neutropenia (preferred term), and ocular disorders (AEs within Eye Disorder SOC).

To be evaluable for DLT, patients must have experienced a DLT or received all planned doses during the 28-day assessment period (regardless of dose and schedule). During the 28-day assessment period, occurrence of any AEs from a predefined list of hematologic and nonhematologic AEs considered related to study treatment constituted a DLT (detailed criteria on defined events are presented in Appendix Table A1). Per protocol, no routine prophylactic antiemetics, granulocyte colony-stimulating factor (G-CSF) for management of neutropenia, or other premedications were allowed during the DLT evaluation period. MTD was defined as the highest dose level at which fewer than 33% of patients experienced a DLT.

Preliminary antitumor activity and PK characterization were secondary objectives. Efficacy end points (per RECIST v1.1) included objective response rate (ORR), duration of response (DOR), clinical benefit rate (CBR) (complete response [CR] + partial response [PR] + stable disease ≥16 weeks), and progression-free survival (PFS). The safety population was defined as all patients who received ≥1 dose of study treatment; the efficacy-evaluable (EE) population
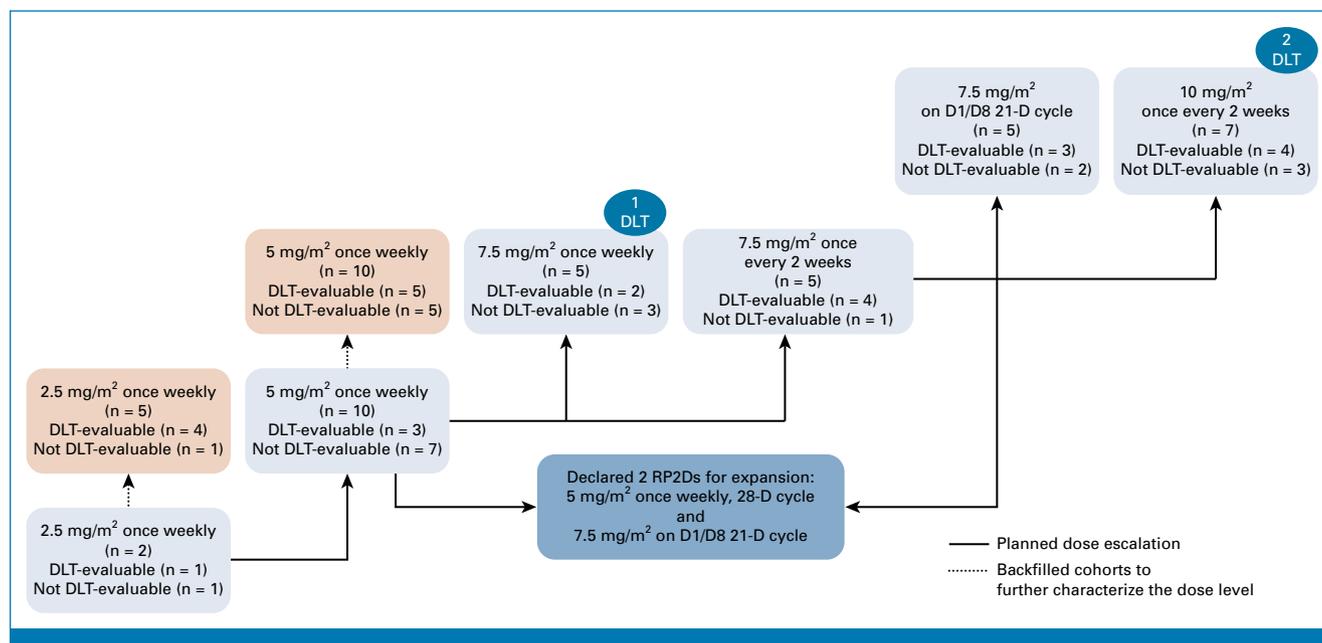

FIG 1. Dose-escalation study schematic of Duravelo-1 (BT8009-100): zelenectide pevedotin monotherapy in patients with advanced solid tumors associated with Nectin-4 expression. The figure shows sequential dosing from bottom to top; dose regimens on the same level were tested in parallel. All cycles were 28 days in length, unless otherwise noted. D, day; DLT, dose-limiting toxicity; RP2D, recommended phase 2 doses.

was defined as all patients who received any dose of study drug and had ≥1 adequate postbaseline response assessment.

Additional details regarding the assessment of DLTs, efficacy and PK end points, and statistical analysis are provided in Appendix 1.

RESULTS

Patient Population

Forty-nine patients were enrolled in Part A-1 from 13 academic centers. UC was the most common tumor type (n = 25 of 49, 51%), including cancers of the bladder (n = 19), urethra (n = 2), renal pelvis (n = 1), and ureter (n = 1); two patients had UC of unknown primary location.

Baseline demographics and clinical characteristics were similar among patients with UC and non-UC patients (Table 1). Briefly, across the total population, the median age was 66 years (range, 35-83), 59% was male, and 61% had an Eastern Cooperative Oncology Group performance status of 1. Patients had received a median of 3 (range, 1-15) previous lines of therapy.

For patients with UC, the median age was 68 years, 80% was male, and all patients had previously received platinum-based therapy and a checkpoint inhibitor. One patient with UC had previously received sacituzumab govitecan, and none had received previous enfortumab vedotin (EV) although this was allowed per protocol.

Nectin-4 expression data were available for 41 of 49 patients (19 of 25 patients with UC), of which 29 (71%) and 13 (68%) had medium-high expression (H-score ≥ 100), respectively. Representative immunohistochemistry images of Nectin-4 staining in breast, lung, and bladder cancers are shown in Appendix Figure A2.

Safety

Safety data were pooled across all doses. All 49 patients treated with zelenectide pevedotin experienced at least one AE, and 46 patients (94%) experienced a treatment-related AE (TRAE; Table 2). The most common TRAEs were nausea (49%), fatigue (39%), and diarrhea and decreased appetite (29% each; Table 3). Grade ≥3 TRAEs occurred in 19 patients (39%), with neutropenia reported most often (16%; Table 3).

AEs led to dose modifications in 63% of cases, most commonly because of neutropenia (n = 8, 16%) and fatigue (n = 6, 12%). Dose interruptions were more frequent than dose reductions (n = 30, 61% and n = 9, 18%, respectively). Dose interruptions were most commonly due to neutropenia (n = 7, 14%) and fatigue (n = 6, 12%). Dose reductions were most commonly due to neutropenia (n = 4, 8%) and asthenia (n = 2, 4%). The median time to first dose reduction was 1.4 months (range, 0.7-14.1). Only two patients (4%)

discontinued zelenectide pevedotin because of AEs (idiopathic intracranial hypertension [grade 3], 2%; sepsis [grade 4], 2%; Table 3). No fatal (grade 5) TRAEs were reported.

TRAEs of clinical interest included peripheral neuropathy (33% [grade ≥2 20%; grade 3/4 2%]), neutropenia (22% [grade 3/4 16%]), skin reactions (22% [grade 3/4 2%]), hyperglycemia/new-onset diabetes mellitus (20% [grade 3/4 2%]; related hyperglycemia [preferred term] 8%), and ocular disorders (10% [grade 3/4 2%]). No patients experienced pneumonitis related to treatment with zelenectide pevedotin. The incidence and time to onset for TRAEs of clinical interest are reported in Appendix Table A2.

Of the 26 patients who were evaluable for DLT, 3 (12%) experienced a DLT: one patient in the 7.5 mg/m² once weekly cohort experienced grade 3 asthenia (ongoing at the time of study withdrawal); one patient in the 10.0 mg/m² once every 2 weeks cohort experienced grade 3 fatigue, decreased appetite, and febrile neutropenia, which led to dose interruption (all events resolved); and a second patient in the 10.0 mg/m² once every 2 weeks cohort experienced grade 4 sepsis for which zelenectide pevedotin was discontinued (event resolved). Twenty-three patients were nonevaluable for DLT, mostly for not receiving all scheduled doses of zelenectide pevedotin in the 28-day evaluation period (Appendix Tables A3 and A4).

