First-in-Human, Phase 1 Dose-Escalation Study of Biparatopic Anti-HER2 Antibody–Drug Conjugate MEDI4276 in Patients with HER2-positive Advanced Breast or Gastric Cancer

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ABSTRACT

MEDI4276 is a biparatopic tetravalent antibody targeting two nonoverlapping epitopes in subdomains 2 and 4 of the HER2 ecto-domain, with site-specific conjugation to a tubulysin-based microtubule inhibitor payload. MEDI4276 demonstrates enhanced cellular internalization and cytolysis of HER2-positive tumor cells *in vitro*. This was a first-in-human, dose-escalation clinical trial in patients with HER2-positive advanced or metastatic breast cancer or gastric cancer. MEDI4276 doses escalated from 0.05 to 0.9 mg/kg (60- to 90-minute intravenous infusion every 3 weeks). Primary endpoints were safety and tolerability; secondary endpoints included antitumor activity (objective response, progression-free survival, and overall survival), pharmacokinetics, and immunogenicity. Forty-seven patients (median age 59 years; median of seven prior treatment regimens) were treated. The maximum tolerated dose was exceeded at 0.9 mg/kg with two patients experiencing dose-limiting toxicities (DLTs) of grade 3 liver function test (LFT) increases, one of whom also had grade 3 diarrhea, which resolved. Two additional patients reported DLTs of grade 3 LFT increases at lower doses (0.4 and 0.6 mg/kg). The most common (all grade) drug-related adverse events (AEs) were nausea (59.6%), fatigue (44.7%), aspartate aminotransferase (AST) increased (42.6%), and vomiting (38.3%). The most common grade 3/4 drug-related AE was AST increased (21.3%). Five patients had drug-related AEs leading to treatment discontinuation. In the as-treated population, there was one complete response (0.5 mg/kg; breast cancer), and two partial responses (0.6 and 0.75 mg/kg; breast cancer)—all had prior trastuzumab, pertuzumab, and ado-trastuzumab emtansine (T-DM1). MEDI4276 has demonstrable clinical activity but displays intolerable toxicity at doses >0.3 mg/kg.

Introduction

In human mammary carcinoma, amplification of *ERBB2*, encoding transmembrane receptor tyrosine kinase HER2, was first reported by King and colleagues in 1985 (1). Subsequently, *ERBB2* gene amplification has been shown to correlate with shortened time to relapse and lower survival rates in women with breast cancer (2). HER2 is a key regulator of cell proliferation and survival (3), and is overexpressed in 15% to 20% of primary human breast cancers (4). HER2 is also overexpressed in approximately 20% of metastatic gastric cancers, most commonly in intestinal-type and gastroesophageal junction (GEJ) cancers (5). Chromoanasynthesis is a common mechanism leading to amplification of the *ERBB2* gene locus (6). Chromoanasynthesis events frequently involve the *NRG1* gene locus, in some cases resulting in *NRG1* gene fusions, that are associated with upregulation of *ERBB3* expression (6). These findings underscore the importance of ligand-activated HER2:HER3 heterodimers in the pathogenesis of *ERBB2*-amplified breast cancer, and present a therapeutic opportunity for antibody-based therapeutics that block the extracellular subdomain 2 dimerization interface of the HER2 receptor (e.g., pertuzumab; refs. 7–12).

Multiple HER2-targeting therapies, including trastuzumab, pertuzumab, margetuximab, lapatinib, neratinib, tucatinib, antibody–drug conjugates (ADCs) ado-trastuzumab emtansine (T-DM1), and fam-trastuzumab deruxtecan-nxki (T-DXd) are approved in the United States for the treatment of HER2 overexpressing/amplified (HER2-positive) breast cancer, whereas trastuzumab is the only approved HER2-targeting therapy for HER2-positive advanced gastric cancer and GEJ cancer (13–21). Despite availability of these agents, most patients with advanced-stage, metastatic HER2-positive cancers experience disease progression, resulting from a myriad of proposed drug resistance mechanisms to HER2-targeting therapies (22, 23). Consequently, there is a need for therapies that improve outcomes for patients with HER2-overexpressing advanced breast cancer and gastric cancer who have progressed after treatment with available therapies. T-DM1 has been shown to bypass a common mechanism of resistance to HER2-targeted therapies, namely activating mutations of *PIK3CA* (24). However, acquired resistance to T-DM1 can occur,

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possibly caused by mechanisms including (i) antigen loss and/or downregulation, (ii) increased expression of drug transporters MDR1 (*ABCB1*) and MRP1 (*ABCC1*), (iii) defects in ADC trafficking, and/or (iv) changes in receptor and signaling pathways (25, 26). In addition, aberrations in lysosomal pH and proteolytic activity and loss of the lysosomal transporter solute carrier family 46, member 3 (SLC46A3) have been observed in T-DM1–resistant cell lines (26–29). Among newer HER2-directed ADCs with novel payloads are [fam-] trastuzumab deruxtecan (T-DXd, formerly known as DS-8201a; Enhertu; approved by the FDA in 2019), (vic-) trastuzumab duocarmazine (SYD985), and ZW49. T-DXd is composed of trastuzumab, conjugated to a novel topoisomerase I inhibitor payload [drug-to-antibody ratio (DAR) 8] via an enzymatically cleavable tetrapeptide-linker, whereas SYD985 has a cleavable DAR 2–4 linker-duocarmycin (a minor groove DNA binder, leading to irreversible alkylation of DNA) payload conjugated to trastuzumab (30, 31). ZW49 is a novel N-acyl sulfonamide auristatin cytotoxin conjugated by a proprietary cleavable linker to ZW25—a novel bispecific antibody targeting HER2 extracellular domains (ECD) 2 and ECD4, resulting in multiple differentiated mechanisms of action including increased tumor cell binding, blockade of ligand-dependent and independent growth, and improved receptor internalization and downregulation relative to trastuzumab (32).

XMT-1522 is also a novel anti-HER2 ADC, which contains a human immunoglobulin G1 (IgG1) anti-HER2 mAb (HT-19) and binds to domain IV of HER2 (33). Each XMT-1522 antibody has an average of 12 auristatin F-hydroxypropylamide (AF-HPA) moieties attached to HT-19 via a cysteine linkage using a biodegradable hydrophilic polymer, which facilitates high AF-HPA loading and inhibition of tubulin polymerization (33). XMT-1522 was evaluated in patients with HER2-positive advanced breast cancer, gastric cancer, and non–small cell lung cancer (NCT02952729). Of note, the FDA placed a partial clinical hold on the study (that was subsequently lifted) following the death of one patient that was thought to be drug-related (34). The safety and efficacy of ADCT-502, an ADC comprising an engineered version of trastuzumab directed against HER2 conjugated to a pyrrolobenzodiazepine dimer cytotoxin, was evaluated in patients with HER2-positive advanced solid tumors (NCT03125200). However, the study was terminated during the dose escalation phase due to safety concerns (35). Many other anti-HER2 ADCs are currently in clinical development, including A166, ALT-P7, ARX788, RC-48, and PF-06804103. A166 was shown to be well-tolerated with promising antitumor activity in patients with heavily pretreated HER2-positive tumors (36, 37). ALT-P7, an ADC with two molecules of monomethyl auristatin E (MMAE) site-specifically conjugated to a cysteine-containing peptide motif of a trastuzumab variant, was evaluated in patients with HER2-positive advanced breast cancer who had received at least two prior anti-HER2 treatment strategies (38). ALT-P7 was well tolerated up to a dose of 4.2 mg/kg (38), and phase 2 studies are ongoing (NCT03281824; ref. 39). ARX788, an ADC linked to a noncleavable amberstatin (AS269) cytotoxic payload, was evaluated in patients with metastatic HER2-positive breast cancer (NCT02512237) and was well tolerated (40, 41). RC48 selectively delivers MMAE into HER2-expressing tumor cells, was well-tolerated, and demonstrated promising efficacy in patients with HER2-positive metastatic breast cancer (42). PF-0684103, an anti-HER2 mAb conjugated to Aur0101, demonstrated a manageable safety profile and promising antitumor activity in patients with advanced breast cancer and gastric cancer (43).

