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A Phase I study of DLYE5953A, an anti-LY6E antibody covalently linked to monomethyl auristatin E, in patients with refractory solid tumors

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Abstract

Purpose: DLYE5953A is an antibody-drug conjugate consisting of an anti-LY6E antibody covalently linked to the cytotoxic agent monomethyl auristatin E. This study characterized the safety, pharmacokinetics, immunogenicity, potential biomarkers, and anti-tumor activity of DLYE5953A in patients with metastatic solid tumors.

Experimental design: This was a phase I, open-label, 3+3 dose-escalation, and dose expansion study of DLYE5953A administered intravenously every 21 days (Q3W) in patients with locally advanced or metastatic solid malignancies.

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Disclaimer

The authors take full responsibility for the design of the study, the collection of the data, the analysis and interpretation of the data, the decision to submit the article for publication, and the writing of the article.

Results: Sixty-eight patients received DLYE5953A (median: 4 cycles; range: 1–27). No dose limiting toxicities were identified during dose escalation (0.2–2.4 mg/kg; *n*=20). The recommended phase II dose (RP2D) of 2.4 mg/kg Q3W was based on overall safety and tolerability. Dose expansion cohorts for HER2-negative metastatic breast cancer (HER2-negative MBC) (*n*=23) and non-small cell lung cancer (NSCLC) (*n*=25) patients were enrolled at the RP2D. Among patients receiving DLYE5953A 2.4 mg/kg (*n*=55), the most common (≥30%) related adverse events (AEs) included alopecia, fatigue, nausea, and peripheral neuropathy. Grade ≥3 related AEs occurred in 14/55 (26%) of patients, with neutropenia being the most common (13%). DLYE5953A demonstrated linear total antibody pharmacokinetics at doses of ≥0.8 mg/kg with low unconjugated MMAE levels in blood. Partial response was confirmed in 8/68 (12%) patients, including 3/29 MBC (10%) and 5/25 NSCLC (20%) patients at the RP2D. Stable disease was the best response for 37/68 (54%) patients.

Conclusions: DLYE5953A administered at 2.4 mg/kg has acceptable safety. Preliminary evidence of anti-tumor activity in HER2-negative MBC and NSCLC patients supports further investigation of LY6E as a therapeutic target.

Keywords

LY6E; antibody-drug conjugate; clinical trial; solid tumors; monomethyl auristatin E

INTRODUCTION

The lymphocyte antigen 6 complex locus E gene (*LY6E*) encodes an interferon inducible, glycophosphatidylinositol-anchored, surface-membrane protein known as LY6E, that has been identified as a promising target for antibody-drug conjugates (ADCs) (1). Characterization of the function of LY6E remains incomplete, with potential roles in immune regulation, viral infection, and oncogenesis (2–5). While there is limited LY6E expression in most normal tissues, frequently there is high LY6E expression (IHC 2/3+) in common epithelial cancers, including breast cancer (64%), pancreatic cancer (63%), non-small cell lung cancer (56%), ovarian cancer (53%), head and neck squamous cell carcinoma (42%), and gastric cancer (31%), thus providing a strong rationale to develop an LY6E-targeting ADC for the treatment of LY6E-expressing solid tumors (1).

DLYE5953A is an ADC that comprises a humanized anti-LY6E monoclonal antibody (IgG_1: MLYE4489A) linked through a protease labile linker (maleimidocaproyl-valine-citrulline p-aminobenzyloxycarbonyl) (6) to a potent anti-mitotic agent (monomethyl auristatin E, or MMAE) (7). Upon binding to LY6E on the cell surface, DLYE5953A is internalized and delivered to lysosomes, where MMAE or MMAE-containing catabolites induce cell death (1). In this manner, ADCs have the potential to increase drug exposure, particularly to cancer cells exhibiting relatively high target expression, while minimizing exposure to normal tissue. In preclinical studies, DLYE5953A showed potent and selective inhibition of cell proliferation in LY6E-expressing cancer cell lines and mice xenograft models (1). Based on acceptable nonclinical pharmacokinetics (PK) and safety profiles, this first-in-human study was designed to characterize the safety, tolerability, and PK parameters of DLYE5953A in patients with solid tumors refractory to standard treatments.

Preliminary assessments of DLYE5953A anti-tumor activity, its association with tumor LY6E expression, and acquired resistance were also performed.

METHODS

Study design

This was an open-label, multi-center, phase I study using a 3 + 3 dose escalation design to evaluate the safety, tolerability, RP2D, PK, anti-drug antibody (ADA) formation, preliminary anti-tumor activity, and potential biomarkers for DLYE5953A. Patients with advanced or metastatic solid tumors received DLYE5953A (supplied by Genentech, Inc.) intravenously at doses of 0.2, 0.4, 0.8, 1.6, or 2.4 mg/kg once every 3 weeks (Q3W). Dose-expansion cohorts were enrolled at the RP2D to further characterize the safety profile and assess the preliminary activity of DLYE5953A in metastatic breast cancer (HER2-negative MBC) and non-small cell lung cancer (NSCLC).