Based on all available safety and efficacy data, the Safety Review Committee (SRC) recommended two doses for phase II: 5.0 mg/m² once weekly on a 28-day cycle and 7.5 mg/m² on days 1 and 8 of a 21-day cycle. The MTD was 7.5 mg/m² once every 2 weeks.

PK Profiles

PK parameters and concentration-time profiles for zelenectide pevedotin and MMAE on C1D1 are shown in Appendix Table A5 and Appendix Figure A3, respectively. Across all patients treated with zelenectide pevedotin (N = 49), the median duration of exposure was 13.0 weeks (range, 1.6-101.4) and the median relative dose intensity was 90.5% (range, 46.8-132.4).

Mean exposures (AUC and maximum plasma drug concentration [C_{max}]) of zelenectide pevedotin and MMAE increased in a generally dose-proportional manner with ascending dose. Time to maximum plasma concentration (T_{max}) for zelenectide pevedotin occurred at approximately 1 hour (end of infusion), and the $t_{1/2}$ value ranged from 0.42 to 0.91 hours. For MMAE, T_{max} occurred at approximately 2-3 hours postinfusion, and the $t_{1/2}$ value ranged from 37 to 50 hours. There was no or limited MMAE accumulation in the plasma after once weekly or once every 2 weeks administration of zelenectide pevedotin (C_{max} and AUC for zelenectide pevedotin and MMAE were similar between C1D1 and C1D15 [data not shown]).

TABLE 1. Baseline Demographics and Clinical Characteristics

Characteristic	Overall, N = 49	Non-UC, n = 24	UC, n = 25
Age, years, median (range)	66 (35-83)	62 (35-83)	68 (47-81)
Sex, No. (%)			
Female	20 (41)	15 (63)	5 (20)
Male	29 (59)	9 (37)	20 (80)
Race/ethnicity, No. (%)			
White	31 (63)	19 (79)	12 (48)
Black or African American	1 (2)	1 (4)	0
Other/missing[a]	17 (35)	4 (17)	13 (52)
ECOG PS, No. (%)			
0	19 (39)	7 (29)	12 (48)
1	30 (61)	17 (71)	13 (52)
Smoking status, No. (%)			
Never	16 (33)	10 (42)	6 (24)
Current	4 (8)	2 (8)	2 (8)
Former	28 (57)	11 (46)	17 (68)
Missing	1 (2)	1 (4)	0
Nectin-4 status, No. (%)			
Data available	41 (84)	22 (92)	19 (76)
Positive (medium-high expression)	29 (59)	16 (67)	13 (52)
Negative	12 (25)	6 (25)	6 (24)
Not evaluable	8 (16)	2 (8)	6 (24)
Primary site of diagnosis, No. (%)			
Urothelial system	25 (51)	NA	25 (100)
Bladder	19 (39)	NA	19 (79)
Urethra	2 (4)	NA	2 (8)
Renal pelvis	1 (2)	NA	1 (4)
Ureter	1 (2)	NA	1 (4)
Unknown/missing[b]	2 (4)	NA	2 (8)
Breast	7 (14)	7 (29)	NA
Lung	6 (12)	6 (25)	NA
Pancreas	6 (12)	6 (25)	NA
Head and neck	3 (6)	3 (13)	NA
Esophageal	1 (2)	1 (4)	NA
Ovary	1 (2)	1 (4)	NA
Primary site of metastasis,[c] No. (%)			
Lung	25 (51)	12 (50)	13 (52)
Local/regional lymph nodes	19 (39)	10 (42)	9 (36)
Liver	17 (35)	12 (50)	5 (20)
Bone	17 (35)	9 (38)	8 (32)
Distant lymph nodes	14 (29)	5 (21)	9 (36)
Breast	2 (4)	1 (4)	1 (4)
Skin/subcutaneous	2 (4)	2 (8)	0
Brain	1 (2)	1 (4)	0
Other	18 (37)	10 (42)	8 (32)
Previous lines of therapy, median (range)	3 (1-15)	3.5 (1-15)	3 (1-7)
Types of previous therapies, No. (%)			
Platinum-based[d]	43 (88)	18 (75)	25 (100)
Checkpoint inhibitor[e]	33 (67)	8 (33)	25 (100)
Pan-FGFR inhibitor[f]	3 (6)	0	3 (12)
	(continued on following page)		

TABLE 1. Baseline Demographics and Clinical Characteristics (continued)

Characteristic	Overall, N = 49	Non-UC, n = 24	UC, n = 25
Sacituzumab govitecan	1 (2)	0	1 (4)
Enfortumab vedotin	0	0	0

Abbreviations: ECOG, Eastern Cooperative Oncology Group; FGFR, fibroblast growth factor receptor; NA, not applicable; PS, performance status; UC, urothelial carcinoma.

[a]Because of French ethics laws, data on race/ethnicity are recorded as other for patients enrolled in France; no Asian patients were enrolled in the study.
[b]Contains primary sites listed as urothelial (n = 1) and UC (n = 1).
[c]Forty-seven patients (96%) had locally advanced, recurrent, or metastatic disease at study entry.
[d]Carboplatin, cisplatin, oxaliplatin, and carboplatin/gemcitabine.
[e]Atezolizumab, avelumab, cetrelimab, durvalumab, ezabenlimab, ipilimumab, nivolumab, and pembrolizumab.
[f]Erdafitinib and futibatinib.

Efficacy

As of the data cutoff date of March 04, 2024, the median duration of follow-up was 7.4 months (range, 0.4-31.8) for the overall population and 12.4 months (range, 0.5-28.6) for the UC population.

Of the total 49 patients treated with zelenectide pevedotin, 10 achieved a confirmed response, including 1 CR (UC) and 9 PR (n = 7 UC; n = 1 breast; n = 1 lung), with an ORR of 20% (10 of 49; 95% CI, 10 to 34) and a CBR of 41% (20 of 49). One additional patient with UC had an unconfirmed PR. In 42 EE patients, an ORR of 24% (10 of 42; 95% CI, 12 to 40) and a CBR of 48% (20 of 42) were observed (Table 4). The median DOR was 11.1 months (range, 4.5-21.6) in patients who achieved a confirmed PR or CR. The median PFS for all patients was 3.6 months (95% CI, 1.8 to 6.5) with the 3- and 6-month PFS being 56% (95% CI, 40.0 to 68.7) and 37% (95% CI, 23.2 to 51.1), respectively.

For EE patients with UC (21 of 25), the ORR was 38% (8 of 21; 95% CI, 18 to 62, including one confirmed CR and 7 PR); the CBR was 57% (12 of 21; Appendix Table A6). The median DOR for patients with UC was 13.1 months (n = 8; range, 4.5-21.6), and the median PFS was 7.4 months (95% CI, 1.8 to 12.4), with the 3- and 6-month PFS being 71% (95% CI, 47.2 to 86.0) and 56% (95% CI, 32.5 to 74.3), respectively.

Among all EE patients with medium-high Nectin-4 expression, the ORR was 25% (7 of 28) and the CBR was 61% (17 of 28). Among EE patients with UC who had medium-high Nectin-4 expression, the ORR was 38% (5 of 13) and 33% (1 of 3) in patients with low-negative Nectin-4, suggesting that the relationship between response and membranous Nectin-4 expression may be more complex.

Best overall response is shown in Figure 2. Representative computed tomography scans of one patient demonstrating a 90% reduction in tumor size from baseline are shown in Appendix Figure A4.