Compared with ZW25 and ZW49, which are biparatopic monoclonal ADCs with two binding domains, MEDI4276 is an investigational ADC comprised of a biparatopic tetravalent mAb that binds to two distinct HER2 epitopes (44). The antibody backbone is a fully human (XenoMouse-derived) antibody 39S–directed against subdomain 2 of the HER2 ECD. Like pertuzumab, it is capable of blocking HER2/HER3 receptor phosphorylation in recombinant heregulin-β1-stimulated cancer cells. MEDI4276 was constructed from 39S by genetically linking the scFv of trastuzumab (which binds HER2 ECD subdomain 4 with high affinity) to the amino terminus of the 39S IgG1 heavy chain. The resulting construct contains two antigen-binding units on each arm, capable of interacting with two different epitopes on the HER2 ectodomain (subdomains 2 and 4, for 39S and trastuzumab scFv, respectively; refs. 44, 45). MEDI4276 blocks HER2:HER3 heterodimerization in the presence of heregulin β-1. Based on the co-crystal structure of the 39S Fab-HER2 complex, the 39S and trastuzumab epitopes are located at the opposite ends of HER2 ECD at a distance >90 Å from each other (45). Consequently, the C-terminal residue of trastuzumab scFv and the N-terminal amino acid of 39S heavy chain are unable to bind simultaneously to the same HER2 receptor molecule. Rather, the biparatopic construct crosslinks adjacent HER2 receptors, resulting in receptor clustering at the cell surface (44, 45). Such clustering results in rapid receptor internalization, inhibition of recycling, and promotes intracellular trafficking towards lysosomal degradation. A tubulysin warhead (AZ13599185; ref. 46), which inhibits microtubule polymerization during mitosis to induce apoptotic cell death (44), is conjugated via a maleimidocaproyl linker via site-specific conjugation to two cysteines introduced at heavy chain residues 239 and 442, resulting in an average DAR of approximately 4 (47). In contrast with T-DM1, MEDI4276 kills neighboring HER2-positive and -negative tumor cells (within a heterogeneous tumor cell population), via a potent bystander killing effect (44). Efflux pumps responsible for drug maytansinoid 1 (DM1) resistance do not effectively transport tubulysin; accordingly, there is potential for non-cross resistance between DM1 and tubulysin (44). Moreover, MEDI4276 demonstrated *in vivo* activity in T-DM1-resistant preclinical models (44). Finally, a lysine-to-phenylalanine substitution at residue 234 was introduced in MEDI4276, which (together with the drug being conjugated at S239) ablates Fcγ receptor interactions (47, 48). Here, we report a first-in-human, phase 1, multicenter, open-label, dose-escalation study to evaluate the safety, pharmacokinetics (PK), immunogenicity, and antitumor activity of MEDI4276 in patients with HER2-positive advanced breast cancer or gastric cancer (NCT02576548).

Materials and Methods

Study design and treatment

A study flow diagram for the dose-escalation sequence is shown in **Fig. 1**. Patients with HER2-positive breast cancer or gastric cancer refractory to standard therapy were enrolled, using a 3 + 3 design with a 21-day dose-limiting toxicity (DLT) evaluation period. The starting dose selection was targeted to be one-sixth of the human equivalent dose of the highest nonseverely toxic dose in a repeat-dose good laboratory practice nonhuman primate study. MEDI4276 was infused intravenously over 60 to 90 minutes at 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.75, or 0.9 mg/kg every 3 weeks. Any dose level not exceeding the MTD during escalation could be expanded to up to 18 patients to provide additional pharmacodynamics, PK, and safety data to inform dose selection. Patients were permitted to receive MEDI4276 until disease progression for up to 2 years.

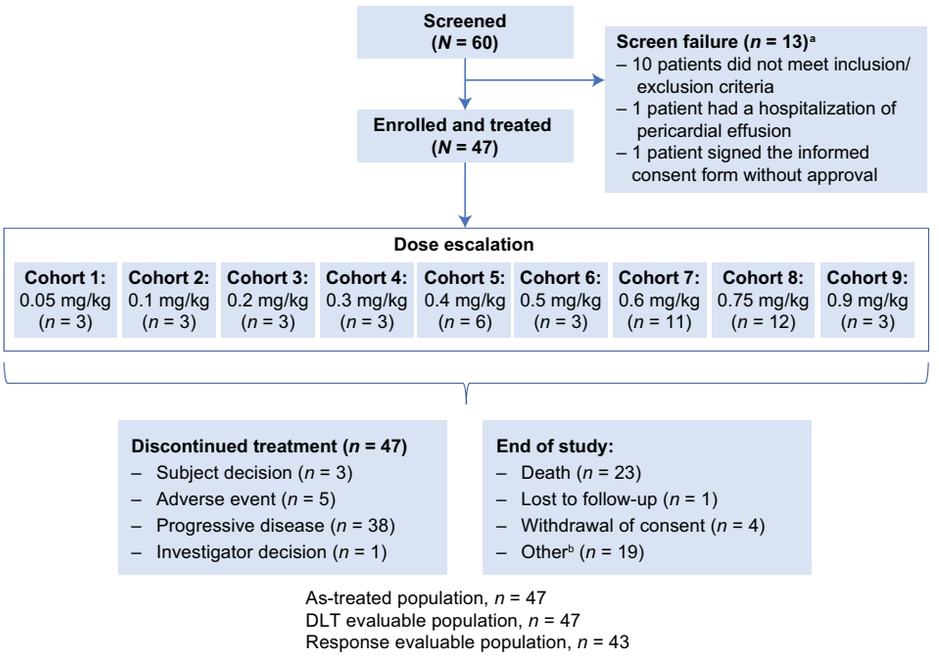

Figure 1.
Study flow diagram–dose-escalation. [a]Screen failure unknown for one patient. [b]For all patients in this category, this was reported as "Other (sponsor decision)" and was due to the sponsor's decision to end the study.

Eligibility criteria

Patients were ≥18 years of age, with histologically or cytologically documented unresectable, locally advanced or metastatic breast cancer or gastric cancer refractory to standard therapy. HER2-positive disease was documented as fluorescence *in situ* hybridization-positive and/or 3+ by IHC on previously collected tumor tissue, per American Society of Clinical Oncology/College of American Pathologists HER2 testing clinical practice guidelines (49); and at least one lesion measurable by RECIST version 1.1. Patients with breast cancer were required to have previously been treated with trastuzumab, pertuzumab, and T-DM1, either alone or in combination; whereas patients with gastric cancer were required to have previously received a trastuzumab-containing chemotherapy regimen. There was no limit to the maximum number of prior treatment regimens allowed before study entry. All patients were required to have left ventricular ejection fraction ≥50% by either echocardiogram or multigated acquisition scan; an Eastern Cooperative Oncology Group performance status of 0 or 1; and adequate bone marrow and organ function. Patients were not permitted to be concurrently enrolled in another clinical study (unless it was an observational study), or to have received any conventional or investigational anticancer treatment within 28 days before the first dose of MEDI4276; and no hormone therapy during the 14 days prior to receiving the first dose of MEDI4276. Patients were excluded if they had a history of exposure to specified cumulative doses of anthracyclines (≥350 mg/m^2 doxorubicin); had unresolved toxicities from previous anticancer therapies; diarrhea of any grade within 14 days before the first dose of MEDI4276; had a history of (or with current symptomatic) congestive heart failure or serious cardiac arrhythmia requiring treatment; had a history of myocardial infarction or unstable angina; or cardiac troponin I ≥0.2 ng/mL within 28 days before the first dose of MEDI4276; known brain metastases that were untreated, symptomatic, or required therapy to control symptoms; severe or uncontrolled nonmalignant systemic disease.

