Results From First-in-Human Phase I Dose-Escalation Study of a Novel Bicycle Toxin Conjugate Targeting EphA2 (BT5528) in Patients With Advanced Solid Tumors

Babar Bashir, MD[1,2]; Judy S. Wang, MD[2,3]; Gerald Falchook, MD[4]; Elisa Fontana, MD[5]; Hendrik-Tobias Arkenau, MD[5]; Louise Carter, MBBS, PhD[6]; Rachel Galot, MD[7]; Bristi Basu, BMBCh, FRCP, PhD[8]; Alastair Greystoke, MD[9]; Vivek Subbiah, MD[2]; Debra L. Richardson, MD[2,10]; Hanna Orr, MD, MFPM[11]; Gavin Bennett, PhD[11]; Rajiv Sharma, MBBS, MSc[11]; Hongmei Xu, PhD[12]; Paola Paganoni, MS[12]; Cong Xu, PhD[12]; Carly Campbell, BS[12]; and Meredith McKean, MD[2]

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ABSTRACT

PURPOSE BT5528 is a Bicycle Toxin Conjugate, a novel class of chemically synthesized molecules, comprising a bicyclic peptide targeting EphA2 tumor antigen, linked to a cytotoxin (monomethyl auristatin E [MMAE]). EphA2 is overexpressed in many solid tumors and contributes to oncogenesis, tumor-associated angiogenesis, and metastasis.

MATERIALS AND METHODS The primary objectives were to investigate the safety and tolerability of BT5528 and to define the maximum-tolerated dose, if observed, and recommended phase II dose (RP2D)/expansion dose. Dose escalation exploring once every week or once every 2 weeks administration of BT5528 employed a 3 + 3 dose-escalation design for the first two dose levels, followed by a Bayesian logistic regression model. Secondary and exploratory end points included preliminary efficacy and the pharmacokinetics of BT5528 and MMAE.

RESULTS Forty-five patients were enrolled and received BT5528 doses between 2.2 mg/m² once every week to 10.0 mg/m² once every 2 weeks within the dose-escalation stage of the study. The most frequent BT5528-related adverse events (AEs) were nausea (44.4%), diarrhea (35.6%), and fatigue (33.3%), and the most common grade ≥3 BT5528-related AE was neutropenia/neutrophil count decrease (22.2%). Dose level 6.5 mg/m² once every 2 weeks was selected as a RP2D. At 6.5 mg/m² once every 2 weeks, the overall response rate was 6.7%, and the disease control rate was 20.0%. BT5528 and MMAE pharmacokinetics are generally dose proportional. BT5528 has a short half-life (0.4-0.7 hours), and the half-life of MMAE is longer (35-47 hours).

CONCLUSION BT5528 was well tolerated and demonstrated favorable and preliminary anti-tumor activity. We believe these data provide preliminary validation of a Bicycle Toxin Conjugate approach to EphA2 tumor antigen. The study is ongoing and is evaluating BT5528 as monotherapy at a RP2D of 6.5 mg/m² once every 2 weeks.

ACCOMPANYING CONTENT

Appendix

Protocol

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INTRODUCTION

The receptor tyrosine kinase, erythropoietin-producing hepatocellular receptor A2 (EphA2), plays a role in oncogenesis, tumor-associated angiogenesis, and metastasis. Intracellular Eph signaling converges on pathways integral to cell growth, proliferation, migration, and invasion.[1] Outside the developing nervous system and vasculature,[2,3] EphA2 is expressed at relatively low levels in normal adult tissues[4,5]; however, it is overexpressed in numerous tumor types including non–small cell lung cancer, ovarian cancer, triple-negative breast cancer, and gastric/upper GI, pancreatic, and urothelial cancers.[6-10] EphA2-mediated oncogenic signaling has been correlated with poor patient outcome in several cancer types.[11-14]

Several therapeutic strategies directed against EphA2 have been investigated, including inhibition of kinase activity or modulation of signaling (using ligands including natural ligand derivatives, peptides, small molecules, antibodies, and siRNA). One approved therapy (dasatinib) inhibits EphA2; however, the drug targets multiple tyrosine kinases and thus clinical experience may not be solely due to its inhibition of EphA2.[15,16] Other early-phase clinical trials targeting EphA2 include the use of CAR-T cells in EphA2-positive recurrent and metastatic malignant glioma

CONTEXT

Key Objective

Is a novel Bicycle Toxin Conjugate (BTC) targeting EphA2, BT5528, tolerated and effective in patients with advanced solid malignancies?

Knowledge Generated

The recommended phase II dose of 6.5 mg/m^2 BT5528 administered once every 2 weeks was well tolerated and did not cause the bleeding events seen with other entities targeting EphA2. Clinical activity was more pronounced in tumors expressing EphA2 (urothelial and ovarian cancers) offering a preliminary validation of the BTC approach to EphA2 tumor antigen targeting in solid tumors.

Relevance (*R.G. Maki*)

The short half life of BT5528 may have mitigated some of the toxicity issues facing other agents that have attempted to target EphA2, which is often up-regulated in cancer versus normal cells. As appears to be the case for other targets such as MDM2, the pharmacodynamic features of target engagement appear to be important when the target is present in both cancer and normal cells.*

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

(NCT02575261) and an EphA2 gene targeting using neutral liposomal small interfering RNA delivery in solid tumors (NCT01591356). Both studies were terminated early but no data have been released to date. An anti-EphA2 antibody, DS-8895a, was studied in patients with advanced or metastatic EphA2-positive solid tumors. The maximum-tolerated dose (MTD) was not reached, and safety data included thrombocytopenia, hypoesthesia, hypotension, peripheral coldness, nausea, vomiting, and infusion reactions.[17] Further development of the antibody was halted because of poor tumor uptake.[18] A study of MM-310, an EphA2 antibody-targeted nanoliposome containing docetaxel, was terminated because of cumulative peripheral neuropathy.[19] Finally, a study of MEDI-547, an antibody-drug conjugate (ADC) of auristatin (cytotoxin) and EphA2 monoclonal antibody, was terminated because of treatment-related bleeding and coagulation defects.[20]

BT5528 is a Bicycle Toxin Conjugate (BTC), a novel class of agents containing bicyclic peptides developed by Bicycle Therapeutics. The molecule is composed of the company's proprietary EphA2 targeting Bicycle peptide, a valine-citrulline (val-cit) tumor microenvironment cleavable linker and a monomethyl auristatin E (MMAE) cytotoxin payload (Appendix Fig A1, online only). BT5528 is approximately 40× smaller than an ADC and has potential to readily penetrate solid tumors.[21] Once within the tumor, the MMAE cytotoxin is released and retained in tumor cells, resulting in tumor cell death and bystander killing. The pharmacokinetics (PK) profile of BT5528 is distinct from ADCs, and the drug has fast distribution and elimination.[21] While the in vivo and clinical toxicity profile of ADCs targeting EphA2, including MEDI-547, included bleeding events, the preclinical profile of BT5528 showed no such issues.

This first-in-human (FIH) study of BT5528 investigates the safety and preliminary efficacy in patients with advanced solid tumors.

MATERIALS AND METHODS

Patients and Eligibility Criteria

The study was registered with ClinicalTrials.gov (identifier: NCT04180371) and was conducted according to the applicable regulatory guidelines, the International Conference on Harmonization Guidelines for Good Clinical Practice, and the Declaration of Helsinki. Institutional review boards at all participating sites approved the study, and all patients provided written informed consent.

Eligibility

Eligible patients were age ≥18 years with histologically confirmed advanced solid malignancy, previously treated with one or more prior lines of anticancer therapy with documented disease progression (PD) on their most recent anticancer therapy. Patients had no other standard-of-care therapies deemed appropriate for their treatment. Additional eligibility criteria included Eastern Cooperative Oncology Group (ECOG) performance status 0-1, adequate bone marrow, organ function, and normal serum chemistry.

Pretreatment Evaluation

EphA2-positive tumor expression was required for some cohorts. Patients provided either archival tumor tissue or a predose tumor biopsy for EphA2 expression analysis by immunohistochemistry.[22] Tumor assessment was performed with computed tomography or magnetic resonance imaging.