DISCUSSION

Nectin-4 is expressed in multiple tumor types, including UC, and represents a promising target for anticancer

TABLE 2. Safety Summary of Zelenectide Pevedotin Monotherapy

Event	2.5 mg/m² Once Weekly (n = 7), No. (%)	5.0 mg/m² Once Weekly (n = 20), No. (%)	7.5 mg/m² Once Weekly (n = 5), No. (%)	7.5 mg/m² Once Every 2 Weeks (n = 5), No. (%)	7.5 mg/m² on D1/8 21-D Cycle (n = 5), No. (%)	10.0 mg/m² Once Every 2 Weeks (n = 7), No. (%)	Overall (N = 49), No. (%)
Any AE	7 (100)	20 (100)	5 (100)	5 (100)	5 (100)	7 (100)	49 (100)
Any TRAEs	7 (100)	17 (85)	5 (100)	5 (100)	5 (100)	7 (100)	46 (94)
AE leading to dose modification	4 (57)	11 (55)	4 (80)	1 (20)	5 (100)	6 (86)	31 (63)
AEs leading to dose interruption	4 (57)	11 (55)	4 (80)	1 (20)	5 (100)	5 (71)	30 (61)
AEs leading to dose reduction	0	4 (20)	2 (40)	0	2 (40)	1 (14)	9 (18)
AEs leading to withdrawal of study treatment	0	0	0	0	1 (20)	1 (14)	2 (4)
Grade ≥3 AEs[a]	4 (57)	12 (60)	4 (80)	2 (40)	5 (100)	6 (86)	33 (67)
Grade ≥3 TRAEs	1 (14)	3 (15)	4 (80)	1 (20)	4 (80)	6 (86)	19 (39)
Any SAE	2 (29)	5 (25)	0	1 (20)	3 (60)	3 (43)	14 (29)
SAEs related to study treatment	0	1 (5)	0	0	2 (40)	3 (43)	6 (12)

Abbreviations: AE, adverse event; D, day; SAE, serious adverse event; TRAE, treatment-related adverse event.
[a]No grade 5 AEs were reported.

TABLE 3. Zelenectide Pevedotin–Related AEs Occurring in ≥10% of Patients and in Patients With Any/All AEs of Grade ≥3

	Overall (N = 49)				
	Any Grade	Grade 1	Grade 2	Grade 3	Grade 4[a]
Patients With Any TRAE	46 (94)	7 (14)	20 (41)	12 (25)	7 (14)
Preferred term, No. (%)					
Nausea	24 (49)	15 (31)	8 (16)	1 (2)	0
Fatigue	19 (39)	9 (18)	7 (14)	3 (6)	0
Decreased appetite	14 (29)	7 (14)	6 (12)	1 (2)	0
Diarrhea	14 (29)	10 (20)	3 (6)	1 (2)	0
Pyrexia	13 (27)	12 (25)	1 (2)	0	0
Alopecia	11 (22)	7 (14)	4 (8)	0	0
Asthenia	11 (22)	4 (8)	5 (10)	2 (4)	0
Neutropenia	11 (22)	2 (4)	1 (2)	5 (10)	3 (6)
Neutrophil count decreased	11 (22)	3 (6)	5 (10)	1 (2)	2 (4)
Peripheral sensory neuropathy	10 (20)	6 (12)	3 (6)	1 (2)	0
Vomiting	8 (16)	5 (10)	2 (4)	1 (2)	0
Anemia	7 (14)	1 (2)	5 (10)	1 (2)	0
ALT increased	6 (12)	5 (10)	0	1 (2)	0
AST increased	6 (12)	5 (10)	1 (2)	0	0
Dyspepsia	6 (12)	3 (6)	3 (6)	0	0
Pruritus	6 (12)	3 (6)	3 (6)	0	0
Constipation	5 (10)	2 (4)	3 (6)	0	0
Hyperglycemia	4 (8)	0	3 (6)	1 (2)	0
Hypokalemia	3 (6)	0	1 (2)	1 (2)	1 (2)
Febrile neutropenia	1 (2)	0	0	1 (2)	0
Hypertension	1 (2)	0	0	1 (2)	0
Idiopathic intracranial hypertension	1 (2)	0	0	1 (2)	0
Intracranial pressure increased	1 (2)	0	0	1 (2)	0
Keratitis	1 (2)	0	0	1 (2)	0
Sepsis	1 (2)	0	0	0	1 (2)
Stomatitis	1 (2)	0	0	1 (2)	0
Ulcerative keratitis	1 (2)	0	0	1 (2)	0

Abbreviations: AE, adverse event; TRAE, treatment-related adverse event.
[a]No grade 5 AEs were reported.

therapies in patient populations with few available treatment options.[8,25] After the recent approval of the MMAE-conjugated Nectin-4–targeting ADC EV by the US Food and Drug Administration, Nectin-4 has become an established therapeutic target for UC.[7,18] ADCs have several limitations, however, including poor tumor penetration and prolonged systemic exposures leading to off-tumor on-target and off-target toxicities.[26,27] BDCs offer a novel therapeutic option to treat Nectin-4–expressing tumors and are distinct from ADCs in both structure and PK properties, with the potential to minimize payload exposure to healthy tissues.[2,4] The dose-escalation part of this study identified two RP2Ds for zelenectide pevedotin of 5.0 mg/m² once weekly on a 28-day cycle and 7.5 mg/m² on days 1 and 8 of a 21-day cycle. At these doses, zelenectide pevedotin showed promising efficacy and a generally well-tolerated safety profile.

Overall, DLTs were experienced by three patients: one patient in the 7.5 mg/m² once weekly cohort and two patients in the 10.0 mg/m² once every 2 weeks cohort. Of note, no anti-emetics, G-CSF, or premedications were allowed during the 28-day DLT evaluation period, which might have contributed to increased rates and severity of AEs. Twenty-three patients were nonevaluable for DLTs in this study; 5 of 23 were enrolled to further characterize doses previously deemed safe and thus were not required to be DLT-evaluable, per protocol. The remainder of nonevaluable patients did not receive all doses within the first 28-day cycle, which was largely attributed to protocol-required dose interruptions for AEs that did not meet the defined DLT criteria.

Ocular disorders,[28,29] skin reactions,[30-32] and peripheral neuropathy[33-35] are known toxicities associated with ADCs containing an auristatin-based payload (such as MMAE). In a

TABLE 4. Efficacy Summary of EE Patients Treated With Zelenectide Pevedotin Monotherapy

Best Confirmed Overall Response	2.5 mg/m² Once Weekly (n = 7), No. (%)	5.0 mg/m² Once Weekly (n = 16), No. (%)	7.5 mg/m² Once Weekly (n = 3), No. (%)	7.5 mg/m² Once Every 2 Weeks (n = 5), No. (%)	7.5 mg/m² on D1/8 21-D Cycle (n = 5), No. (%)	10.0 mg/m² Once Every 2 Weeks (n = 6), No. (%)	Overall (n = 42), No. (%)
CR	0	1 (6)	0	0	0	0	1 (2)
PR	1 (14)	3 (18)	0[a]	0	2 (40)	3 (50)	9 (21)
SD	4 (57)	6 (38)	2 (67)	1 (20)	3 (60)	0	16 (38)
PD	2 (29)	6 (38)	0	4 (80)	0	3 (50)	15 (36)
ORR	1 (14)	4 (25)	0	0	2 (40)	3 (50)	10 (24)
CBR[b]	3 (43)	7 (44)	2 (67)	0	5 (100)	3 (50)	20 (48)

Abbreviations: C1D1, cycle 1 day 1; CBR, clinical benefit rate; CR, complete response; D, day; EE, efficacy-evaluable; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease.