Ethical conduct of the study and patient informed consent

The study protocol, protocol amendments, and patient informed-consent documents were approved by the Institutional Review Board (IRB) or Independent Ethics Committee (IEC) at each site. This study was conducted in accordance with the Declaration of Helsinki, the International Council for Harmonization Guidance for Good Clinical Practice (Topic E6), any applicable laws and requirements, and any conditions required by a regulatory authority and/or IRB/IEC that approved this study to be conducted in its territory. All patients provided written informed consent before conduct of any protocol specific activity or study entry.

Objectives and endpoints

The primary objective of the study was to assess the safety, describe the DLTs, determine the MTD or the maximum administered dose (in the absence of exceeding the MTD), for MEDI4276 administered as a single agent. Safety endpoints included adverse events (AE), serious AEs (SAEs), AEs of special interest (AESI), DLTs, changes in laboratory parameters, vital signs, and electrocardiogram results. Secondary objectives were to: (i) evaluate the preliminary antitumor activity of MEDI4276, (ii) determine the PK of MEDI4276 administered as a single agent [i.e., maximum observed concentration (C_{max}), area under the drug concentration–time curve [AUC], clearance [CL], and terminal half-life ($t_{½}$)], and (iii) assess the immunogenicity potential of MEDI4276.

Statistical analysis

The as-treated population included all patients who received any investigational product analyzed according to the treatment actually received. The DLT-evaluable population was defined as all patients enrolled in the dose-escalation phase who received ≥1 dose of MEDI4276 and completed the safety follow-up through the 21-day DLT-evaluation period or experienced any DLT during the DLT-evaluation period. In terms of safety considerations, the MTD evaluation was based on the DLT-evaluable population; all other safety analyses were based on the as-treated population. AEs were coded by

Medical Dictionary for Regulatory Activities (MedDRA) version 17.1 or newer and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events 4.03. AEs and SAEs were collected from the time of informed consent through the end of the follow-up period.

Efficacy assessment

Clinical efficacy was based on the as-treated population, by investigator assessment according to RECIST v1.1. Time to event data were summarized using Kaplan–Meier estimates. Disease assessments were performed every 6 weeks during the first year of treatment, and then every 12 weeks after the first year of treatment.

Pharmacokinetics

One of the major catabolic processes for ADCs is the deconjugation of the warhead. Thus, deconjugation of the ADC *in vivo* must be characterized to evaluate ADC catabolism upon administration in patients. In addition, preclinical data revealed that the tubulysin warhead AZ13599185 (abbreviated as AZ9185) undergoes deacetylation to free deacetylated tubulysin (AZ13687308, abbreviated as AZ7308). Tubulysin has the potential to deacetylate as either a free molecule or when conjugated to antibody. MEDI1498 is a deacetylated version of MEDI4276 (conjugated AZ7308). To comprehensively characterize the PK of MEDI4276 and its potential catabolites, three bioanalytical methods designed to quantify five different classes of analytes were validated and implemented: method 1 for MEDI4276 Total ADC [Conjugated AZ9185, lower limit of quantification (LLOQ) = 25 ng/mL] and Total Antibody (LLOQ = 45 ng/mL); method 2 for MEDI1498 Total Deacetylated ADC (Conjugated AZ13687308, LLOQ = 25 ng/mL); method 3 for free tubulysin (AZ9185, LLOQ = 50 pg/mL), and free deacetylated tubulysin (AZ7308, LLOQ = 50 pg/mL) concentrations. The hybrid ligand-binding assay-LC-MS/MS (LBA-LC-MS/MS) method employing capture with anti-MEDI4276 antibody, followed by digestion with trypsin, was utilized to quantify Total Antibody and Total Conjugated AZ9185 by measuring the characteristic peptide and tubulysin, respectively. Similarly, MEDI1498 Total ADC concentrations were measured using the LBA-LC-MS/MS method, employing the same capture as for MEDI4276 and Total Antibody, but detecting deacetylated tubulysin after tryptic digestion using LC-MS/MS. The reference standards used in the methods consisted of either MEDI4276 or MEDI1498 serving as representative of either fully acetylated or fully deacetylated ADC with an average DAR of ∼4. These DAR-sensitive methods for the quantification of average DAR of ADCs rely upon the approach where conjugated toxin is liberated via proteolytical digestion and the released toxin, serving as the surrogate analyte, representing the average number of tubulysin molecules conjugated to the antibody, is then detected via LC-MS/MS. One can expect direct proportionality between the moles of conjugated toxin and the assay signal manifested as peak area ratio between the surrogate analyte (released peptide or toxin) and the appropriate internal standard. Thus, both methods for conjugated AZ9185 and conjugated AZ7308 are expected to be DAR-sensitive (50). The methods report average DAR by correlating it back to the concentrations of the reference standard ADC (either MEDI4276 or MEDI1498). Therefore, any changes in the concentrations of MEDI4276 or MEDI1498 when compared with concentrations of Total Antibody indeed encapsulate changes in DAR of this ADC. A multiplex LC-MS/MS method for the quantification of AZ9185 and AZ7308 employed extraction using protein precipitation, followed by Solid-phase Extraction with subsequent analysis by LC-MS/MS. Further method details can be found in the article by Faria and colleagues (51). The analyte concentrations were then analyzed using Phoenix WinNonlin (Pharsight, Mountain View, CA) to generate noncompartmental PK parameters. Plasma samples for PK analysis were collected predose on Day 1, and immediately post end of infusion, 2 hours postinfusion, and 6 hours postinfusion. Plasma samples were also collected on Days 2, 3, 8, and 15. On designated days of MEDI4276 administration between Day 22 (cycle 2) and Day 127 (cycle 7), plasma samples were collected predose and immediately post end of infusion. Starting on Day 211 (cycle 11) and every 12 weeks thereafter on days of MEDI4276 administration, plasma samples were collected immediately post end of infusion.

Immunogenicity

The immunogenic potential of MEDI4276 was evaluated by a validated bridging immunoassay, and a tiered immunogenicity testing approach for screening, confirmation, and titer. The bridging immunoassay utilizes a mixture of equal concentrations of biotinylated MEDI4276, biotinylated deacetylated MEDI4276 (MEDI1498) and biotinylated Her-Bs2-FCC (unconjugated MEDI4276) as capture; and a mixture of equal concentrations of ruthenylated MEDI4276, ruthenylated deacetylated MEDI4276 (MEDI1498), and ruthenylated Her-Bs2-FCC as detection. This novel method ensures detection of antidrug antibodies (ADA) to MEDI4276 and its major metabolites using a single assay for screening, confirmation, and titer. The method was validated to have sufficient sensitivity (105.75 ng/mL) for adequate detection of ADAs, according to FDA guidance (52). Surrogate anti-MEDI4276 antibody-positive controls at 390, 6,250, 25,000, and 100,000 ng/mL were detectable in the presence of up to 100, 1,000, 10,000, and 100,000 ng/mL of MEDI4276, respectively. Serum samples for immunogenicity assessment were collected predose; on days 1, 15, and 22 of cycle 1; every 3 weeks through Day 127; and then every 12 weeks starting on Day 211 of MEDI4276 administration.