Study Design

The study is a phase I/II, open-label, multicenter study to assess the safety, tolerability, PK, and preliminary antitumor activity of BT5528 in patients with advanced solid malignancies. The phase I part reported here is the dose-escalation portion; a dose-expansion portion is ongoing and will be reported at a later date. The primary objectives for the dose-escalation portion were to define the MTD, if observed; select a recommended phase II dose (RP2D); and to investigate the safety and tolerability profile of BT5528. Secondary objectives were to assess preliminary signals of antitumor activity, preliminary PK parameters of BT5528 and MMAE, and to determine incidences of antidrug antibodies (ADAs). A 3 + 3 dose-escalation design[23] was used for the first two dose levels (2.2 and 4.4 mg/m^2 once every week). After evidence of tolerability, all subsequent dose escalations (6.5 mg/m^2 once every week, 6.5 mg/m^2 once every 2 weeks, 8.5 mg/m^2 once every week, etc) were based on a Bayesian logistic regression model (BLRM)[24] incorporating the escalation with overdose control (EWOC) principle.[25] These data were monitored by a safety review committee (SRC), alongside the broader safety profile, PK data, and other relevant information, to recommend closing or opening new cohorts or expanding cohorts at the same dose.

Dose Escalation, Dose-Limiting Toxicities, and MTD

A starting dose of 2.2 mg/m^2 for humans was calculated as 1/10th the severely toxic dose in 10% (STD10) relative to the most sensitive animal, rats. Dose escalation was monitored by an SRC comprising the coordinating Investigator, medical monitor, the sponsor global safety physician, and the Principal Investigator or delegate for each active study center prior to treatment of the first patient in each cohort.

An adverse event (AE) was considered dose-limiting (DLT) if it occurred during the first cycle (28 days) and was considered related to BT5528. Attribution of relatedness was assumed in this FIH study unless it was considered highly unlikely. To be evaluable for a DLT, patients must have either experienced a DLT during the first cycle or received all planned doses during cycle 1. An MTD was to be based on the results from the BLRM (unless escalation was stopped during the 3+3 portion) or if the SRC used their discretion to declare it lower than the BLRM result.

Drug Administration

BT5528 mg/m^2 was administered intravenously (IV) over 1 hour, either once every week or once every 2 weeks. Prophylactic antiemetics or premedications and RBC or platelet transfusions or growth factors (eg, granulocyte colony-stimulating factor) were allowed after the 28-day DLT assessment period. Supportive care and other medications considered necessary for patient safety and well-being could be administered at the discretion of the Investigator. Any patient with a treatment delay of more than 4 weeks due to treatment-related toxicity was discontinued from study treatment, unless they continued treatment at a lower dose or a change in the dosing schedule was in the best interest of the patient.

Toxicity Assessments

AE grades were evaluated using the National Cancer Institute Common Terminology for Adverse Events v5.0 (CTCAE 5.0).[26]

Response Assessments

Tumor responses were assessed by the investigator using RECIST, version 1.1[27] every 8 weeks. Patients were considered evaluable for response if they had measurable disease at baseline, received at least one dose of BT5528, and had at least one adequate postbaseline response assessment.

PK

Blood samples for PK were collected at predose, 20 and 40 minutes after start of infusion, at end of infusion, and 10, 20, and 30 minutes, 1, 2, 3, 6, and 24 hours, and 7 and 14 days after end-of-infusion. Plasma concentrations of BT5528 and MMAE were measured by York Bioanalytical Solutions (Northminster Business Park, Upper Poppleton, York, United Kingdom) using a validated liquid chromatography-tandem mass spectrometry method. The analytical range of the assay was 5 to 2500 ng/mL for BT5528 and 0.05-50 ng/mL for MMAE. The precision and accuracy were all within ±19% and ±8% for the quality control samples of BT5528 and MMAE, respectively. Maximum plasma concentration (C_{max}), time to maximum concentration (T_{max}), terminal half-life ($t_{1/2}$), clearance (CL), terminal volume of distribution (Vz), and area under the concentration-time curve values (AUC) from time zero to infinity (AUC_{0-inf}) were determined for BT5528 and MMAE. All PK calculations were performed using a noncompartmental analysis in Phoenix WinNonlin.

Intratumoral MMAE concentrations were measured using triple quadrupole mass spectrometry, from tumor biopsies collected 24 hours after the end-of-infusion in a small subset of patients. Tumor and plasma concentration ratios were calculated.

Statistical Considerations

The statistical analysis included all patients who received BT5528. Data cutoff was August 1, 2022. No formal sample size calculations were performed, and descriptive statistics were tabulated and reported. After the first two cohorts, BLRM with EWOC principle was applied to cumulative DLT/safety data for considerations on all dose-escalation decisions. Overall response rate (ORR) was defined as the proportion of patients with a best overall response (BOR) of complete response (CR) or partial response (PR). Disease control rate (DCR) was defined as the proportion of patients with a BOR of CR, PR, or stable disease (SD), and DCR at 4 months was defined as the proportion of patients with a BOR of CR, PR, or SD ≥4 months.

TABLE 1. Patient Characteristics

Demographic	All Cohorts (N = 45)	6.5 mg/m² Q2W (n = 15)
Age, years, median (range)	63 (49-76)	61 (51-75)
Sex, No. (%)		
Male	15 (33.3)	9 (60.0)
Female	30 (66.7)	6 (40.0)
ECOG PS at baseline, No. (%)		
0 (good performance status)	18 (40.0)	5 (33.3)
1	27 (60.0)	10 (66.7)
Prior lines of therapies, median (range)	4 (1-13)	4 (2-13)
Primary diagnosis/tumor type, No. (%)		
Ovarian[a]	21 (46.7)	3 (20.0)
Urothelial[b]	8 (17.8)	6 (40.0)
Pancreatic	8 (17.8)	1 (6.7)
Lung[c]	4 (8.9)	2 (13.3)
Other[d]	4 (8.9)	3 (20.0)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; Q2W, once every 2 weeks.
[a]Includes ovarian, fallopian tube.
[b]Includes bladder, urethra, urinary bladder, and urothelial carcinoma.
[c]Includes lung, non–small cell lung cancer.
[d]Includes bone, rectal, stomach, and squamous of unknown origin.

RESULTS

Patient Characteristics

Forty-five patients were enrolled in the monotherapy dose-escalation portion of the study, of which 15 patients were treated at the expansion dose of 6.5 mg/m² once every 2 weeks. Baseline characteristics are presented in Table 1. The median age for the entire dose-escalation population was 63 years (range, 49-76 years), and 30 patients (67%) were female. Most patients (60%) had an ECOG performance status score of 1, and 18 patients (40%) had a score of 0. The median number of prior lines of systemic therapies was 4 (1-13). The most common tumor types were ovarian (46.7%), urothelial (17.8%), and pancreatic (17.8%).

Treatment

Forty patients (88.9%) had discontinued study treatment at the time of data cutoff with PD as the most common reason (30 patients [66.7%]). Five patients (11.1%) discontinued because of either patient or physician decision, three patients (6.7%) because of an AE, one patient (2.2%) died on study of an unrelated AE, and one patient (2.2%) discontinued for a reason categorized as other (inability to gain IV access).

Dose Escalation

There were no DLTs at 2.2 mg/m² once every week (three patients, all DLT-evaluable) or 4.4 mg/m² once every week (3 patients, all DLT-evaluable). At 8.5 mg/m² once every week, none of the four patients was evaluable for DLTs because of drug interruptions for non-DLT AEs; however, the overall safety profile led the SRC to conclude the dose was not tolerated. At 6.5 mg/m² once every week (8 patients, 4 DLT-evaluable), two patients experienced grade 3 DLTs: one tumor lysis syndrome and one ileus. At 10 mg/m² once every 2 weeks (two patients, 2 DLT-evaluable), both patients experienced DLTs: one grade 4 neutropenia and one grade 3 fatigue not recovered after 5 days. At 8.5 mg/m² once every 2 weeks (10 patients, 8 DLT-evaluable), two patients experienced grade 3 DLTs: one dehydration and one fatigue. At 6.5 mg/m² once every 2 weeks (15 patients, 14 DLT-evaluable), one patient experienced a DLT of grade 3 hyperglycemia (Appendix Table A1). On the basis of the dose-escalation data, the SRC declared 6.5 mg/m² once every 2 weeks to be a RP2D/recommended expansion dose.