[a]One patient in this cohort had an unconfirmed partial response.

[b]CBR = CR + PR + SD, where SD must be at least 16 weeks from C1D1.

phase III study of EV, 19% of patients reported treatment-related ocular AEs, 45% reported treatment-related rash (6% grade ≥3), and 46% reported treatment-related peripheral neuropathy (2% of patients discontinued for this reason).[7,25] Although the current trial sample size is small, it is notable in this phase I/II study that only 10% (4 of 49 patients; 95% CI, 3.4 to 22.2) of zelenectide pevedotin–treated patients reported any treatment-related ocular AEs (1 at grade 3). Treatment-related skin reactions occurred in 22%, and treatment-related peripheral neuropathy occurred in 33% of patients (20% grade ≥2). Of note, 29% (14 of 49) of patients had grade 1 peripheral neuropathy at study entry and no patients discontinued zelenectide pevedotin because of peripheral neuropathy. The incidence of MMAE-associated AEs with zelenectide pevedotin was generally lower than has been observed with EV, suggesting reduced systemic exposure to MMAE and minimal toxicity to healthy tissues with BDCs.

The PK characteristics of zelenectide pevedotin support the generally well-tolerated safety profile demonstrated in this study. The novel structure and small molecular size of zelenectide pevedotin are anticipated to allow for better penetration of tumors, less toxicity, and fewer off-target interactions than ADCs. Exposure-safety analyses for three MMAE-containing ADCs evaluating both conjugated and unconjugated analytes identified positive relationships predominantly associated only with, or more strongly with, the conjugated rather than the unconjugated MMAE, particularly for skin and ocular toxicities.[36] These observations suggest that toxicities after administration of MMAE-containing ADCs could be mitigated by decreasing exposure to the conjugated parent drug. In line with this, the zelenectide pevedotin $t_{1/2}$ (0.42-0.91 hours) is substantially shorter than those of MMAE-containing ADCs, resulting in extensive elimination of conjugate within hours of dose administration, rather than weeks.[6,7,37-39]

Zelenectide pevedotin demonstrated promising preliminary antitumor activity in this heavily pretreated population at a median follow-up of 11.1 months, with a CBR of 48% and a confirmed ORR of 24% in EE patients with advanced solid tumors. In the subset of patients with UC, the CBR was 57% and the ORR was 38%, with one patient achieving a CR. The ORR in this study is encouraging, particularly for patients with UC, as similar[25] or lower[40] response rates have been reported in larger populations of patients with UC treated with available ADCs. A median PFS of 7.4 months among patients with UC is also promising for these heavily pretreated patients. All patients in this analysis were EV-naïve. An initial eligibility criterion for Duravelo-1 excluded Nectin-4–targeting agents, and although this was updated, all, but one, were consented and enrolled before EV availability.

Because of the high prevalence of Nectin-4 expression in UC and the early implementation of Nectin-4 selection in non-UC tumor types, most patients enrolled in this study had Nectin-4–expressing tumors. Overall, the ORR for all EE patients was 24% (10 of 42) and 25% for those with medium-high Nectin-4 expression (H score ≥ 100; 7 of 28). For patients with UC in this study, the overall ORR and the ORR among patients with medium-high Nectin-4 expression were the same at 38%. Data suggest that the target expression level does not necessarily correlate with response to EV[41] or sacituzumab govitecan[42] in patients with UC or metastatic bladder cancer, respectively.[43] Given the small sample size and the limited number of Nectin-4–low/Nectin-4–negative tumors in this study, the relationship between the expression level of Nectin-4 and efficacy of zelenectide pevedotin must be evaluated further at the RP2Ds. We have previously identified that the *NECTIN4* gene is amplified in certain patients and that the presence of *NECTIN4* amplification increases the probability of identifying Nectin-4–expressing tumors.[44] More recently, Klumper et al have confirmed that *NECTIN4* amplification may be an effective predictive biomarker for response to Nectin-4–targeted therapy.[45] Exploratory analyses of patients treated in Duravelo-1 support future investigation of *NECTIN4* amplification in a larger data set to identify patients who might have enhanced benefit of zelenectide pevedotin therapy.[46,47]
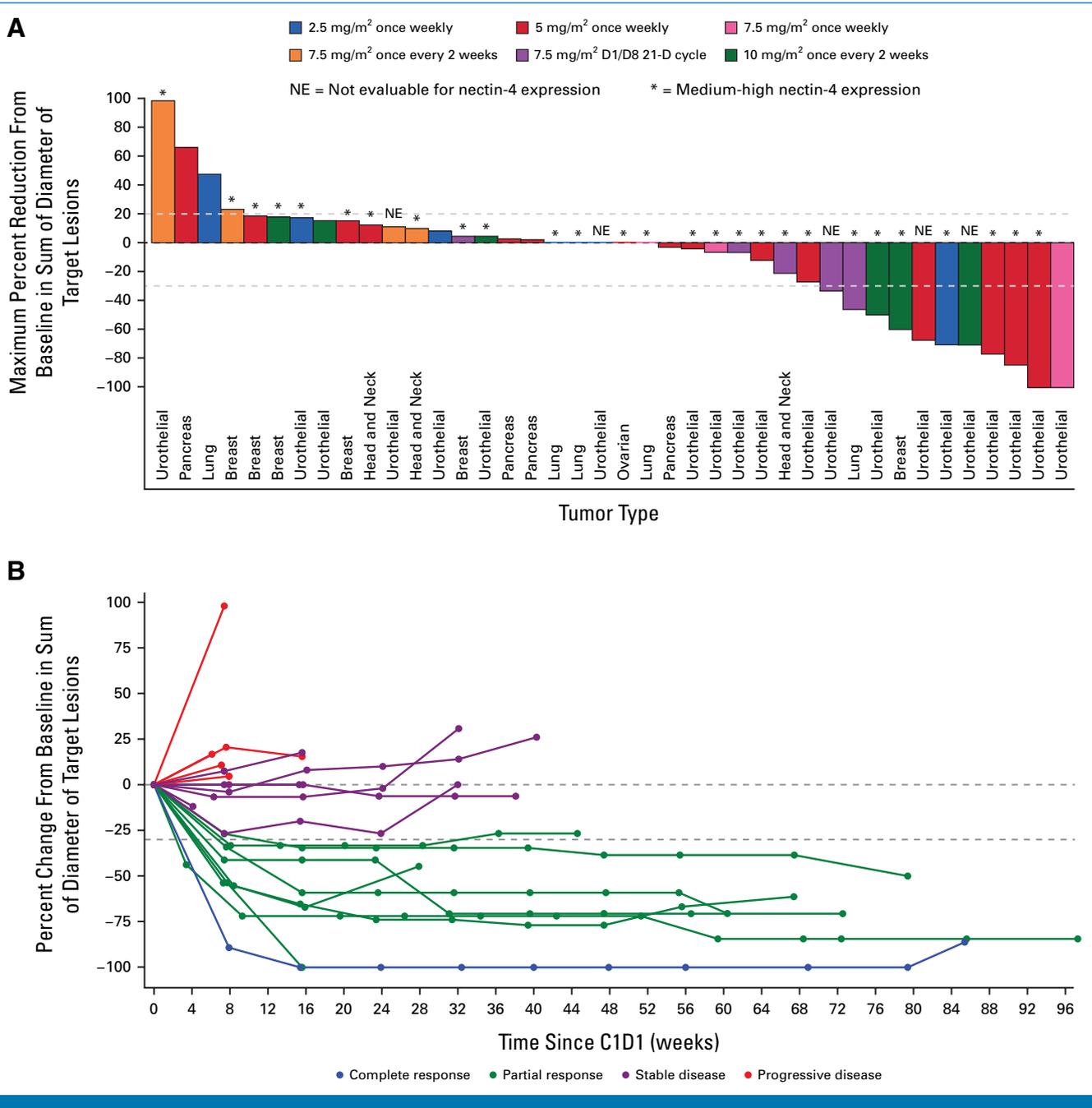
FIG 2. (A) Best overall response for the total EE population (n = 40[a,b,c]) and (B) best overall response and duration of response for EE patients with urothelial carcinoma (n = 21[a,b]) treated with zelenectide pevedotin monotherapy. [a]Data include one unconfirmed response. [b]Two patients (n = 1, 5.0 mg/m² once weekly; n = 1, 7.5 mg/m² once every 2 weeks) had an overall response of PD because of the presence of a new lesion; however, the original baseline target lesion was NE (eg, poor-quality image), so the postbaseline assessment could not be calculated. [c]Patients who did not have a postbaseline assessment were not included. C1D1, cycle 1 day 1; D, day; EE, efficacy-evaluable; NE, not evaluable.