Results

Patient disposition, demographics, and exposure

The first patient signed informed consent on 23 September 2015, and the final visit for the last patient was 23 May 2018. All 47 patients had metastatic disease; 32 patients had breast cancer, and 15 patients had gastric cancer. The median duration of follow-up was 8 months (range, 0.7–30.6 months). Among the 32 patients with breast cancer, the majority were female (96.9%) and white (81.3%); the median age was 58 years (range, 33–75 years). Patients had a median of 8 prior regimens (range, 3–23), and received the following HER2-targeting therapies (**Table 1**): median of 3 (range, 1–11) prior trastuzumab-containing regimens; median of 1 (range, 1–2) prior T-DM1-containing regimen; and median of 1 (range, 1–3) prior pertuzumab-containing regimen. Among the 15 patients with gastric cancer, the majority were male (86.7%) and white (93.3%); the median age was 66 years (range, 44–76 years). Patients had a median of 4 prior regimens (range, 2–8) and received a median of 1.5 (range, 1–4) prior trastuzumab-containing regimens (**Table 1**). Across the dose-escalation cohorts, patients received a median of 2 cycles of treatment (range, 1–14 cycles) corresponding to a median of 6.14 weeks (range, 3–42 weeks). The 12 patients in the 0.75 mg/kg cohort received a median of 3.5 cycles of treatment.

Safety and tolerability

All adverse events

Across all dose-escalation cohorts, 46 (97.9%) patients had at least one AE of any grade, irrespective of causality. The most frequent AEs

Table 1. Demographics and patient disposition.

	0.05 mg/kg *n* = 3	0.1 mg/kg *n* = 3	0.2 mg/kg *n* = 3	0.3 mg/kg *n* = 3	0.4 mg/kg *n* = 6	0.5 mg/kg *n* = 3	0.6 mg/kg *n* = 11	0.75 mg/kg *n* = 12	0.9 mg/kg *n* = 3	Total *N* = 47
Age, years, median (range)	60.0 (41–75)	66.0 (59–67)	47.0 (44–66)	68.0 (62–69)	62.5 (39–76)	54.0 (37–56)	65.0 (45–72)	56.0 (33–69)	67.0 (59–70)	59.0 (33–76)
<65	2 (66.7)	1 (33.3)	2 (66.7)	1 (33.3)	3 (50.0)	3 (100)	5 (45.5)	11 (91.7)	1 (33.3)	29 (61.7)
≥65	1 (33.3)	2 (66.7)	1 (33.3)	2 (66.7)	3 (50.0)	0	6 (54.5)	1 (8.3)	2 (66.7)	18 (38.3)
Sex										
Female	3 (100)	2 (66.7)	1 (33.3)	1 (33.3)	4 (66.7)	3 (100)	8 (72.7)	9 (75.0)	2 (66.7)	33 (70.2)
Male	0	1 (33.3)	2 (66.7)	2 (66.7)	2 (33.3)	0	3 (27.3)	3 (25.0)	1 (33.3)	14 (29.8)
ECOG performance status										
0	0	0	2 (66.7)	0	3 (50.0)	1 (33.3)	2 (18.2)	3 (25.0)	1 (33.3)	12 (25.5)
1	3 (100)	3 (100)	1 (33.3)	3 (100)	3 (50.0)	2 (66.7)	9 (81.8)	9 (75.0)	2 (66.7)	35 (74.5)
Breast cancer, *n*	2	2	1	1	4	3	7	10	2	32
ER/PR positive	2 (100)	2 (100)	1 (100)	1 (100)	3 (75.0)	2 (66.7)	4 (57.1)	6 (60.0)	2 (100)	23 (71.9)
HER2 positive	2 (100)	2 (100)	1 (100)	0	4 (100)	3 (100)	7 (100)	10 (100)	2 (100)	31 (96.9)
IHC 3+	1 (50.0)	2 (100)	1 (100)	0	2 (50.0)	1 (33.3)	3 (42.9)	7 (70.0)	1 (50)	18 (56.3)
FISH positive	1 (50.0)	0	0	0	4 (100)	2 (66.7)	4 (57.1)	4 (40.0)	1 (50.0)	16 (50.0)
Prior breast cancer treatment, median number of regimens (range)										
Overall	10.0 (5–15)	16.0 (10–22)	3.0 (3–3)	7.0 (7–7)	8.5 (7–15)	5.0 (5–10)	6.0 (5–17)	8.0 (4–22)	16.5 (10–23)	8.0 (3–23)
Trastuzumab	3.5 (3–4)	6.5 (2–11)	1.0 (1–1)	2.0 (2–2)	4.5 (3–5)	3.0 (2–4)	3.0 (1–5)	3.0 (1–5)	4.5 (4–5)	3.0 (1–11)
Pertuzumab	1.0 (1–1)	1.0 (1–1)	1.0 (1–1)	1.0 (1–1)	2.0 (1–2)	2.0 (1–2)	1.0 (1–2)	1.0 (1–3)	1.0 (1–1)	1.0 (1–3)
Ado-trastuzumab emtansine	1.0 (1–1)	1.0 (1–1)	1.0 (1–1)	1.0 (1–1)	1.0 (1–2)	1.0 (1–1)	1.0 (1–1)	1.0 (1–2)	1.0 (1–1)	1.0 (1–2)
Gastric cancer, *n*	1	1	2	2	2	0	4	2	1	15
HER2 positive	1 (100)	1 (100)	2 (100)	2 (100)	2 (100)	—	4 (100)	2 (100)	1 (100)	15 (100)
IHC 3+	1 (100)	1 (100)	1 (50)	1 (50)	0	—	2 (50)	2 (100)	1 (100)	9 (60)
FISH positive	0	0	1 (50)	1 (50)	2 (100)	—	2 (50)	0	0	6 (40)
Prior gastric cancer treatment, median number of regimens (range)										
Overall	7.0 (7–7)	8.0 (8–8)	4.0 (4–4)	4.0 (3–5)	3.5 (2–5)	—	3.5 (2–8)	3.0 (2–4)	2.0 (2–2)	4.0 (2–8)
Trastuzumab	4.0 (4–4)	2.0 (2–2)	1.5 (1–2)	3.0 (3–3)	1.5 (1–2)	—	1.0 (1–4)	1.5 (1–2)	1.0 (1–1)	1.5 (1–4)

Note: Data are shown as *n* (%) unless otherwise noted.

Abbreviations: ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; PR, progesterone receptor.

Table 2. Adverse events.