Safety

All patients experienced at least 1 AE, and 41 patients (91.1%) experienced at least one treatment-related AE (TRAE). At the expansion dose of 6.5 mg/m² once every 2 weeks, all patients experienced at least one TRAE, the majority of which were grade ≤2. The most frequent TRAEs (≥15%), including those related to the MMAE component, were nausea (44.4%), diarrhea (35.6%), fatigue (33.3%), neutropenia/neutrophil count decrease (33.3%), vomiting (26.7%), anemia (22.2%), decreased appetite, alopecia, and peripheral neuropathy (15.6% each). No new safety signals were identified during the ongoing expansion phase (Table 2).

The most common grade ≥3 TRAE overall was neutropenia/neutrophil count decrease (11 events in 10 patients [22.2%]). These events were managed with weekly (Cycles 1-4) and then once every 2 weeks (Cycles 5+) blood counts, use of granulocyte colony-stimulating factor, and drug interruptions or reductions. None led to treatment discontinuation. As of data cutoff, the median (range) duration of nine resolved events was 5 (range of 3-9) days.

Two patients (6.7%) discontinued because of a TRAE: grade 3 ileus and grade 1 lower respiratory tract infection/pleuritic pain. One patient discontinued because of an unrelated AE: grade 1 hematuria.

Fourteen patients (31.1%) experienced serious AEs (SAEs), including eight patients (17.8%) who experienced treatment-related SAEs. Two patients died of AEs: one acute kidney injury because of dehydration from persistent treatment-related nausea and vomiting (8.5 mg/m² once every 2 weeks) and one intestinal ischemia that was considered unrelated to treatment (6.5 mg/m² once every week). An additional 21 patients died of PD during the study period, after treatment discontinuation.

Rates of hemorrhages, skin toxicity, and eye disorders were low and manageable (Appendix Table A2). Five patients

TABLE 2. Most Common (≥15%) Treatment-Related AEs (N = 45)

Related AE	All Cohorts (N = 45), All Grades, No. (%)	All Cohorts (N = 45), Grade ≥3, No. (%)	6.5 mg/m² Q2W (n = 15), All Grades, No. (%)	6.5 mg/m² Q2W (n = 15), Grade ≥3, No. (%)
Nausea	20 (44.4)	1 (2.2)	8 (53.3)	0
Diarrhea	16 (35.6)	1 (2.2)	7 (46.7)	1 (6.7)
Fatigue	15 (33.3)	2 (4.4)	6 (40.0)	0
Neutrophil count decrease[a]	15 (33.3)	10 (22.2)	2 (13.3)	0
Vomiting	12 (26.7)	1 (2.2)	3 (20.0)	0
Anemia	10 (22.2)	4 (8.9)	4 (26.7)	2 (13.3)
Decreased appetite	7 (15.6)	0	4 (26.7)	0
Alopecia	7 (15.6)	0	1 (6.7)	0
Peripheral neuropathy[b]	7 (15.6)	0	2 (13.3)	0

Abbreviations: AE, adverse event; Q2W, once every 2 weeks.

[a]Neutrophil count decrease also includes neutropenia.

[b]Peripheral neuropathy events include neuropathy peripheral, muscular weakness, peripheral sensory neuropathy, gait disturbance, neuralgia, and paresthesia.

experienced bleeding events, all because of underlying cancer and all grade 1 with the exception of one grade 3 gastric hemorrhage. Seven patients experienced skin AEs including maculo-papular rash, contact dermatitis, or skin blistering. All events were grade ≤2. Only two patients experienced skin AEs that were considered related to treatment; these were both maculopapular rash (6.5 mg/m² once every week cohort). Four patients overall (two patients at the expansion dose) experienced eye disorders including photophobia, visual impairment, dry eye, blurred vision, or eye pain. All events were grade ≤2 with two considered treatment-related (1 at 6.5 mg/m² once every 2 weeks and one at 8.5 mg/m² once every week). None of these patients required treatment modifications.

PK

Forty-five patients' plasma PK data were available for analysis; two patients were excluded from summary tables and figures because of an incomplete PK profile.

Plasma PK

BT5528 and MMAE plasma PK parameters of BT5528 are summarized in Tables 3 and 4 and in Appendix Figures A2 and A3. After a single IV dose, BT5528 exposure increased in a dose proportional manner from 2.2 to 8.5 mg/m² once every week. There were minimal differences of BT5528 exposure (C_{max} and AUCs) when administered once every week versus once every 2 weeks at 6.5 mg/m². BT5528 has a short $t_{1/2}$, ranging from 0.38 to 0.71 hours. CL and the V_z ranged from 7.7 to 11.7 L/hour and 4.9 to 7.6 L, respectively. Interindividual variability of BT5528 AUC and C_{max} ranged from 5.8% to 66%. No BT5528 accumulation was observed after multiple doses. Conversely, MMAE has longer $t_{1/2}$, ranging from 35 to 47 hours. Extravascular drug clearance (CL/F) and the apparent extravascular volume of distribution during terminal phase (Vz/F) ranged from 2.1 to 4.6 L/hour and 106 to 250 L, respectively. With once every week or once every 2 weeks dosing on Day 15, accumulation was low, ranging from 0.808 to 2.05.

Tumor PK

Two fresh tumor biopsy samples were obtained 24-hour postdose on Cycle 1 Day 15 from patients at 4.4 mg/m² once every week (Table 5). The tumor to plasma concentration ratio was 9.3-10.1 for the 24-hour postdose sample.

Efficacy

In the overall population, the ORR was 8.9% (two patients with ovarian cancer, two patients with urothelial cancer, all EphA2 positive), and the DCR was 44.4%. At the RP2D of 6.5 mg/m², ORR was 6.7% and DCR was 20.0% (Appendix Table A3). One patient with metastatic ovarian cancer (with peritoneal disease and nontarget peritoneum lesions), initially treated at 8.5 mg/m² once every 2 weeks, but dose reduced to 6.5 mg/m² once every 2 weeks on Cycle 13 Day 1 because of grade 1 nausea, had a CR (at end of Cycle 12) and remained on treatment for over 17 cycles. The percentage changes in tumor size for the 35 patients evaluable for response and a complete set of target lesion measurements at the postbaseline scan regardless of cancer type are shown in Figure 1A and for those with ovarian and urothelial cancers (n = 23) in Figure 1B.

DISCUSSION

BT5528 is a novel conjugate of an EphA2-binding bicyclic peptide and the cytotoxin MMAE. BT5528 administered once every week or once every 2 weeks in a 28-day treatment cycle to adult patients with refractory solid tumors was well tolerated with a manageable safety profile across all dose

TABLE 3. Summary BT5528 Pharmacokinetic Parameters After a Single IV Infusion of BT5528 as Monotherapy—Part A1—Cycle 1 Day 1

	BT5528 Monotherapy						
PK Parameter[a]	2.2 mg/m^2 QW (n = 3)	4.4 mg/m^2 QW (n = 3)	6.5 mg/m^2 QW (n = 8)	6.5 mg/m^2 Q2W (n = 14)[d]	8.5 mg/m^2 QW (n = 4)	8.5 mg/m^2 Q2W (n = 9)[c]	10 mg/m^2 Q2W (n = 2)
$AUC_{0\text{-inf},}$[b] ng × hours/mL	367 (8.1); 3	1,060 (29.4); 3	1,160 (26.2); 8	1,200 (31.8); 13	1,660 (20.7); 4	1,990 (42.8); 8	2,830 (NC); 2
$C_{max,}$ ng/mL	277 (5.8); 3	853 (24.0); 3	942 (27.8); 8	957 (32.0); 14	1,220 (24.5); 4	1,490 (34.3); 9	1,700 (NC); 2
$T_{max,}$ hours[c]	0.67 (0.67; 1.18); 3	1.05 (0.72; 1.27); 3	0.96 (0.33; 1.45); 8	0.92 (0.62; 1.33); 14	1.12 (1.00; 1.22); 4	0.98 (0.60; 1.53); 9	1.14 (0.92; 1.37); 2
$t_{1/2}$, hours	0.382 (11.4); 3	0.461 (25.9); 3	0.516 (49.3); 8	0.467 (34.5); 13	0.495 (5.3); 4	0.562 (42.0); 8	0.710 (NC); 2
CL, L/hours	10.4 (7.8); 3	7.86 (36.0); 3	10.8 (34.8); 8	11.7 (42.0); 13	10.7 (36.7); 4	8.54 (34.5); 8	7.72 (NC); 2
Vz, L	5.72 (8.1); 3	4.93 (11.5); 3	7.27 (27.5); 8	7.39 (37.0); 13	7.55 (33.6); 4	6.43 (27.6); 8	7.45 (NC); 2

Abbreviations: CL, clearance; C_{max}, maximum plasma concentration; NC, not calculated; PK, pharmacokinetics; QW, once every week; Q2W, once every 2 weeks; $t_{1/2}$, terminal half-life; T_{max}, time to maximum concentration; V_z, volume of distribution during terminal phase.