Based on all available data, the SRC determined 5.0 mg/m² once weekly and 7.5 mg/m² on days 1 and 8 of a 21-day cycle to be the RP2Ds. The MTD was calculated to be 7.5 mg/m² once every 2 weeks based on the highest dose intensity below the doses where DLTs were reported. These data support further investigation in expansion cohorts of solid tumors associated with Nectin-4 expression; the phase II expansion portion of the study is ongoing.

Recent data have shown that combining a Nectin-4–targeting agent with pembrolizumab yielded significantly better outcomes (PFS and overall survival) than chemotherapy in patients with untreated locally advanced or metastatic UC,[17] suggesting that combining treatments in this way may be a valuable therapeutic approach. A phase II/III randomized, multicenter, open-label study is now enrolling (Clinical-Trials.gov identifier: NCT06225596) to assess the efficacy

and safety of zelenectide pevedotin as monotherapy and zelenectide pevedotin in combination with pembrolizumab versus chemotherapy in patients with locally advanced/metastatic UC.

In conclusion, zelenectide pevedotin is a novel agent with a generally well-tolerated safety profile that has demonstrated preliminary efficacy, especially in patients with UC at the RP2Ds of 5.0 mg/m² once weekly and 7.5 mg/m² on days 1 and 8 of a 21-day cycle. Zelenectide pevedotin represents a promising treatment option in the therapeutic pathway for this patient population and a potentially well-suited partner for combination therapies in earlier lines of treatment. The results presented herein are promising but must be validated in future planned prospective studies.

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First-in-Human, Phase I/II Dose Escalation and Expansion Study of Zelenectide Pevedotin in Patients With Advanced Solid Tumors: Results From Monotherapy Dose Escalation

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Research Funding: Repare Therapeutics (Inst), Bicycle Therapeutics (Inst), Artios (Inst), Seagen (Inst), Amgen (Inst), Nurix (Inst), BioNTech SE (Inst), Relay Therapeutics (Inst), Taiho Pharmaceutical (Inst), Pfizer (Inst), Roche (Inst), Daiichi Sankyo (Inst), Gilead Sciences (Inst), Basilea (Inst), Jiangsu Hengrui Medicine (Inst), Mereo Biopharma (Inst), HUTCHMED (Inst), Merus (Inst), Crescendo Biologics (Inst), GlaxoSmithKline (Inst), BeiGene (Inst), Turning Point Therapeutics (Inst), Sapience Therapeutics (Inst), Acerta Pharma (Inst), ADC Therapeutics (Inst), Arcus Biosciences (Inst), Astellas Pharma (Inst), AstraZeneca (Inst), Bayer (Inst), Blueprint Medicines (Inst), Boehringer Ingelheim (Inst), Calithera Biosciences (Inst), Carrick Therapeutics (Inst), CASI Pharmaceuticals (Inst), Clovis Oncology (Inst), CytomX Therapeutics (Inst), Deciphera (Inst), Lilly (Inst), Ellipses Pharma (Inst), Exelixis (Inst), Fore Biotherapeutics (Inst), G1 Therapeutics (Inst), Genentech (Inst), H3 Biomedicine (Inst), Ignyta/Genentech/Roche (Inst), Immunocore (Inst), Immunomedics (Inst), Incyte (Inst), Instil Bio (Inst), Iovance Biotherapeutics (Inst), Janssen (Inst), Kronos Bio (Inst), Lupin Pharmaceuticals (Inst), Macrogenics (Inst), Menarini (Inst), Merck KGaA (Inst), Millennium Pharamceuticals (Inst), Nerviano Medical Sciences (Inst), Oxford VacMedix (Inst), Plexxikon (Inst), QED Therapeutics (Inst), Ribon Therapeutics (Inst), Servier (Inst), Stemline Therapeutics (Inst), Synthon (Inst), Tesaro (Inst), PMV Pharma (Inst),

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APPENDIX 1. SUPPLEMENTAL METHODS

Nectin-4 Expression

Nectin-4 expression was assessed retrospectively by immunohistochemistry (IHC) for all patients with urothelial carcinoma and prospectively in most patients with other tumor types, except for an initial single-patient accelerated cohort that was not restricted to this criterion. Nectin-4 protein levels were assessed by IHC using a proprietary rabbit monoclonal anti–Nectin-4 primary antibody (YMW-1-58; Abcam, Burlingame, CA) and a BOND Polymer Refine detection kit (Leica Biosystems, Switzerland AG). Histology (H)-scores (calculated by stain intensity on a scale of 0-3 × % positive tumor cells) were generated by a pathologist. The tumor cell membrane and tumor cell cytoplasm were evaluated independently, generating a unique H-score for each compartment.

Dose Escalation

At all dose levels, the first patient was observed for ≥48 hours before subsequent patients were dosed. The Safety Review Committee (SRC) met regularly, assessing all relevant safety, tolerability, and available pharmacokinetics (PK) data after each dose level. The SRC consisted of the coordinating investigator, the principal investigator, or a delegate from the investigational sites, a medical monitor for the study, and clinical and safety representatives from the responsible contract research organization and the Sponsor.

The zelenectide pevedotin starting dose of 2.5 mg/m^2 once weekly was determined based on preclinical safety analyses accounting for the International Council for Harmonization S9 guidance (Food and Drug Administration, HHS: International Conference on Harmonisation, Fed Regist, Mar 8, 2010, 75:10487-8; International Council for Harmonisation: Harmonised tripartite guideline, 2009, https://database.ich.org/sites/default/files/S9_Guideline.pdf) and first-in-human principles (European Medicines Agency, Committee for Medicinal Products for Human Use [CHMP]: 2017, https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-strategies-identify-and-mitigate-risks-first-human-and-early-clinical-trials-investigational-medicinal-products-revision-1_en.pdf). The clinical exposures of zelenectide pevedotin were predicted using single species allometric scaling from nonhuman primate PK data. A starting dose of 2.5 mg/m^2 once weekly was below the predicted fully efficacious range and below the predicted partial range across numerous cell line– and patient-derived xenograft models tested.

Assessment of Dose-Limiting Toxicities

To be evaluable for dose-limiting toxicity (DLT), patients must have experienced a DLT or received all planned doses during the 28-day assessment period (regardless of dose and schedule). During the 28-day assessment period, occurrence of any adverse events (AEs) from a predefined list of hematologic and nonhematologic AEs considered related to study treatment constituted a DLT (detailed criteria on defined events are presented in Appendix Table A1). Because of the first-in-human nature of this study, attribution of "related" to zelenectide pevedotin was presumed, unless relatedness to the study drug was highly unlikely and plausible alternative etiology was documented. If a patient withdrew from treatment during C1 for any reason other than DLT and did not meet the minimum requirements for inclusion in the maximum tolerated dose–determining population (ie, were declared not DLT evaluable), that patient was replaced.