	Treatment-emergent AEs in ≥5% of patients					Treatment-related grade 3 or grade 4 AEs				
Preferred term, *n* (%)	**0.05–0.5 mg/kg, *n* = 21**	**0.6 mg/kg, *n* = 11**	**0.75 mg/kg, *n* = 12**	**0.9 mg/kg, *n* = 3**	**Total, *N* = 47**	**0.05–0.5 mg/kg, *n* = 21**	**0.6 mg/kg, *n* = 11**	**0.75 mg/kg, *n* = 12**	**0.9 mg/kg, *n* = 3**	**Total, *N* = 47**
Any event	20 (95.2)	11 (100)	12 (100)	3 (100)	46 (97.9)	3 (14.3)	4 (36.4)	8 (66.7)	2 (66.7)	17 (36.2)
Nausea	15 (71.4)	5 (45.5)	10 (83.3)	3 (100)	33 (70.2)	0	0	0	1 (33.3)	1 (2.1)
Fatigue	10 (47.6)	4 (36.4)	9 (75.0)	3 (100)	26 (55.3)	0	1 (9.1)	0	0	1 (2.1)
Vomiting	9 (42.9)	6 (54.5)	5 (41.7)	2 (66.7)	22 (46.8)	0	0	0	0	0
AST increased	5 (23.8)	5 (45.5)	8 (66.7)	2 (66.7)	20 (42.6)	3 (14.3)	3 (27.3)	3 (25.0)	1 (33.3)	10 (21.3)
Diarrhea	4 (19.0)	5 (45.5)	8 (66.7)	3 (100)	20 (42.6)	0	0	1 (8.3)	2 (66.7)	3 (6.4)
ALT increased	4 (19.0)	5 (45.5)	8 (66.7)	2 (66.7)	19 (40.4)	2 (9.5)	2 (18.2)	2 (16.7)	1 (33.3)	7 (14.9)
Decreased appetite	4 (19.0)	4 (36.4)	9 (75.0)	1 (33.3)	18 (38.3)	0	0	0	0	0
Alopecia	0	4 (36.4)	6 (50.0)	2 (66.7)	12 (25.5)	0	0	0	0	0
Constipation	5 (23.8)	4 (36.4)	2 (16.7)	1 (33.3)	12 (25.5)	0	0	0	0	0
Weight decreased	6 (28.6)	1 (9.1)	3 (25.0)	2 (66.7)	12 (25.5)	0	0	0	0	0
Abdominal pain	2 (9.5)	1 (9.1)	6 (50.0)	1 (33.3)	10 (21.3)	0	0	0	0	0
ALP increased	3 (14.3)	4 (36.4)	1 (8.3)	2 (66.7)	10 (21.3)	2 (9.5)	0	0	0	2 (4.3)
Dehydration	2 (9.5)	3 (27.3)	3 (25.0)	2 (66.7)	10 (21.3)	0	0	1 (8.3)	0	1 (2.1)
Dyspnea	1 (4.8)	1 (9.1)	4 (33.3)	2 (66.7)	8 (17.0)	0	0	0	0	0
Hypokalemia	0	1 (9.1)	4 (33.3)	3 (100)	8 (17.0)	0	0	1 (8.3)	0	1 (2.1)
Urinary tract infection	1 (4.8)	2 (18.2)	4 (33.3)	1 (33.3)	8 (17.0)	0	0	0	0	0
Blood bilirubin increased	2 (9.5)	2 (18.2)	0	1 (33.3)	5 (10.6)	1 (4.8)	1 (9.1)	0	1 (33.3)	3 (6.4)
Neuropathy peripheral	0	1 (9.1)	2 (16.7)	0	3 (6.4)	0	1 (9.1)	1 (8.3)	0	2 (4.3)
Peripheral sensory neuropathy	2 (9.5)	0	1 (8.3)	0	3 (6.4)	0	0	1 (8.3)	0	1 (2.1)

(>20%) were nausea, fatigue, vomiting, increased aspartate aminotransferase (AST), diarrhea, increased alanine aminotransferase (ALT), decreased appetite, alopecia, constipation, decreased weight, abdominal pain, increased blood alkaline phosphatase (ALP), and dehydration (**Table 2**).

Treatment-related AEs

Overall, 43 (9.15%) patients had at least one AE (any grade) that was considered treatment-related by the investigator. The most frequent treatment-related AEs (>10%) were nausea (59.6%), fatigue (44.7%), AST increased (42.6%), ALT increased (40.4%), vomiting (38.3%), diarrhea and decreased appetite (29.8% each); alopecia (25.5%), blood ALP increased (21.3%); dyspepsia and dysgeusia (12.8% each); and increased blood bilirubin, weight decreased, and dehydration (10.6% each). Seven (14.9%) patients in the 0.75 mg/kg cohort had treatment-related AEs leading to a dose reduction of MEDI4276.

Adverse events of special interest

AEs of special interest (AESI) were defined as elevations in liver biochemistry that met Hy's law [defined as AST or ALT ≥3 × the upper limit of normal (ULN) together with total bilirubin levels ≥2 × ULN, where no reason other than the study drug is found to explain the combined increases]; potential Hy's law (defined as AST or ALT ≥3 × ULN together with total bilirubin levels ≥2 × ULN); grade 3 elevations in ALT, AST, or total bilirubin level; and diarrhea that was recurrent or persistent despite maximal medical therapy and led to permanent treatment discontinuation. Diarrhea is an AE of special interest because it was observed in the good laboratory practice toxicology study for MEDI4276 and patients with breast cancer tend to have a higher risk of all-grade diarrhea when receiving HER2-targeted agents compared with patients who have other types of cancer (53). In total, there were three patients who experienced AESIs. One patient in the 0.75 mg/kg cohort had treatment-related grade 3 diarrhea and grade 3 ALT increased which were considered AESIs. One patient in the 0.9 mg/kg cohort had increased ALT/AST with concurrent elevated bilirubin (also considered an SAE and DLT); bilirubin levels in this patient returned to within normal limits by Day 29, but ALT/AST levels remained slightly elevated (1.83 × ULN and 1.29 × ULN, respectively). There was no evidence of hemolysis, virology testing was negative, and the patient was not known to have liver metastases. All events for this patient were considered by the investigator to be related to MEDI4276 treatment. A second patient in the 0.9 mg/kg cohort experienced grade 3 increased ALT and AST, which were related to MEDI4276 and led to study-drug discontinuation.

Treatment-related grade 3 or 4 adverse events

Overall, 17 (36.2%) patients had at least one treatment-related grade 3 or 4 AE. The most frequent treatment-related grade 3 or 4 AEs (>5%) were increased AST (21.3%), increased ALT (14.9%); and diarrhea and increased blood bilirubin (6.4% each; **Table 2**). Events of grade 3/4 diarrhea and increased bilirubin all resolved, except for one case of increased bilirubin which resolved to grade 2 and was still ongoing at the end of the study.

AEs leading to treatment discontinuation

Five (10.6%) patients had treatment-related AEs leading to discontinuation; three of these patients (one in the 0.4 mg/kg cohort, and two in the 0.9 mg/kg cohort) had liver function test (LFT) elevations, which were also considered DLTs. The other two patients (one in the 0.6 mg/kg, and one in the 0.75 mg/kg cohort) had grade 3 peripheral neuropathy, and grade 2 peripheral sensory neuropathy, respectively. Two additional patients discontinued treatment due to investigator or patient decision.

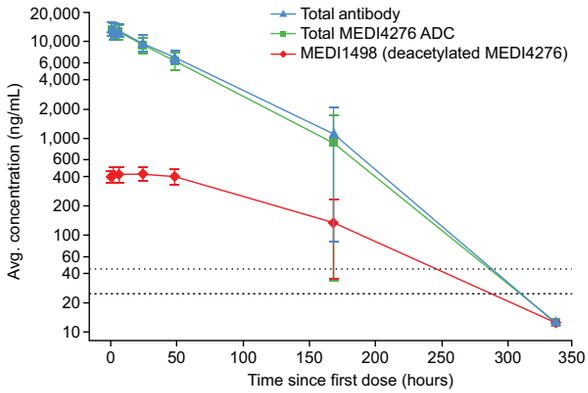

Figure 2.

Mean (SD) plasma concentration-time profiles of MEDI4276 (total ADC), reflecting average DAR, total mAb and MEDI1498 after first intravenous dose of 0.75 mg/kg MEDI4276.