[a]Arithmetic mean (arithmetic CV%); No.

[b]For a single dose of BT5528.

[c]Median (Min, Max); No. T_{max} value is time after start of infusion.

[d]Terminal life parameters ($AUC_{0\text{-inf}}$, $t_{1/2}$, CL and V_z) excluded from summary because of nonreliable terminal phase.

TABLE 4. Summary Monomethyl Auristatin E Pharmacokinetic Parameters After a Single IV Infusion of BT5528 as Monotherapy—Part A1—Cycle 1 Day 1

	BT5528 Monotherapy						
PK Parameter[a]	2.2 mg/m² QW (n = 3)[c]	4.4 mg/m² QW (n = 3)[c]	6.5 mg/m² QW (n = 8)[c]	6.5 mg/m² Q2W (n = 14)[c]	8.5 mg/m² QW (n = 4)[c]	8.5 mg/m² Q2W (n = 9)[c]	10 mg/m² Q2W (n = 2)
$AUC_{0\text{-inf}}$, ng × hours/mL	NC	560 (NC); 1	710 (29.7); 6	752 (54.7); 13	682 (33.2); 3	1,070 (63.2); 8	2,120 (NC); 2
C_{max}, ng/mL	9.95 (45.1); 3	18.0 (33.5); 3	20.0 (15.9); 8	19.3 (40.7); 14	22.8 (26.3); 4	27.8 (43.3); 9	36.9 (NC); 2
T_{max}, hours[b]	2.27 (1.75; 2.27); 3	2.25 (2.08; 3.25); 3	2.05 (1.95; 2.55); 8	2.15 (1.82; 3.28); 14	2.18 (2.03; 2.3); 4	2.10 (1.87; 3.3); 9	3.38 (2.92; 3.83); 2
$t_{1/2}$, hours	NC	34.8 (NC); 1	45.4 (6); 6	43.8 (25.6); 13	38.2 (9.9); 3	41.7 (16.4); 8	46.7 (NC); 2
CL/F, L/h	NC	2.11 (NC); 1	2.88 (36.8); 6	3.92 (72.1); 13	4.60 (39.3); 3	2.88 (41.8); 8	2.10 (NC); 2
V_z/F, L	NC	106 (NC); 1	186 (32.9); 6	230 (62.1); 13	250 (35.0); 3	166 (38.9); 8	141 (NC); 2

Abbreviations: CL/F, extravascular drug clearance; C_{max}, maximum plasma concentration; IV, intravenous; NC, not calculated; PK, pharmacokinetics; QW, once every week; Q2W, once every 2 weeks; $t_{1/2}$, terminal half-life; T_{max}, time to maximum concentration; V_z/F, extravascular volume of distribution during terminal phase.

[a]Arithmetic mean (arithmetic CV%); No.

[b]Median (Min, Max); No.

[c]Terminal life parameters ($AUC_{0\text{-inf}}$, $t_{1/2}$, CL, and V_z) excluded from summary because of nonreliable terminal phase.

TABLE 5. Tumor/Plasma Monomethyl Auristatin E Concentration Ratio

Patient	Tumor Type	Dose, mg/m²	Schedule	Cycle/ Day	Time of Sample	Plasma Concentration, nM	Tumor Concentration, nM	Tumor/Plasma Ratio
1	Soft tissue (Ewing sarcoma)	4.4	QW	C1D15	24 h postdose	8.70	87.5	10.1
2	Ovary	4.4	QW	C1D15	24 h postdose	21.2	197	9.3

Abbreviations: C1D15, Cycle 1 Day 15; h, hour; QW, once every week.

levels. Previous clinical trials of other EphA2-targeting ADC molecules reported high rates of vascular hemorrhages, which precluded further development.[20] In this study, bleeding events were rare and were assessed as unrelated to the study drug. The most common AEs were nausea, diarrhea, fatigue, and neutrophil count decreased. No specific signal of renal toxicity was observed. This FIH study established 6.5 mg/m² once every 2 weeks as a RP2D of BT5528. At that dose level, AEs were manageable.

BT5528 has a short half-life (0.4-0.7 hours) and short systemic exposure. MMAE has a longer half-life (35-47 hours) with measurable concentrations 1-2 weeks postdose. There was minimum accumulation of MMAE in plasma after multiple doses at the RP2D. MMAE accumulation in the tumor was more pronounced than systemic plasma exposure. The MMAE tumor to plasma ratio was 9.3-10.1 in the tumor biopsy samples approximately 24 hours after dosing. Despite limited numbers of biopsy samples, these data suggest rapid delivery of the payload and retention at the site of action within the tumor. This is, to our knowledge, the first direct demonstration in the clinic of cytotoxic payload delivery by targeted conjugates. This observation is consistent with in vivo data. The BT5528 and MMAE clinical PK and preclinical PK data demonstrated tumor penetration and tumor internalization, supporting the mechanism of action of BT5528 and the BTC platform.

BT5528 exhibits a shorter $t_{1/2}$ (<1 hour) in comparison with that seen for MMAE-ADCs ($t_{1/2}$ 3.4-12 days),[28-30] despite delivering sustained plasma MMAE concentrations similar to ADCs and evidence of tumor penetration.

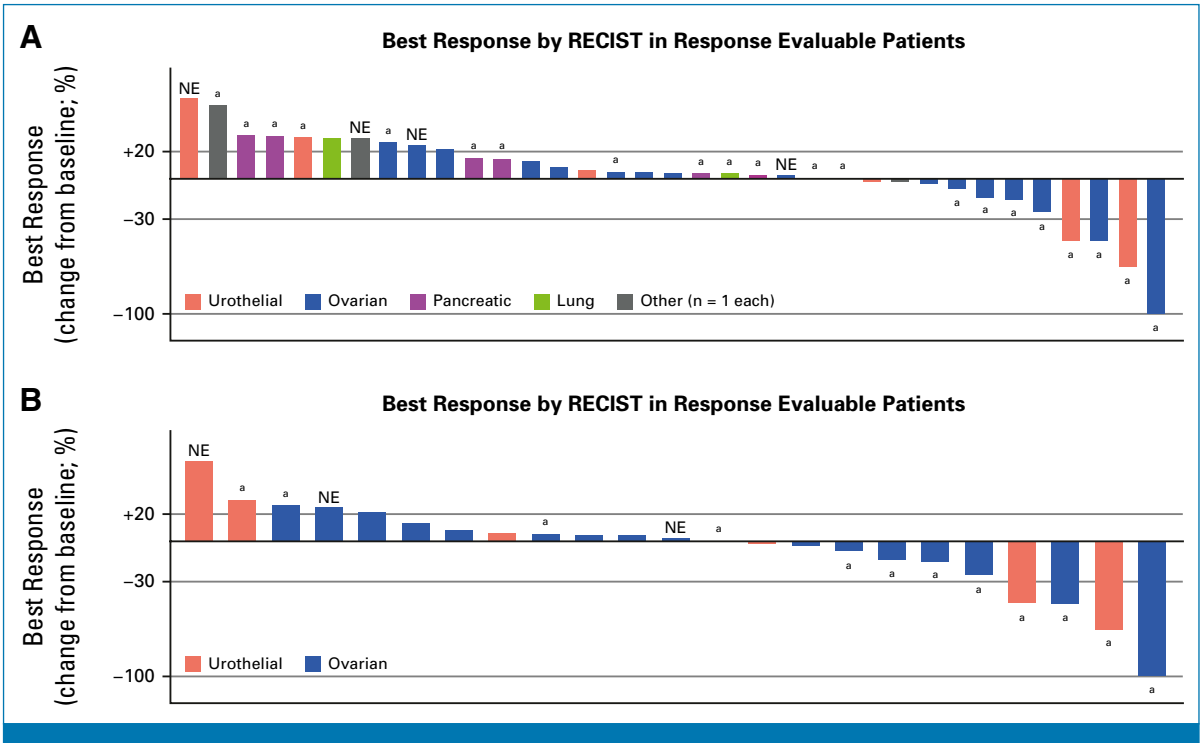

FIG 1. (A) Percentage change in tumor size. The change in reference tumor size of target lesions compared with baseline during treatment is shown as a percent change. Figure shows patients with target lesions and adequate postbaseline assessment (n = 35). Eight patients discontinued early from treatment and did not have a postbaseline response assessment and two patients had an uninterpretable postbaseline assessment. (B) Percentage change in tumor size for patients with urothelial or ovarian cancer. The change in reference tumor size of target lesions compared with baseline during treatment is shown as a percent change. [a]EphA2 positive = IHC TPS >1%; NE for EphA2 status. Figure shows only patients with an evaluable response. IHC, immunohistochemistry; NE, not evaluated; TPS, tumor proportion score.