Efficacy End Points

Response assessments were performed via radiographic imaging at 8-week intervals for the first 12 months and then every 12 weeks thereafter per RECIST v1.1.

PK End Points

PK end points included maximum plasma concentration (C$_{max}$) of zelenectide pevedotin and monomethyl auristatin E (MMAE), AUC, and drug elimination half-life (t$_{1/2}$). Blood samples for PK assessment were collected before dosing and throughout the infusion and up to 6 hours postinfusion on C1D1 and C1D15.

Statistical Analyses

Descriptive statistics, including median and range, were calculated for all continuous measures; proportions and frequencies were calculated for all categorical measures; time-to-event end points were assessed using Kaplan-Meier estimates and 95% CIs. Plasma concentrations of zelenectide pevedotin and MMAE were used to calculate PK parameters via noncompartmental analyses.

TABLE A1. AE Severity Thresholds for Defining DLTs

Type of DLT	Severity Threshold
Hematologic AEs	Grade ≥4 neutropenia lasting >7 days Febrile neutropenia (ANC <1,000/mm³ with a single temperature of 101°F (38.3°C) or a sustained temperature of 100.4°F (38°C) for >1 hour) Grade ≥4 thrombocytopenia or grade 3 thrombocytopenia associated with clinically significant bleeding Grade ≥4 anemia unexplained by underlying disease
Nonhematologic AEs	Grade ≥3 fatigue lasting ≥5 days Any grade ≥3 nonhematologic AE of any duration occurring from the infusion start in C1D1 through predose C2D1 (except for grade ≥3 nausea, vomiting, or diarrhea, ie, responsive to supportive care and lasts ≤3 days) Grade ≥3 hypertension that cannot be returned to grade ≤2 within 72 hours Grade ≥3 infusion-related reaction that does not resolve within 24 hours Potential Hy's law (elevated ALT or AST by >3 times the ULN; TBIL >2 times ULN; ALP <2 times ULN; no other reason for increased ALT/AST)
Events that will not be considered a DLT include the following: Grade ≤3 nausea, vomiting, or diarrhea lasting ≤72 hours Grade ≤3 electrolyte events lasting <72 hours and do not require hospitalization Grade ≤3 ALT/AST increases lasting <5 days and with total bilirubin ≤twice the upper limit of normal Grade 3 laboratory abnormalities that are not considered clinically relevant or clinically significant in the opinion of the investigator AEs clearly related to disease, pre-existing conditions, or environmental factors	

Abbreviations: AE, adverse event; ALP, alkaline phosphatase; ANC, absolute neutrophil count; C1D1, cycle 1 day 1; C2D1, cycle 2 day 1; DLT, dose-limiting toxicity; TBIL, total bilirubin; ULN, upper limit of normal.

TABLE A2. AEs of Clinical Interest for Zelenectide Pevedotin

Event	Overall (N = 49)	
	Any Grade, No. (%) [95% CI]	Grade 3, No. (%)
Peripheral neuropathy[a]	19 (39) [25.2 to 53.8]	1 (2)
Related to zelenectide pevedotin	16 (33) [19.9 to 47.5]	1 (2)
Time to onset of related events, months, median (range)	2.2 (0.1-14.1)	
Hyperglycemia[b]	17 (35) [21.7 to 49.6]	1 (2)
Related to zelenectide pevedotin	10 (20) [10.2 to 34.3]	1 (2)
Time to onset of related events, months, median (range)	1.4 (0.1-3.5)	
Skin reactions[c]	16 (33) [19.9 to 47.5]	1 (2)
Related to zelenectide pevedotin	11 (22) [11.8 to 36.6]	1 (2)
Time to onset of related events, months, median (range)	0.3 (0.1-6.3)	
Neutropenia[d]	11 (22) [11.8 to 36.6]	5 (10)[e]
Related to zelenectide pevedotin	11 (22) [11.8 to 36.6]	5 (10)[e]
Time to onset of related events, months, median (range)	0.5 (0.2-4.6)	
Ocular disorders[f]	8 (16) [7.3 to 29.7]	1 (2)
Related to zelenectide pevedotin	5 (10) [3.4 to 22.2]	1 (2)
Time to onset of related events, months, median (range)	2.6 (0.4-13.6)	

Abbreviations: SMQs, Standardized MedDRA Queries; SOC, system organ class.
[a]MedDRA peripheral neuropathy SMQs (broad).
[b]MedDRA hyperglycemia/new-onset diabetes mellitus SMQs.
[c]MedDRA Severe Cutaneous Adverse Reactions SMQs and events that fell into MedDRA SOC of Skin and Subcutaneous Tissue disorders, excluding alopecia.
[d]MedDRA preferred term.
[e]Three patients experienced grade 4 events that were deemed treatment-related.
[f]Ocular disorders were adverse events that fell into the MedDRA SOC of eye disorders.

TABLE A3. Patient Disposition—DLTs Associated With Zelenectide Pevedotin

Patient	2.5 mg/m² Once Weekly (n = 7), No.	5.0 mg/m² Once Weekly (n = 20), No.	7.5 mg/m² Once Weekly (n = 5), No.	7.5 mg/m² Once Every 2 Weeks (n = 5), No.	7.5 mg/m² on D1/8 21-D Cycle (n = 5), No.	10.0 mg/m² Once Every 2 Weeks (n = 7), No.
DLT-evaluable	5	8	2	4	3	4
DLT nonevaluable	2	12[a]	3	1	2	3
Reason nonevaluable						
Dose interruption because of non-DLT AE[b]	2	7	2	1	2	3
Missed visit for nonmedical reason	0	1	0	0	0	0
Progressive disease	0	3	0	0	0	0
Delayed data entry	0	1	0	0	0	0
Patient withdrew consent during DLT period	0	0	1	0	0	0

Abbreviations: AE, adverse event; D, day; DLT, dose-limiting toxicity.
[a]Five of the 12 nonevaluable patients enrolled after the safety review committee reviewed the safety and pharmacokinetics data to further characterize the 5.0 mg/m² once weekly dose.
[b]AE did not meet the threshold for DLT.

TABLE A4. AEs Leading to Dose Interruption During the DLT Evaluable Period (Cycle 1)

AE	No.	Grade	Related or Unrelated
2.5 mg/m² once weekly			
Hypertension	1	3	Unrelated
Pulmonary embolism	1	3	Unrelated
5.0 mg/m² once weekly			
Neutropenia	2	2	Related
		4	Related
Anemia	2	3	Unrelated
		3[a]	Unrelated
Fatigue	2	2	Related
		2[a]	Related
Asthenia	1	3	Related
Transaminitis	1	2	Related
7.5 mg/m² once weekly			
Neutropenia	2	3	Related
		3	Related
7.5 mg/m² once every 2 weeks			
Anemia	1	3	Unrelated
7.5 mg/m² on D1/D8 21-day cycle[b]			
Appetite reduced	2	2	Related
Constipation	1	2	Related
Dehydration	1	2	Related
Dyspepsia	1	2[c]	Related
Hypokalemia	1	2[c]	Related
10.0 mg/m² once every 2 weeks			
Neutropenia	2	3	Related
	1	4	Related
Fatigue	1	3[d]	Related
Nausea	1	3[d]	Related
Vomiting	1	3[d]	Related
Overdose[d]	1	NA	NA

Abbreviation: AE, adverse event.

[a]One patient experienced both grade 3 anemia and grade 2 fatigue.

[b]One patient experienced general malaise and the three AEs reported.

[c]Patient also experienced grade 1 AST/ALT elevation, dysgeusia, and arthralgia.

[d]Patient received a protocol deviation for overdose that was not considered clinically significant and had dose interruption because of the reported AEs.