The top dotted line indicates the total antibody LLOQ; the bottom dashed line indicates the ADC LLOQ.

DLTs

Four patients experienced DLTs during the study. One patient each in the 0.4 and 0.6 mg/kg cohort had LFT elevations (increased blood ALP, increased AST, increased ALT, increased total bilirubin level). LFT elevations in the 0.4 mg/kg cohort were also SAEs. One patient in the 0.9 mg/kg cohort had LFT elevations and an SAE of diarrhea. One patient in the 0.9 mg/kg cohort had LFT elevation (also an SAE). The majority of DLTs were grade 3 in severity and resolved. The MTD was exceeded at 0.9 mg/kg every three weeks due to the occurrence of DLTs in two out of three patients. The MTD was defined to be 0.75 mg/kg every 3 weeks.

Mortality

Twenty-two patients died due to underlying malignant disease under treatment, and one patient's cause of death was not available to the site or sponsor. There were no grade 5 drug-related AEs reported.

Pharmacokinetics and immunogenicity

Concentration–time profiles and PK parameters for MEDI4276 and Total Antibody measurement, respectively, were similar (**Fig. 2**; Supplementary Table S1). The methods employed for the quantification of Total Conjugated MEDI4276 and MEDI1498 utilized *DAR-sensitive* mass spectrometry-based approaches (50) that measured average DAR by interrelating the moles of conjugated toxin released using trypsin and then quantified using LC-MS/MS from unknown samples against a standard curve generated using respective ADC reference standards. Comparison of MEDI4276 concentrations with Total Antibody (**Fig. 2**; Supplementary Table S1; Supplementary Figs. S1 and S2) suggest *minimal* deconjugation and, thus, minimal changes in average DAR over time. This is further supported by the observation of low levels of free tubulysin (AZ9185, Supplementary Fig. S3A). The LLOQ for these highly sensitive assays was 50 pg/mL, indicating that the low levels detected were not related to poor assay sensitivity. Comparison of MEDI4276

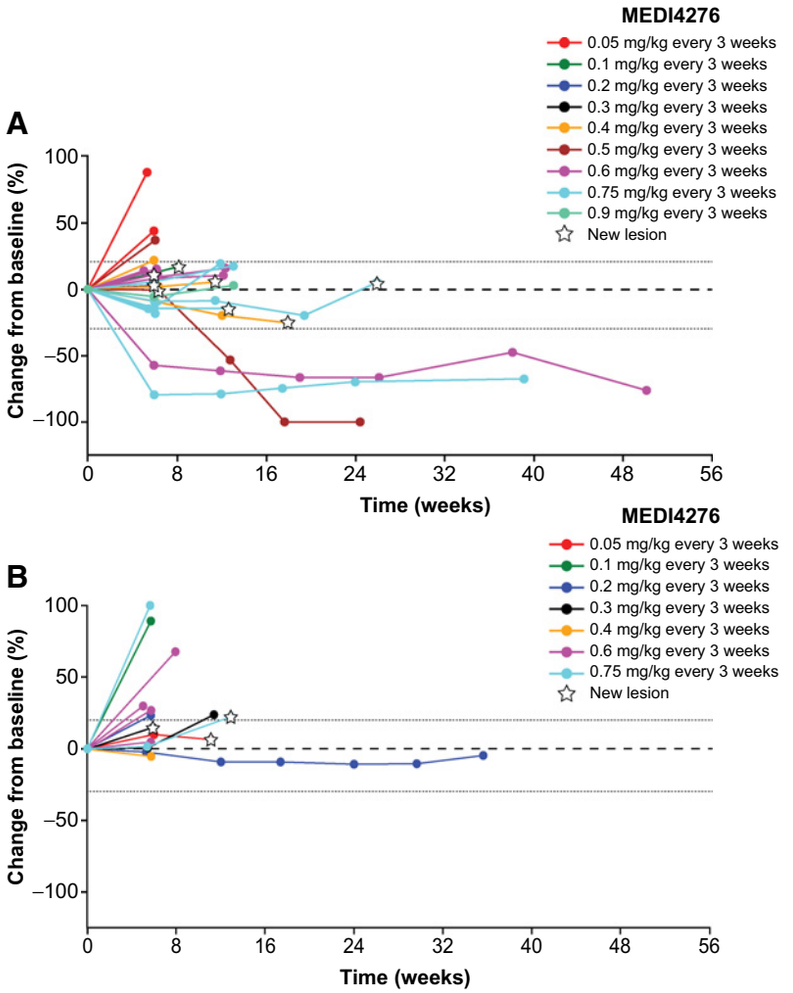

Figure 3.

Change in tumor size from baseline for patients with (**A**) breast cancer[a] or (**B**) gastric cancer[b]. [a]CR (0.5 mg/kg, $n = 1$); PR (0.6 and 0.75 mg/kg, $n = 1$ each, respectively); SD (0.05–0.5 mg/kg, $n = 2$; 0.6 mg/kg, $n = 2$; 0.75 mg/kg, $n = 5$; 0.9 mg/kg, $n = 1$); PD (0.05–0.5 mg/kg, $n = 9$; 0.6 mg/kg, $n = 3$; 0.75 mg/kg, $n = 2$). [b]SD (0.05–0.5 mg/-kg, $n = 3$; 0.6 mg/kg, $n = 1$); PD (0.05–0.5 mg/kg, $n = 4$; 0.6 mg/kg, $n = 3$; 0.75 mg/kg, $n = 2$).
PD, progressive disease; SD, stable disease.

and MEDI1498 demonstrated that deacetylated MEDI4276 exposure was <10% of MEDI4276 (Supplementary Fig. S2), indicating limited deacetylation of MEDI4276, enabling *quantitative* assessment of this catabolic process. This was further confirmed by virtually undetectable levels of deacetylated tubulysin (AZ7308; Supplementary Fig. S3B). Taken together, these data suggest that MEDI4276 remains stable upon administration to patients and that deacetylation of circulating MEDI4276 is a minor catabolic process. Following intravenous administration of MEDI4276 to patients, plasma concentrations of MEDI4276 declined rapidly with average terminal half-life ($t_{½}$) values increasing with dose from 0.5 to 2 days (Supplementary Fig. S1; Supplementary Table S1). No MEDI4276 accumulation was observed with multiple every-3-weeks dosing. All trough concentration (C_{trough}) levels were below the LLOQ, as expected from its relatively short $t_{½}$ values. Systemic clearance (CL) decreased (90.8–22.5 mL/kg/day) with increases in dose, indicating nonlinear PK at the evaluated dose range (0.05–0.9 mg/kg).

ADAs were positive at baseline in nine of 43 (20.9%) patients and were of low titer. Postbaseline, ADAs were positive in 14 of 44 (31.8%) patients. Treatment-induced (ADA-negative at baseline and ADA-positive postbaseline at any time) ADAs were observed in 4 of 41 (9.8%) patients. MEDI4276 peak exposure stratified by ADA status was compared (Supplementary Fig. S4), and there was no clear indication that PK was affected by ADA formation.