On the basis of exposure-safety analyses for three MMAE-containing ADCs, including enfortumab vedotin, a positive relationship between safety and exposure was predominantly associated with the conjugates rather than with the free analyte, particularly for nonhematologic toxicity.[31,32] The significantly reduced BT5528 conjugate exposure compared with ADCs may contribute to an improved safety profile, including minimal skin and eye toxicities.

It appeared that the patients most likely to experience response or durable disease control in this study were those whose tumors expressed EphA2, suggesting a potential association between EphA2 expression and a positive outcome, although no significant trend was observed between the relationship of tumor proportion score (TPS) and disease worsening (percent change in tumor size) or for the relationship between TPS and DCR at the first postbaseline assessment. Further exploration of EphA2 expression in an expanded population is warranted to better understand the potential for BT5528 in specific indications and its use as a predictive biomarker for BT5528 efficacy. While the number of responses in this phase I study are small, the data support the possibility that clinical activity is related to expression of the EphA2 target in tumor tissue.

The BTC system for the targeted delivery of MMAE to EphA2-expressing tumors may offer advantages over ADCs. While BT5528 and MEDI-547 both target EphA2 to deliver auristatin payloads, in vitro assays have shown key advantages of BT5528. When compared with an EphA2 ADC synthesized according to the published information for MEDI-547, both performed similarly in binding EphA2, with low nanomolar affinity to EphA2 protein and EphA2-expressing cells.[21] The similarities between the two molecules were limited thereafter mainly due to the differences between the constructs, including the nature of the payload: cell-permeant MMAE for BT5528, cell-impermeant monomethyl auristatin F (MMAF) for MEDI-547, and the structure of the overall construct, with BT5528 being approximately 40 times smaller and containing a single conjugated MMAE, whereas MEDI-547 contains on an average approximately four MMAF per conjugate molecule.[33] The difference in size results in a significant difference in the PK/PD properties of the molecules. BT5528 is able to penetrate tumor tissues and deliver payload within hours and is rapidly cleared from systemic circulation in animal models,[21] consistent with the clinical profile seen in this report. Rapid release/prolonged retention of MMAE in tumors reduces toxin exposure to tissues.

In summary, BT5528 was well tolerated and has shown preliminary activity at a RP2D dose of 6.5 mg/m^2 administered once every 2 weeks. We believe these findings provide preliminary validation of the BTC approach to EphA2 tumor antigen targeting in solid tumors. Unlike earlier attempts to target EphA2, the use of BT5528 has shown preliminary antitumor activity in patients at tolerable dose levels. This justifies the further exploration of efficacy and safety, particularly in the indications where efficacy signals were seen.

AFFILIATIONS

[1]Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA
[2]Sarah Cannon Research Institute, Nashville, TN
[3]Florida Cancer Specialists, Sarasota, FL
[4]Sarah Cannon Research Institute at HealthONE, Denver, CO
[5]Sarah Cannon Research Institute, London, United Kingdom
[6]The University of Manchester and The Christie NHS Foundation Trust, Manchester, UK
[7]Saint Luc Cliniques Universitaires Saint-Luc, Brussels, Belgium
[8]University of Cambridge and Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom
[9]Newcastle University, Newcastle upon Tyne, United Kingdom
[10]Stephenson Cancer Center, University of Oklahoma, Oklahoma City, OK
[11]BicycleTX Ltd, Cambridge, United Kingdom
[12]Bicycle Therapeutics, Cambridge, MA

CORRESPONDING AUTHOR

Meredith McKean, MD; e-mail: Meredith.McKean@scri.com.

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AUTHOR CONTRIBUTIONS

Conception and design: Babar Bashir, Vivek Subbiah, Hanna Orr, Gavin Bennett, Paola Paganoni, Cong Xu, Carly Campbell, Meredith McKean
Administrative support: Vivek Subbiah, Gavin Bennett
Provision of study materials or patients: All authors
Collection and assembly of data: All authors
Data analysis and interpretation: All authors
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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Results from First-in-Human Phase I Dose-Escalation Study of a Novel Bicycle Toxin Conjugate Targeting EphA2 (BT5528) in Patients With Advanced Solid Tumors

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Babar Bashir
Consulting or Advisory Role: Merck/Eisai
Research Funding: Boehringer Ingelheim (Inst), Ikena Oncology (Inst), Bicycle Therapeutics (Inst), Syros Pharmaceuticals (Inst), KAHR Medical (Inst), Tarveda Therapeutics (Inst), Amgen (Inst), RasCal (Inst), Merck (Inst), Artios (Inst), Daiichi Sankyo/Lilly (Inst), Pionyr (Inst), Lyell Immunopharma (Inst), Elucida Oncology (Inst), Gritstone Bio (Inst), Jazz Pharmaceuticals (Inst)
Open Payments Link: https://openpaymentsdata.cms.gov/physician/321442

Judy S. Wang
Consulting or Advisory Role: Kanaph Therapeutics, Fusion Pharmaceuticals
Speakers' Bureau: AstraZeneca, Eisai
Research Funding: AstraZeneca (Inst), Bicycle Therapeutics (Inst), BioNTech (Inst), Boehringer Ingelheim (Inst), Celgene (Inst), cyteir (Inst), Daiichi Sankyo (Inst), Genentech/Roche (Inst), GlaxoSmithKline (Inst), H3 Biomedicine (Inst), Janssen Research & Development (Inst), Klus Pharma (Inst), Kymab (Inst), Loxo (Inst), LSK BioPharma (Inst), Macrogenics (Inst), Merck (Inst), Moderna Therapeutics (Inst), Phoenix Pharmaceuticals (Inst), Prelude Therapeutics (Inst), QiLu Pharmaceutical (Inst), Revolution Medicines (Inst), Ribon Therapeutics (Inst), Syndax (Inst), Xencor (Inst), Artios (Inst), Erasca, Inc (Inst), Immunogen (Inst), Cullinan Oncology (Inst), Immune-Onc Therapeutics (Inst), Bayer Health (Inst), Zymeworks (Inst), BioTheryX (Inst), TeneoBio (Inst), Nurix (Inst), IgM Biosciences (Inst), PureTech (Inst), Forty Seven (Inst), Treadwell Therapeutics (Inst), MabSpace Biosciences (Inst), Olema Oncology (Inst), Seven and Eight Biopharmaceuticals (Inst), Relay Therapeutics (Inst), Jazz Pharmaceuticals (Inst), Adagene (Inst), NGM Biopharmaceuticals (Inst), Agenus (Inst), BeiGene (Inst), Astellas Pharma (Inst), Blueprint Medicines (Inst), Hotspot Therapeutics (Inst), Centessa Pharmaceuticals (Inst), Allorion Therapeutics (Inst), C4 Therapeutics (Inst), Accutar Biotech (Inst), BMS GmbH & Co. KG (Inst), Step Pharma (Inst), Tango Therapeutics (Inst), Vividion Therapeutics (Inst), Georgiamune (Inst), Kura Oncology (Inst), Immunitas (Inst), Coherus Biosciences (Inst), Mersana (Inst), Medikine (Inst), Pyxis (Inst), Kineta (Inst), Biostar (Inst), Ellipses Pharma (Inst), Compugen (Inst), Sanofi (Inst)