TABLE A5. PK Parameters of Zelenectide Pevedotin and MMAE on C1D1

Analyte	Parameter	Mean (CV %) [No.] PK Parameters by Zelenectide Pevedotin Dose Level			
		2.5 mg/m² Once Weekly (n = 7)	5.0 mg/m² Once Weekly (n = 20)	7.5 mg/m² (n = 15)[a]	10.0 mg/m² Once Every 2 Weeks (n = 7)
Zelenectide pevedotin	AUC_{0-24}, ng*h/mL	353 (25.4) [7]	801 (33.5) [20]	1,360 (36.5) [14]	2,030 (38.5) [7]
	AUC_{0-t}, ng*h/mL	346 (25.8) [7]	773 (33.7) [20]	1,400 (38.8) [14]	1,940 (37.1) [7]
	AUC_{0-inf}, ng*h/mL	353 (25.1) [7]	786 (34.2) [19]	1,330 (34.4) [13]	1,970 (41.4) [6]
	C_{max}, ng/mL	306 (24.0) [7]	628 (39.2) [20]	939 (25.1) [14]	1,230 (33.9) [7]
	T_{max}, h[b]	0.98 (0.62; 1.22) [7]	0.96 (0.62; 1.55) [20]	1.17 (0.92; 1.67) [14]	1.17 (0.92; 1.67) [7]
	$t_{1/2}$, h	0.42 (26.6) [7]	0.74 (69.8) [19]	0.74 (34.2) [13]	0.91 (31.6) [6]
	CL, L/h	13.4 (22.3) [7]	13.0 (33.5) [19]	10.8 (24.7) [13]	10.9 (37.9) [6]
	V_z, L	7.95 (26.8) [7]	12.3 (42.7) [19]	11.0 (25.6) [13]	13.0 (22.7) [6]
MMAE	AUC_{0-24}, ng*h/mL	123 (45.9) [7]	248 (60.2) [19]	301 (39.3) [15]	384 (55.5) [7]
	AUC_{0-t}, ng*h/mL	269 (56.3) [7]	674 (78.5) [20]	811 (49.8) [15]	1,140 (64.3) [7]
	AUC_{0-inf}, ng*h/mL	308 (56.3) [6]	814 (78.9) [15]	856 (52.9) [15]	1,430 (60.7) [5]
	C_{max}, ng/mL	9.94 (32.4) [7]	18.2 (45.9) [20]	20.5 (40.9) [15]	22.7 (52.5) [7]
	T_{max}, h[b]	2.02 (1.55; 2.97) [7]	2.40 (1.00; 4.37) [20]	3.00 (1.05; 4.42) [15]	3.03 (2.00; 4.00) [7]
	$t_{1/2}$, h	40.4 (9.60) [6]	46.4 (21.8) [15]	37.3 (18.9) [15]	50.1 (33.1) [5]
	CL, L/h	3.58 (79.7) [6]	2.75 (50.8) [15]	3.19 (37.8) [15]	2.73 (51.5) [5]
	V_z, L	201 (72.3) [6]	174 (45.7) [15]	167 (38.4) [15]	191 (47.8) [5]

Abbreviations: C1D1, cycle 1 day 1; CL, clearance; C_{max}, maximum plasma drug concentration; CV, coefficient of variation; MMAE, monomethyl auristatin E; PK, pharmacokinetic; t½, drug elimination half-life; T_{max}, time to maximum concentration; V_z, volume of distribution.
[a]Consists of samples from all 7.5 mg/m² dosing regimens.
[b]T_{max} is reported as median (min; max).

TABLE A6. Efficacy Summary for Efficacy-Evaluable Patients With Urothelial Carcinoma Treated With Zelenectide Pevedotin

Best Confirmed Overall Response	2.5 mg/m² Once Weekly (n = 4), No. (%)	5.0 mg/m² Once Weekly (n = 7), No. (%)	7.5 mg/m² Once Weekly (n = 2), No. (%)	7.5 mg/m² Once Every 2 Weeks (n = 2), No. (%)	7.5 mg/m² on D1/D8 21-D Cycle (n = 2), No. (%)	10.0 mg/m² Once Every 2 Weeks (n = 4), No. (%)	Overall (n = 21), No. (%)
CR	0	1 (14)	0	0	0	0	1 (5)
PR	1 (25)	3 (43)	0[a]	0	1 (50)	2 (50)	7 (33)
SD	2 (50)	3 (43)	1 (50)	0	1 (50)	0	7 (33)
PD	1 (25)	0	0	2 (100)	0	2 (50)	5 (24)
ORR	1 (25)	4 (57)	0	0	1 (50)	2 (50)	8 (38)
CBR[b]	1 (25)	6 (86)	1 (50)	0	2 (100)	2 (50)	12 (57)

Abbreviations: C1D1, cycle 1 day 1; CBR, clinical benefit rate; CR, complete response; D, day; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease.
[a]One patient in this cohort had an unconfirmed partial response.
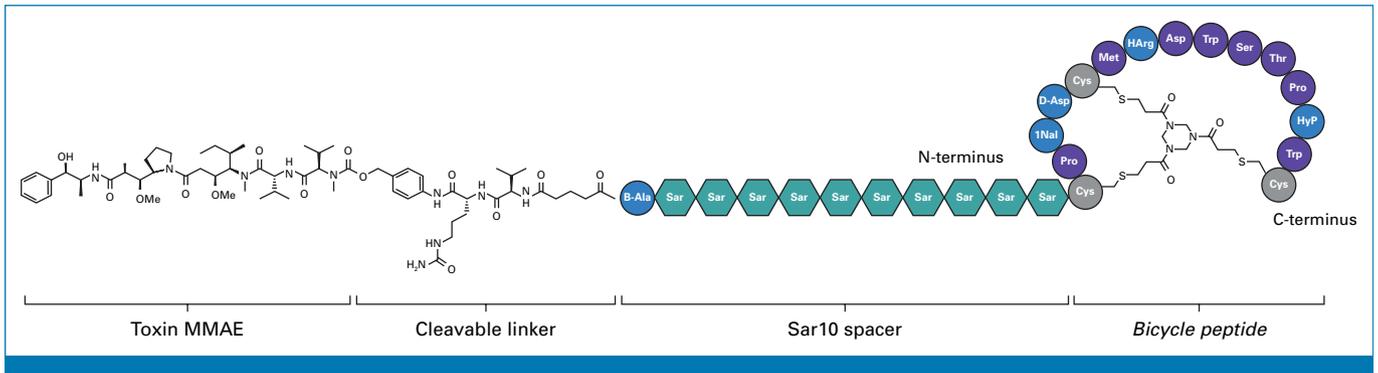[b]CBR = CR + PR + SD, where SD must be at least 16 weeks from C1D1 to be included in the CBR.