Efficacy

Breast cancer

Three patients with metastatic breast cancer, each previously treated with trastuzumab, pertuzumab and T-DM1, had confirmed objective responses; 1 patient in the 0.5 mg/kg cohort had a complete response (CR); and 2 patients in the 0.6 and 0.75 mg/kg cohort, respectively, had a partial response (PR) (**Fig. 3**). The objective response rate (ORR) based on RECIST v1.1 per investigator was 9.4% (3 of 32 patients). Among the 3 patients, the time to response ranged from 1.3 to 2.9 months; duration of response ranged from 4.2+ to 10.2+ months. In patients with breast cancer, the median progression-free survival (PFS) for patients enrolled in the 0.05 to 0.4 mg/kg cohorts ranged from 1.3 to 2.0 months; longer median PFS, ranging from 4.6 to 15.4 months, was observed in the 0.5 to 0.75 mg/kg cohorts (**Fig. 3**). In patients with breast cancer [0.05–0.5 mg/kg ($n = 13$), 0.6 mg/kg ($n = 7$), 0.75 mg ($n = 10$)], the median overall survival (OS) was 19.1 months (range, 0.8+ to 30.6+; 95% CI: 9.6, not estimable). In the higher-dose cohorts (0.5 to 0.9 mg/kg), OS data were not mature; deaths were reported in 4 of 22 patients. Change in tumor size from baseline is depicted in **Fig. 3A**.

Gastric cancer

There were no objective responses in patients with gastric cancer, with a median PFS of 1.8 months (range, 0+ to 10.7; 95% CI, 1.3–3.0).

Across dose cohorts, median PFS ranged from 1.3 to 6.0 months without a clear dose–response relationship. In patients with gastric cancer, median OS was 6.5 months (range, 2.8–16.3; 95% CI, 3.1–16.3). Change in tumor size from baseline is shown in **Fig. 3B**.

Discussion

This was a first-in-human, phase 1 dose-escalation study of the biparatopic anti-HER2 tetravalent ADC, MEDI4276, in patients with HER2-positive advanced breast cancer and gastric cancer. The MTD was defined as 0.75 mg/kg every 3 weeks, with DLTs of LFT elevations and diarrhea observed at 0.9 mg/kg every 3 weeks. Notable toxicities included hepatotoxicity, gastrointestinal toxicity, and peripheral neuropathy. There was a trend for these types of AEs to increase in frequency and severity as the dose increased beyond 0.5 mg/kg. AEs associated with hepatic and gastrointestinal toxicity tended to show acute occurrence, with typical onset within the first week of treatment, and generally resolved before the next scheduled dose. Peripheral neuropathy had an initial onset following three to four doses of MEDI4276 and did not resolve by the end of the study. At the MTD (0.75 mg/kg), MEDI4276 had poor tolerability, as evidenced by the fact that 75.0% of patients experienced ≥1 serious and/or grade ≥3 event.

Based upon clinical data alone, it is difficult to determine which attribute(s) of MEDI4276 (e.g., tubulysin payload, design features, site-specific conjugation chemistry resulting in an average DAR of 4, biparatopic configuration, or combinations thereof) contributes most to its toxicity. Free tubulysin was very low (see Supplementary Fig. S3A), despite a highly sensitive assay with an LLOQ of 50 pg/mL, and exhibited limited deacetylation; thus toxicity caused by free tubulysin in the circulation is unlikely to account for MEDI4276-associated toxicities. MEDI4276 was designed for rapid internalization (54) and has a dissociation constant (K_D) of 137 pmol/L (44). This is a unique design feature of MEDI4276 and may exacerbate the "on-target" toxicity observed, compared with T-DM1, T-DxD, and SYD985, which have a K_D in the nmol/L range [2.7 nmol/L (55), 7.3 ng/mL (30), and 1.1 nmol/L (31), respectively], and are better tolerated.

MEDI4276 contains three site mutations in the Fc region (i.e., L234F, S239C, S442C; ref. 44). The presence of two engineered cysteine residues per heavy chain (i.e., S239C and S442C) facilitates site-specific conjugation of the antibody to the tubulysin warhead (AZ13599185) via a maleimidocaproyl linker thereby forming the biparatopic ADC with an average DAR of 4 (44). Moreover, both the L234F and S239C mutations present in MEDI4276 reduce Fcγ receptor binding, which is proposed to reduce Fcγ receptor-mediated HER2-independent uptake of the ADC by normal tissue, and also decrease off-target toxicity (e.g., thrombocytopenia; refs. 44, 48). Therefore, the site-specific conjugation approach resulting in an average DAR 4 for MEDI4276 provides an advantage compared with ADCs that have random lysine conjugation, for which there is conjugation variability and an increased potential for the formation of high DAR species that can lead to toxicities.

In a phase 1 dose-escalation trial of ZW25 (an anti-HER2 biparatopic antibody) in patients with heavily pretreated HER2-positive advanced cancers, the recommended phase 2 dose was 10 mg/kg weekly or 20 mg/kg biweekly. The most common AEs were diarrhea and infusion reaction, all grade 1 or 2, with no treatment-related discontinuations (32). Preclinical characterization of a novel anti-HER2 biparatopic ADC ZW49, which is generated via conjugation of an N-acyl sulfonamide auristatin payload to the inter-chain disulfide bond cysteines of ZW25, via a protease-cleavable linker, showed that intravenous administration every 2 weeks for three doses was well tolerated, with a highest nonseverely toxic dose of 18 mg/kg (56). In comparison, the highest nonseverely toxic dose of MEDI4276 in nonhuman primates (cynomolgus monkeys) was 1 mg/kg (data on file; AstraZeneca, Gaithersburg, MD), which is an important limitation of this biparatopic ADC. It is interesting to note that lower gastrointestinal tract toxicity is an AE shared by pertuzumab (10), MEDI4276, and ZW25 (32), suggesting this AE may be the result of an "on-target" effect of blocking ECD subdomain 2, the dimerization domain of HER2. Notably, both T-DM1 and MEDI4276 share a potential for hepatotoxicity. Yan and colleagues reported that T-DM1 is internalized upon binding to cell surface HER2 in human hepatocytes and is colocalized with lysosomal-associated membrane protein 1, resulting in DM1-associated cytotoxicity, including disorganized microtubules, nuclear fragmentation/multiple nuclei, and cell growth inhibition (57). However, these published experiments lack a nontargeted DM1 ADC control; consequently, the results should be interpreted with caution as they cannot formally rule out non–HER2-mediated mechanism(s) of T-DM1 uptake by hepatocytes. It is, therefore, not possible, based solely upon our clinical data, to determine with certainty whether MEDI4276-associated hepatotoxicity is a result of an on-target HER2-dependent effect. Based on the available data from ZW25, ZW49, and T-DM1, the biparatopic configuration of MEDI4276 alone is unlikely to account for the magnitude of its toxicity. In support, results from the dose-escalation portion of the phase 1 study of ZW49 demonstrated multiple confirmed responses and stable disease in several tumor types; >90% treatment-related AEs were of grade 1 or 2, and reversible; and no DLTs or treatment-related deaths were observed (58). Although there are differences with respect to valency between MEDI4276 (tetravalent) and ZW49 (bivalent), it is not possible to determine whether variations in valence relate to toxicity based on cross-trial comparisons. Rather, the highly potent tubulysin payload is more likely to account for the MEDI4276 toxicity profile. This finding is also consistent with a recent review of ADC toxicology by the FDA, wherein Saber and Leighton observed that ADC toxicity is largely driven by linker/payload composition, rather than expression/anatomical distribution of the target antigen (59). Interestingly, they noted that ADCs sharing the same linker/payload composition tend to reach approximately the same MTD, even when their target antigens showed endogenous expression in completely different tissue/organ compartments (59). However, additional studies with an ADC containing a comparable linker/payload combination would facilitate determination of whether the toxicities observed with MEDI4276 are primarily linker/payload related. Like MEDI4276, other HER2-targeted ADCs have faced significant toxicity challenges in the clinic. A phase 1 trial of ADCT-502 (composed of trastuzumab with site-specific conjugation to the potent pyrrolobenzodiazepine dimer-based linker-drug tesirine) was terminated because of toxicities of fluid retention and pulmonary edema, the latter presumedly caused by the extensive expression of HER2 in pulmonary tissue (60). Additionally, a partial temporary clinical hold (because of a grade 5 SAE) was imposed by the FDA on a phase 1 dose-escalation study of XMT-1522, a high DAR ∼12 HER2 ADC composed of a proprietary HER2 antibody conjugated with Mersana Therapeutic's Dolaflexin platform—a fleximer polymer linked with an auristatin payload (61).