Gerald Falchook
Employment: Sarah Cannon Research Institute, HealthONE
Honoraria: Clinical Care Options
Consulting or Advisory Role: Fujifilm (Inst), EMD Serono, Silicon Therapeutics (Inst), Navire (Inst), Turning Point Therapeutics (Inst), Silicon Therapeutics (Inst), Predicine (Inst), Inspirna (Inst), Regeneron (Inst), Jubilant Pharmaceuticals (Inst), BostonGene (Inst), BostonGene (Inst), Abbvie (Inst), Teon Therapeutics (Inst), Merck (Inst), Sanofi (Inst), BridgeBio Pharma (Inst)
Speakers' Bureau: Total Health Conferencing
Research Funding: Millennium (Inst), EMD Serono (Inst), Celgene (Inst), MedImmune (Inst), Genmab (Inst), Vegenics (Inst), Novartis (Inst), AstraZeneca (Inst), Incyte (Inst), ARMO BioSciences (Inst), Kolltan Pharmaceuticals (Inst), 3-V Biosciences (Inst), Abbvie (Inst), Aileron Therapeutics (Inst), DelMar Pharmaceuticals (Inst), eFFECTOR Therapeutics (Inst), Strategia Therapeutics (Inst), Fujifilm (Inst), Hutchison MediPharma (Inst), Regeneron (Inst), Biothera (Inst), Curegenix (Inst), Curis (Inst), Lilly (Inst), Jounce Therapeutics (Inst), OncoMed (Inst), Precision Oncology (Inst), Syndax (Inst), Taiho Pharmaceutical (Inst), Tesaro (Inst), Takeda (Inst), BeiGene (Inst), Ignyta (Inst), Merck (Inst), Rgenix (Inst), Tarveda Therapeutics (Inst), Tocagen (Inst), Loxo (Inst), Jacobio (Inst), Ciclomed (Inst), miRNA Therapeutics (Inst), Celldex (Inst), ADC Therapeutics (Inst), Amgen (Inst), Exelixis (Inst), Bioatla (Inst), Turning Point Therapeutics (Inst), Ribon Therapeutics (Inst), Cyteir (Inst), Xencor (Inst), Daiichi (Inst), Epizyme (Inst), Abbisko (Inst), Prelude Therapeutics (Inst), Poseida (Inst), Oncorus (Inst), Synthorx (Inst), BioInvent (Inst), Sapience Therapeutics (Inst), Bicycle Therapeutics (Inst), Silicon Therapeutics (Inst), PureTech (Inst), Immunogen/MacroGenics (Inst), IgM Biosciences (Inst), Navire (Inst), TeneoBio (Inst), Erasca, Inc (Inst), RasCal (Inst), Boehringer Ingelheim (Inst), Pyramid Biosciences (Inst), Samumed (Inst), ABL Bio (Inst), Freenome (Inst), Artios (Inst), NiKang Therapeutics (Inst), Molecular Templates (Inst), Sirnaomics (Inst), Accutar Biotech (Inst), Relay Therapeutics (Inst), Simcha Therapeutics (Inst), Black Diamond Therapeutics (Inst), Seagen (Inst), Jubilant Pharmaceuticals (Inst), Metabomed (Inst), Agenus (Inst), Tallac Therapeutics (Inst), Zhuhai Yufan Biotechnologies (Inst), Mirati Therapeutics (Inst), Immunitas (Inst), Jazz Pharmaceuticals (Inst), Bayer (Inst), Kineta (Inst), NGM Biopharmaceuticals (Inst), Roche (Inst), Tarus Therapeutics (Inst), Pyxis (Inst), Harbor BioMed (Inst), Centessa Pharmaceuticals (Inst), IDEAYA Biosciences (Inst), Tango Therapeutics (Inst), Biomea Fusion (Inst), Conjupro Biotherapeutics (Inst), Phanes Therapeutics (Inst), Sarah Cannon Research Institute (Inst), Nuvectis Pharma (Inst), Kura Oncology (Inst), Eikon Therapeutics (Inst), GlaxoSmithKline (Inst), ASCO (Inst), National Institute of Health (NIH) (Inst), Oncothyreon (Inst), MD Anderson Cancer Center (Inst)
Patents, Royalties, Other Intellectual Property: Handbook of Targeted Cancer Therapy
Travel, Accommodations, Expenses: Millennium, Sarah Cannon Research Institute, EMD Serono, Bristol Myers Squibb, Fujifilm, Amgen, Synthorx/Sanofi

Elisa Fontana
Employment: HCA/Sarah Cannon
Consulting or Advisory Role: Astellas Pharma, Celgene, SERVIER, Bristol Myers Squibb
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)
Patents, Royalties, Other Intellectual Property: Patent No: 1716712.3 pending
Travel, Accommodations, Expenses: Bristol Myers Squibb, SERVIER, Repare Therapeutics, Caris Life Sciences, Seagen, Sapience Therapeutics, Bicycle Therapeutics
Other Relationship: European Organisation for Research and Treatment of Cancer (EORTC)

Hendrik-Tobias Arkenau
Employment: Hospital Corporation of America, Sarah Cannon Research Institute UK, Sarah Cannon Research Institute UK, Ellipses Pharma
Honoraria: Guardant Health, SERVIER, BeiGene
Consulting or Advisory Role: iOnctura, Engitix
Research Funding: Sarah Cannon Research Institute

Louise Carter
Consulting or Advisory Role: Athenex, Bicycle Therapeutics, Boehringer Ingelheim
Research Funding: Sierra Oncology (Inst), Athenex (Inst), Takeda (Inst), CellCentric (Inst), CytomX Therapeutics (Inst), Lilly (Inst), Boehringer Ingelheim (Inst), Bicycle Therapeutics (Inst), Lupin Pharmaceuticals (Inst), Repare Therapeutics (Inst), ADC Therapeutics (Inst), Merck Serono (Inst), Kronos Bio (Inst)

Rachel Galot
Travel, Accommodations, Expenses: MSD, MSD

Bristi Basu
Consulting or Advisory Role: GenMab (Inst), Eisai (Inst), Roche (Inst)
Speakers' Bureau: Eisai Europe, Eisai Europe (Inst)
Research Funding: Celgene (Inst), Varsity Pharmaceuticals (Inst)
Travel, Accommodations, Expenses: Roche

Alastair Greystoke
Consulting or Advisory Role: AstraZeneca, Janssen, Pfizer, Lilly, Boehringer Ingelheim, MSD Oncology, Takeda, BMSi, Foundation Medicine, Guardant Health, Novartis
Speakers' Bureau: AstraZeneca, Pfizer, Bayer, Lilly, Novartis, Foundation Medicine, Roche, Sanofi, Merck Serono, Pfizer, Amgen, Janssen, Janssen, Janssen
Research Funding: AstraZeneca

Vivek Subbiah
Consulting or Advisory Role: Loxo/Lilly, Relay Therapeutics (Inst), Pfizer (Inst), Roche (Inst), Bayer (Inst), Incyte (Inst), Novartis (Inst), Pheon Therapeutics (Inst), Abbvie (Inst), Illumina, AADi, Foundation Medicine
Research Funding: Novartis (Inst), GlaxoSmithKline (Inst), NanoCarrier (Inst), Northwest Biotherapeutics (Inst), Genentech/Roche (Inst), Berg Pharma (Inst), Bayer (Inst), Incyte (Inst), Fujifilm (Inst), PharmaMar (Inst), D3 Oncology Solutions (Inst), Pfizer (Inst), Amgen (Inst), Abbvie (Inst), Multivir (Inst), Blueprint Medicines (Inst), LOXO (Inst), Vegenics (Inst), Takeda (Inst), Alfasigma (Inst), Agensys (Inst), Idera (Inst), Boston Biomedical (Inst), Inhibrx (Inst), Exelixis (Inst), Amgen (Inst), Turning Point Therapeutics (Inst), Relay Therapeutics (Inst)
Other Relationship: Medscape, Clinical Care Options