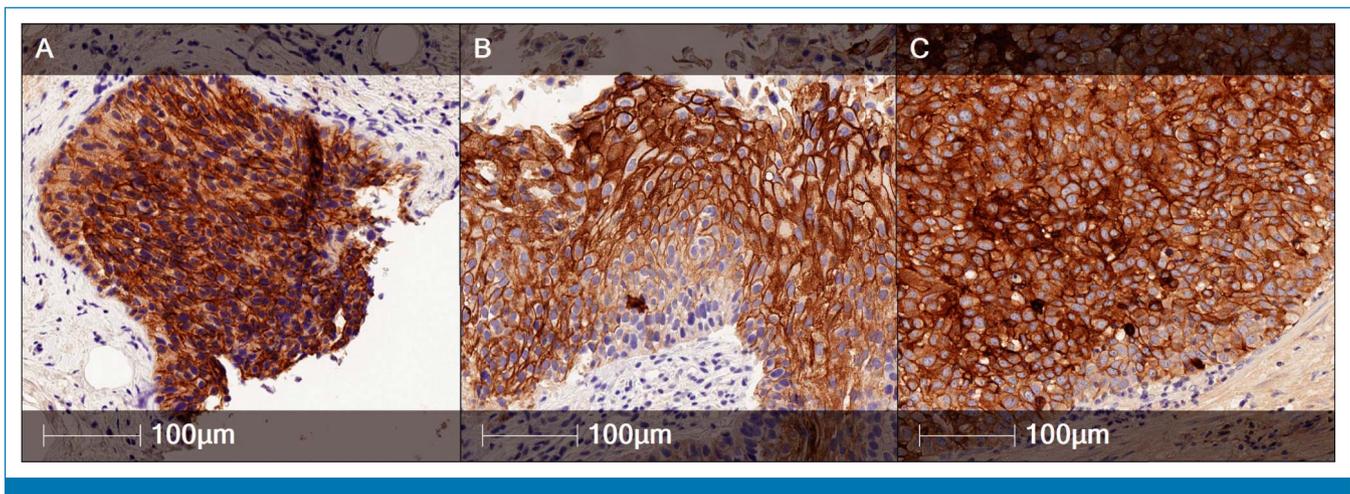**FIG A1.** Structure of zelenectide pevedotin. MMAE, monomethyl auristatin E.

FIG A2. Representative immunohistochemistry images for Nectin-4 staining from (A) breast cancer, (B) lung cancer, and (C) urothelial carcinoma.
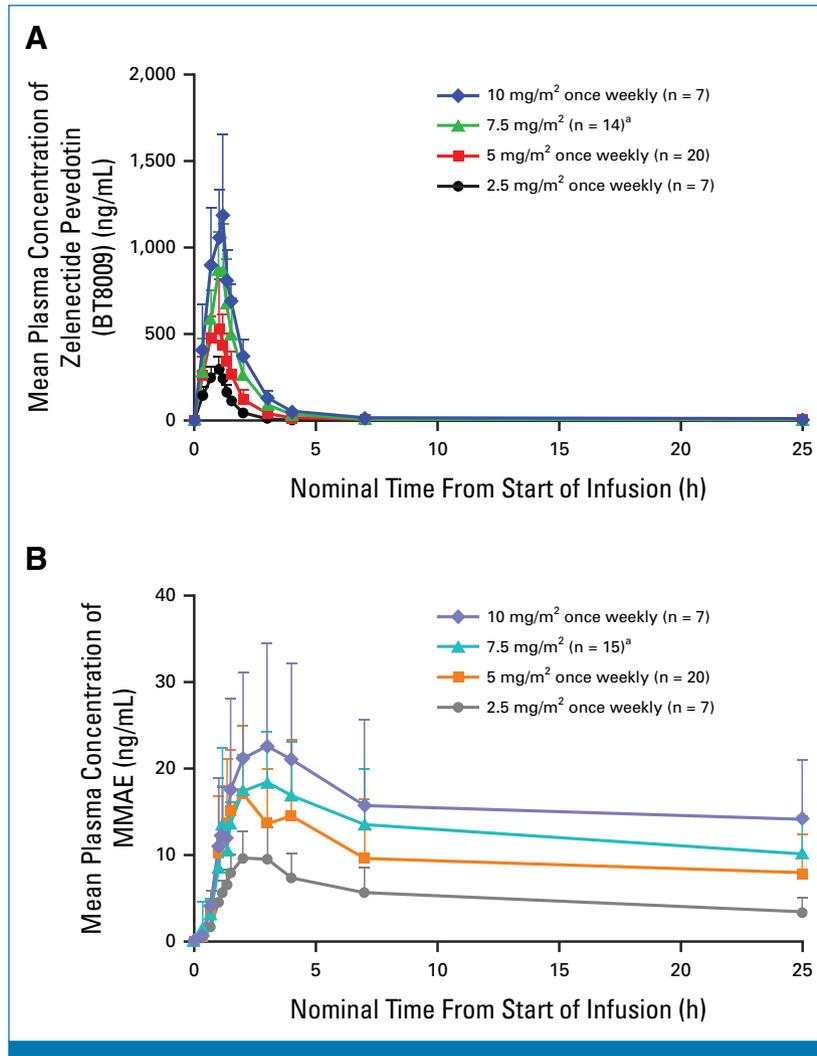
FIG A3. Mean (+standard deviation) plasma concentration of (A) zelenectide pevedotin and (B) MMAE over time (Cycle 1 Day 1). [a]Consists of samples from all 7.5 mg/m^2 dosing regimens: once weekly, once every 2 weeks, and on D1/8 of a 21-day cycle. MMAE, monomethyl auristatin E.

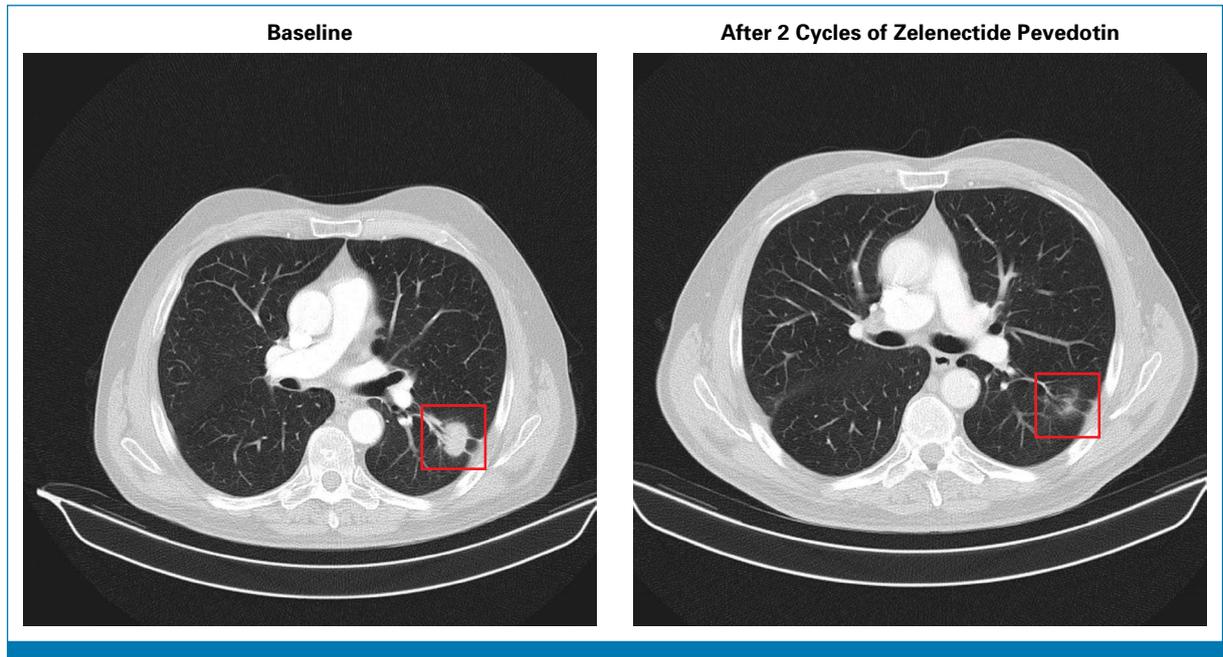| Baseline | After 2 Cycles of Zelenectide Pevedotin |

FIG A4. Representative computed tomography scans show a near CR in a patient with metastatic urothelial carcinoma. Metastatic target lesions in the left lung were reduced by 90% after two cycles of zelenectide pevedotin monotherapy. The patient went on to achieve a CR after four cycles. The CR was maintained for over 18 months as of the data cutoff date of March 4, 2024. CR, complete response.