ADCs are complex biotherapeutic modalities that require sophisticated bioanalytical methods to properly interrogate their PK and catabolism (50, 62). To enable quantitative analysis of changes in DAR over time for MEDI426, and to assess the impact that deacetylation would have on the tubulysin warhead, we employed sophisticated, novel, DAR-sensitive, bioanalytical methods for the assessment of

MEDI4276 PK (51). MEDI4276 exhibited nonlinear PK at the doses evaluated (0.05–0.9 mg/kg), with decreasing clearance with increasing dose (90.8–22.5 mL/kg/day), and increased $t_{1/2}$ (although still short, indicating a target sink) with increased dose (0.509–1.90 days). MEDI4276 and total antibody PK generally overlapped (**Fig. 2**; Supplementary Figs. S1 and S2) indicating limited deconjugation of the tubulysin warhead, thus implying that MEDI4276 exhibited minimal changes in its DAR over time upon administration in patients. This was further supported by low levels of detectable free tubulysin (Supplementary Fig. S3), even when highly sensitive methods were employed. Low levels of deacetylated MEDI4276 (MEDI1498) (**Fig. 2**; Supplementary Figs. S1 and S2), and virtually undetectable levels of AZ7308 (Supplementary Fig. S3B) indicate very low levels of deacetylation of the tubulysin warhead. The relatively low MTD, short $t_{1/2}$, and high CL, at the tolerated doses—compared with either T-DM1 or trastuzumab (63, 64)—could handicap MEDI4276 efficacy if any of the other proposed mechanisms of action of HER2 mAbs (e.g., disruption of HER2:HER3 complexes and inhibition of HER2 C-terminal fragment (p95) generation by proteolysis) are shared by MEDI4276. However, it should be noted that the recommended dose of trastuzumab is much higher, which also impacts its PK. We note that, at the MTD, MEDI4276 has a very short half-life relative to T-DM1 at its MTD; however, it is important to point out that in the phase 1 studies of T-DM1, the clearances at doses <1.2 mg/kg were also faster than at high doses (63). Mean T-DM1 clearance at doses of 0.3 to 1.2 mg/kg ranged from 21.2 to 27.8 mL/d/kg, whereas clearances at doses >1.2 mg/kg were lower (6.9–12.9 mL/d/kg). These results are thought to reflect the saturation of HER2-binding sites at lower T-DM1 doses, whereas above 1.2 mg/kg, MEDI4276 clearance is facilitated by binding to Fc receptors, similar to other systemic antibodies (63). Similar dosage-based differences in clearance have been described for trastuzumab (64).

As predicted, on the basis of preclinical data showing no cross resistance between MEDI4276 and T-DM1, MEDI4276 did show anecdotal evidence of efficacy, even in patients with prior T-DM1 treatment. Confirmed objective responses in heavily pretreated patients with HER2-positive metastatic breast cancer were observed at the higher dose levels, including two PRs (0.6 and 0.75 mg/kg dose levels), and one CR at the 0.5 mg/kg dose level. The response durations in these cases ranged from 4.2+ to 10.2+ months, which were judged to be clinically meaningful in such a heavily pretreated patient population. All three patients had received prior trastuzumab, pertuzumab, and T-DM1 treatment. T-DXd and SYD985 have also showed impressive clinical activity against multiple HER2-positive disease states (33% ORR for SYD985 in heavily pretreated HER2-positive advanced breast cancer, and 64.2% for T-DXd, also in pretreated patients; refs. 65, 66). In a phase 2 study, T-DXd treatment in patients with HER2-positive metastatic breast cancer previously treated with T-DM1, trastuzumab, and pertuzumab resulted in an ORR of 60.9% (112 of 184 patients; 95% CI, 53.4%–68.0%) and disease control rate (DCR) of 97.3% (95% CI, 93.84%–99.1%); median duration of follow-up was 11.1 months (range, 0.7–19.9; ref. 67). Furthermore, these two ADCs have been studied in heavily pretreated "HER2 low" (defined as IHC 1+ or 2+ and *in situ* hybridization-negative for amplification at the ERBB2/HER2 gene locus), where objective clinical response rates were 37.0% (95% CI, 24.3%–51.3%) for T-DXd and 27% (hormone receptor positive) to 40% (triple-negative) for SYD985 (65, 68). Both of these ADCs are arguably better tolerated than MEDI4276, with the most common adverse drug reactions for SYD985 being fatigue, dry eyes, conjunctivitis, and increased lacrimation; whereas for T-DXd, common AEs were nausea 73.5% (3.5% grade ≥3), decreased appetite 59.5% (4.5% grade ≥3), and vomiting 39.5% (1.5% grade ≥3; refs. 65, 66). However, the safety profile of T-DXd includes a number of cases of interstitial lung disease, including grade 5, which have been reported during phase 1 and 2 clinical development (66, 69, 70), prompting close monitoring and early clinical intervention for pulmonary toxicity for all patients receiving T-DXd. Similar to T-DXd and SYD985, MEDI4276 also elicited objective responses in TDM-1-resistant patients (two achieved a PR; and one achieved a CR).

In summary, MEDI4276 shows evidence of non–cross-resistance to T-DM1 with durable objective clinical responses observed in this first-in-human phase 1 study, confirming clinical observations made with other newer HER2 ADCs. To what extent, if any, more rapid internalization (and trafficking to lysosomes) plays a role in either the efficacy or toxicity of MEDI4276 remains unclear, particularly on clinical time scales of weeks to months, as opposed to short-term (minutes to hours) laboratory assays used to measure internalization and/or endosomal/lysosomal trafficking rates. We conclude that despite clinical activity in breast cancer (however limited), further clinical development of MEDI4276 is challenged by an unfavorable PK profile (insufficient to overcome a potential antigen sink) and high toxicity. Given the observed toxicities, we believe that the MTD achieved may be too low to saturate the antigen sink. We posit that a better tolerated payload may have been able to achieve a higher dose (i.e., 3–5 mg/kg), potentially enabling tissue (and circulating HER2 ECD) sink saturation and improved therapeutic index.

Authors' Disclosures

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Authors' Contributions

M.D. Pegram: Formal analysis, investigation, writing–original draft. **E.P. Hamilton:** Data curation, supervision, investigation. **A.R. Tan:** Data curation, supervision, investigation. **A.M. Storniolo:** Data curation, supervision, investigation. **K. Balic:** Resources, formal analysis, writing–review and editing. **A.I. Rosenbaum:** Resources, formal analysis, funding acquisition, methodology, validation, and visualization, writing–review and editing. **M. Liang:** Resources, formal analysis, writing–review and editing. **P. He:** Resources, formal analysis, funding acquisition, writing–review and editing. **S. Marshall:** Resources, formal analysis, writing–review and editing. **A. Scheuber:** Resources, supervision, writing–review and editing. **M. Das:** Resources,

formal analysis, funding acquisition, writing–review and editing. **M.R. Patel:** Formal analysis, investigation, writing–review and editing.

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