Debra L. Richardson
Consulting or Advisory Role: AstraZeneca, Mersana, GlaxoSmithKline, Immunogen, Eisai, ProfoundBio
Research Funding: Genentech (Inst), AstraZeneca (Inst), Syros Pharmaceuticals (Inst), Innovent Biologics (Inst), Deciphera (Inst), ArQule (Inst), Roche (Inst), Tesaro (Inst), Merck (Inst), Karyopharm Therapeutics (Inst), Aravive (Inst), Mersana (Inst), Harpoon therapeutics (Inst), Hookipa Biotech (Inst), Five Prime Therapeutics (Inst), Fujifilm (Inst), Clovis Oncology (Inst), Arch Oncology (Inst), Plexxikon (Inst), GlaxoSmithKline (Inst), Lilly (Inst), Celsion (Inst), Shattuck Labs (Inst)

Hanna Orr
Employment: Bicycle Therapeutics
Stock and Other Ownership Interests: Bicycle Therapeutics

Gavin Bennett
Employment: Bicycle Therapeutics
Stock and Other Ownership Interests: Bicycle Therapeutics

Rajiv Sharma
Employment: Bicycle Therapeutics
Stock and Other Ownership Interests: Bicycle Therapeutics, Roche

Hongmei Xu
Employment: Bicycle Therapeutics
Stock and Other Ownership Interests: AstraZeneca, Karyopharm Therapeutics, Bicycle Therapeutics
Travel, Accommodations, Expenses: Bicycle Therapeutics

Paola Paganoni
Employment: Bicycle Therapeutics

Cong Xu
Employment: Bicycle Therapeutics
Stock and Other Ownership Interests: Vertex, Bicycle Therapeutics

Carly Campbell
Employment: Bicycle Therapeutics
Stock and Other Ownership Interests: Bicycle Therapeutics, Epizyme

Meredith McKean
Consulting or Advisory Role: Pfizer, (Inst), Castle Biosciences (Inst), Moderna Therapeutics (Inst), IQVIA (Inst), Merck (Inst)
Research Funding: Prelude Therapeutics (Inst), Genentech (Inst), Tizona Therapeutics, Inc. (Inst), GlaxoSmithKline (Inst), IDEAYA Biosciences (Inst), Exelixis (Inst), Jacobio (Inst), Moderna Therapeutics (Inst), Regeneron (Inst), Epizyme (Inst), Top Alliance BioScience (Inst), Ascentage Pharma Group (Inst), Oncorus (Inst), Ikena Oncology (Inst), Bicycle Therapeutics (Inst), Tmunity Therapeutics, Inc (Inst), Sapience Therapeutics (Inst), NBE Therapeutics (Inst), Dragonfly Therapeutics (Inst), Infinity Pharmaceuticals (Inst), Novartis (Inst), Plexxikon (Inst), Seagen (Inst), Alpine Immune Sciences (Inst), Arcus Biosciences (Inst), Arvinas (Inst), Bayer (Inst), BioMed Valley Discoveries (Inst), BioNTech (Inst), EMD Serono (Inst), Erasca, Inc (Inst), Foghorn Therapeutics (Inst), Gilead Sciences (Inst), Immvira (Inst), Kechow Pharma (Inst), Kezar Life Sciences (Inst), Kinnate Biopharma (Inst), MedImmune (Inst), Mereo BioPharma (Inst), Metabomed (Inst), Nektar (Inst), PACT Pharma (Inst), Pfizer (Inst), Pyramid Biosciences (Inst), Scholar Rock (Inst), Synthorx (Inst), TeneoBio (Inst), Tempest Therapeutics (Inst), Xilio Therapeutics (Inst), AADi (Inst), Astellas Pharma (Inst), G1 Therapeutics (Inst), OncoC4 (Inst), Poseida (Inst), ASCO (Inst), Aulos Bioscience (Inst), Bristol-Myers Squibb (Inst), C4 Therapeutics (Inst), NucMito Pharmaceuticals (Inst), OnKure (Inst)

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APPENDIX

TABLE A1. Dose Escalation and DLTs (N = 45)

Dose	Treated Patients (N = 45), No.	Evaluable Patients[a] (n = 34), No.	Patients with DLT (n = 7), No.	DLT Description	Reason for DLT Non-evaluable
2.2 mg/m^2 QW	3	3	0		
4.4 mg/m^2 QW	3	3	0		
6.5 mg/m^2 QW	8	4	2	Tumor lysis syndrome (G3) Ileus (G3)	Drug interrupted: Patient skipped dose (n = 1) Drug interrupted: Non-DLT AE (n = 1) Dose reduced: Reason unknown (n = 1) Drug interrupted: Patient discontinuation from study (n = 1)
6.5 mg/m^2 Q2W	15	14	1	Hyperglycemia (G3)	Dosing error at one visit (n = 1)
8.5 mg/m^2 QW	4	0	0		Drug interrupted: Non-DLT AE (n = 4)
8.5 mg/m^2 Q2W	10	8	2	Dehydration (G3) Fatigue (G3)	Drug interrupted: Non-DLT AE (n = 2)
10 mg/m^2 Q2W	2	2	2	Neutropenia (G4) Fatigue (G3)	

Abbreviations: AE, adverse event; G, grade; DLT, dose-limiting toxicity; QW, once every week; Q2W, once every 2 weeks.
[a]Patients unevaluable for DLTs did not experience a DLT and did not receive all planned doses within the 28-day assessment period.

TABLE A2. Vascular, Skin, and Eye Toxicity (N = 45)

	All AEs				Related AEs			
AE of Interest	All Cohorts (N = 45) All Grades, No. (%)	All Cohorts (N = 45) Grade ≥3, No. (%)	6.5 mg/m^2 Q2W (n = 15) All Grades, No. (%)	6.5 mg/m^2 Q2W (n = 15) Grade ≥3, No. (%)	All Cohorts (N = 45) All Grades, No. (%)	All Cohorts (N = 45) Grade ≥3, No. (%)	6.5 mg/m^2 Q2W (n = 15) All Grades, No. (%)	6.5 mg/m^2 Q2W (n = 15) Grade ≥3, No. (%)
Skin rash	7 (15.6)	0	3 (20.0)	0	2 (4.4)	0	0	0
Hemorrhage	5 (11.1)	1 (2.2)	1 (6.7)	1 (6.7)	0	0	0	0
Eye disorders	4 (8.9)	0	1 (6.7)	0	2 (4.4)	0	1 (6.7)	0

Abbreviations: AE, adverse event; Q2W, once every 2 weeks.

TABLE A3. Response Assessment

Best Overall Response	All Patients (N = 45), No. (%)	6.5 mg/m^2 Q2W (n = 15), No. (%)	Ovarian EphA2+ (n = 9), No. (%)	Urothelial EphA2+ (n = 3), No. (%)
CR	1 (2.2)	0	1 (11.1)[a]	0
PR	3 (6.7)	1 (6.7)	1 (11.1)[b]	2 (66.7),[c,d]
SD	16 (35.6)	4 (26.7)	4 (44.4)	0
Progressive disease	17 (37.8)	8 (53.3)	3 (33.3)	1 (33.3)
Not evaluable	8 (17.8)[e]	2 (13.3)	0	0
ORR (CR + PR)	4 (8.9)	1 (6.7)	2 (22.2)	2 (66.7)
DCR at 4 months (CR + PR + SD ≥4 months)	9 (20.0)	3 (20.0)	6 (66.7)	2 (66.7)
DCR (CR + PR + SD)	20 (44.4)	5 (33.3)	6 (66.7)	2 (66.7)

Abbreviations: AE, adverse event; CR, complete response; DCR, disease control rate; ORR, overall response rate; PR, partial response; Q2W, once every 2 weeks; SD, stable disease.

[a]Ovarian CR patient started at 8.5 mg/m^2 once every 2 weeks and reduced to 6.5 mg/m^2 once every 2 weeks after 12 cycles. Patient remains on therapy >16 months.

[b]Ovarian PR patient started at 6.5 mg/m^2 once every 2 weeks and remains on therapy >4 months.

[c]A urothelial responder started at 8.5 mg/m^2 once every 2 weeks and reduced to 6.5 mg/m^2 once every 2 weeks after one dose. They remained on therapy approximately 6 months.

[d]A urothelial responder started at 10 mg/m^2 once every 2 weeks and reduced to 6.5 mg/m^2 once every 2 weeks after one dose. They remained on therapy approximately 3 months.

[e]All eight patients discontinued early from treatment and did not have a postbaseline response assessment.

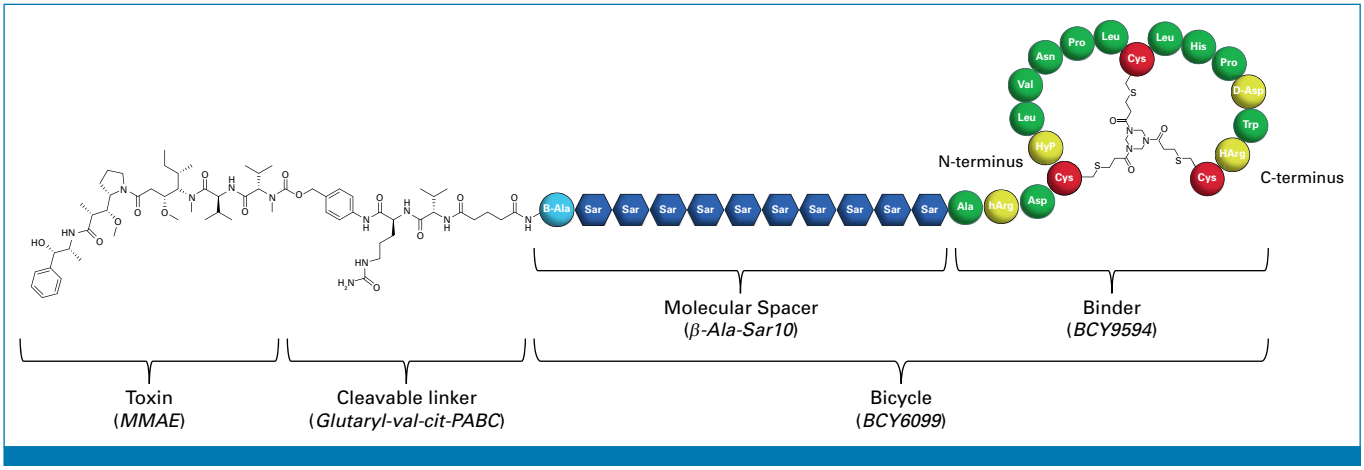

FIG A1. BT5528: EphA2 BTC chemical structure. BTC, Bicycle Toxin Conjugate; MMAE, monomethyl auristatin E.

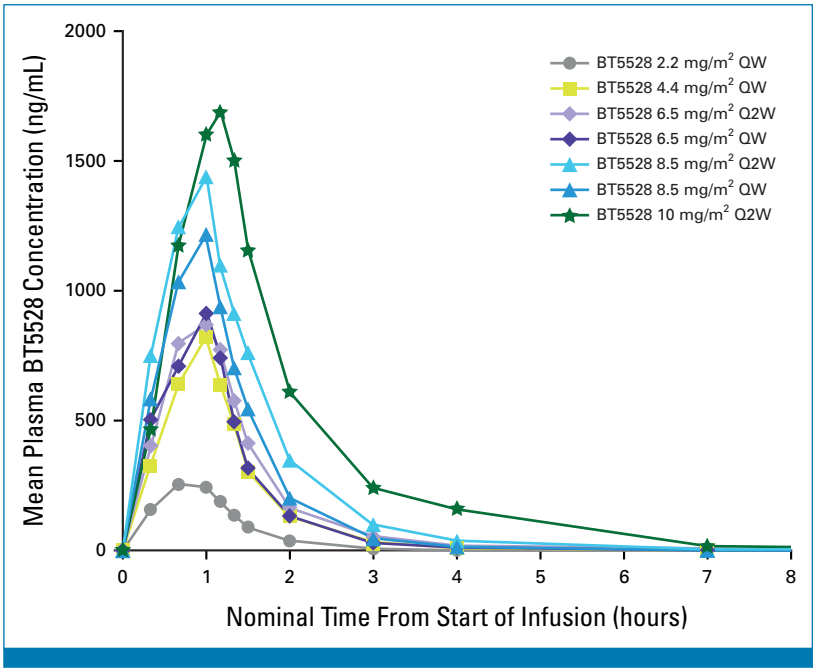

FIG A2. Mean plasma concentration of BT5528 versus time after a single IV infusion of BT5528 as monotherapy—Cycle 1 Day 1. QW, once every week; Q2W, once every 2 weeks.

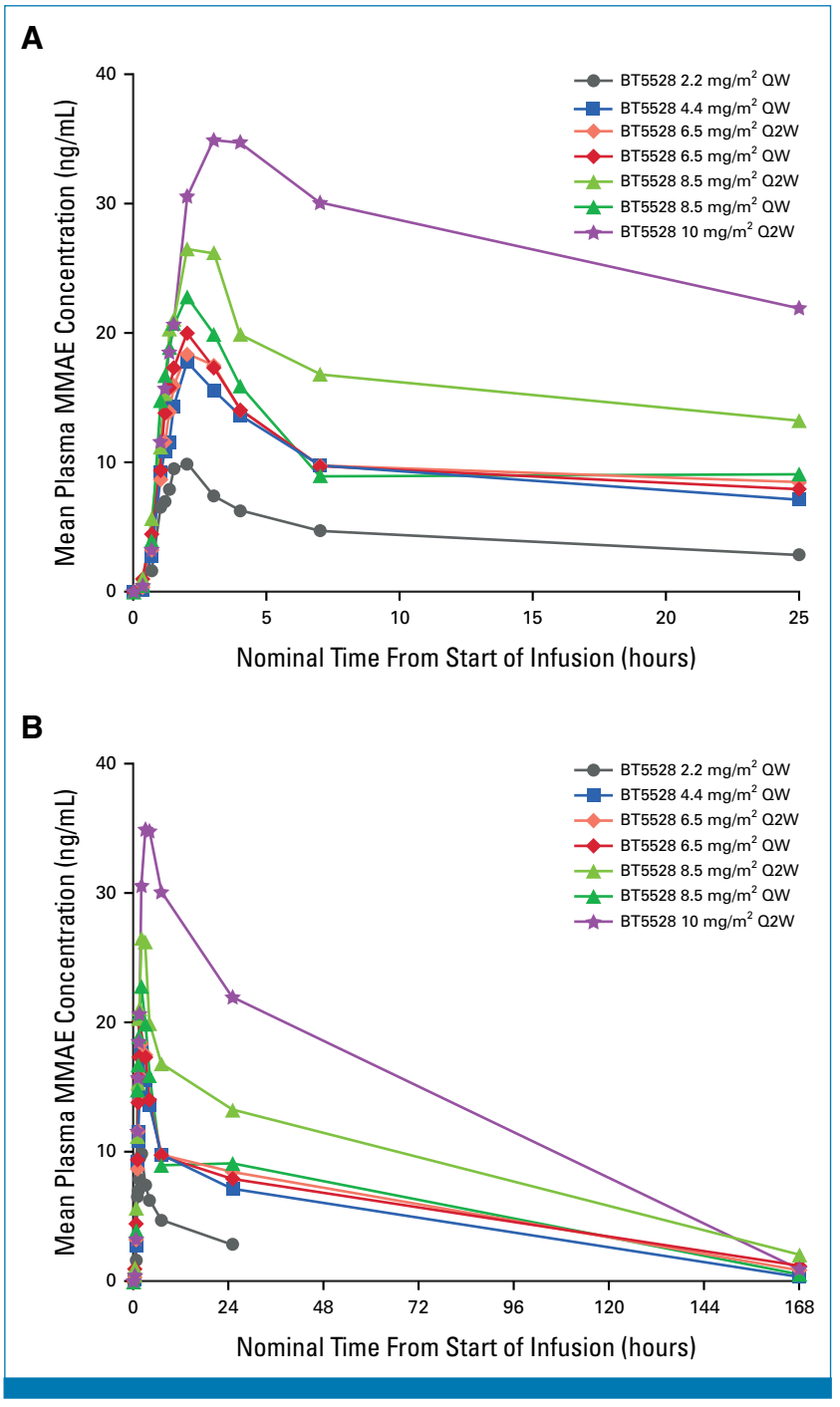

FIG A3. Mean plasma concentration of MMAE versus time after a single IV infusion of BT5528 as monotherapy—Cycle 1 Day 1. MMAE, monomethyl auristatin E. QW, once every week; Q2W, once every 2 weeks.