The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. Approval from the institutional review boards and ethics committees was obtained before study start. Written informed consent was obtained from patients prior to enrollment. The study was registered on ClinicalTrials.gov (NCT02092792).

Patients

Key eligibility criteria for patients included age ≥18 years, Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, histologically or cytologically documented advanced or metastatic breast, ovarian, pancreatic, NSCLC, head and neck squamous cell carcinoma, or gastric cancer in dose escalation, adequate hematologic and end organ function, and measurable disease per RECIST v1.1. In addition, tissue samples for assessment of LY6E were required of patients dosed at ≥1.6 mg/kg DLYE5953A. There were no eligibility restrictions with regards to prior therapies in the dose escalation portion of the study, except patients who had received treatment with another ADC containing MMAE were excluded.

The dose expansion cohorts included patients with HER2-negative MBC and NSCLC who had received ≤2 prior lines of chemotherapy in the advanced and/or metastatic setting. Eligibility was not limited with regards to the number of prior non-chemotherapeutic agents (e.g. endocrine therapy, CDK4/6 inhibitors, mTOR inhibitors). Patients with HER2-negative MBC were required to have received a taxane and/or anthracycline and at least one cytotoxic regimen in the metastatic setting. Patients with *EGFR* mutation-positive NSCLC or *ALK* rearrangement-positive NSCLC were required to have received an EGFR inhibitor or an ALK inhibitor, respectively. Patients with grade ≥2 toxicity from prior therapy or grade ≥2 peripheral neuropathy regardless of causality were excluded.

Safety assessments

All patients who received DLYE5953A were assessed for safety based on reports of adverse events (AEs), clinical laboratory testing, vital signs, physical examination, and

electrocardiography. AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, v4.0. Infusion-related reactions (IRRs) were defined as any infusion related sign/symptom that occurred during or within 24 h of DLYE5953A infusion. AEs meeting criteria of an IRR were reported and graded as individual signs and symptoms rather than IRR and grade ≥2 AEs related to an IRR were considered an adverse event of special interest. A dose limiting toxicity (DLT) was defined as any grade ≥3 non-hematologic study drug-related AE occurring within the first 21 days with certain exceptions including grade 3 nausea, vomiting, or diarrhea that resolved to grade ≤1 within 7 days and infusion related reactions that did not limit re-treatment (Supplementary Table S1 and S2, complete definition of DLTs and AEs of special interest [AESI]). The maximum tolerated dose (MTD) was defined as the highest dose level at which less than one-third of a minimum of 6 patients experienced a DLT.

Pharmacokinetic and immunogenicity assessments

Samples for PK evaluation were collected at multiple time points following the first dose of DLYE5953A and less frequently in subsequent cycles. Concentration-time profiles were determined by dose level using a validated enzyme-linked immunosorbent assay (ELISA) for DLYE5953A with a minimum quantifiable concentration in serum of 50 ng/mL. Unconjugated MMAE analyte concentrations in plasma were determined using a validated liquid chromatographic-tandem mass spectrometry (LC/MS-MS) with electrospray ionization assay, with a lower limit of quantitation of 0.036 ng/mL. PK parameters for total antibody and unconjugated MMAE were determined using non-compartmental approach based on concentration-time profile in cycle 1.

For immunogenicity assessments, a validated antibody bridging ELISA (8) was used to detect ADA formation, characterize the ADA domain specificity, and determine the titer of confirmed ADAs in patient samples.

Clinical activity

Disease status was assessed using the Response Evaluation Criteria in Solid Tumors, (RECIST version 1.1). Patients underwent tumor assessments at screening, during the final week of every even numbered cycle until cycle 8 and every 3 cycles thereafter, and at study discontinuation. Best overall response, objective response, and time on study were evaluated by indication and dose level. Objective response was defined as a complete response (CR) or partial response (PR) that was confirmed ≥4 weeks after initial documentation of response. Time on treatment was defined as time from first treatment dose to 21 days after the last dose of DLYE5953A or date of death, whichever came first. Patients with no post-baseline tumor response assessment were considered non-responders.

Biomarker analyses

NK cells—Given that LY6E is expressed on subsets of human peripheral blood leukocytes (Genentech, data on file), blood samples were collected to monitor circulating T cells, B cells, and NK cells (TBNK) prior to DLYE5953A administration on day 1 of cycles 1, 5, 9, and every 4 cycles thereafter, and at discontinuation of study participation. Quantification of cells in blood samples was performed at a central lab using a standard BD Biosciences (San

Jose, CA) TBNK flow cytometry panel including markers for B cells (CD19), T cells (CD3), T helper cells (CD4), cytotoxic T cells (CD8), and NK cells (CD16/CD56).

LY6E *characterization*—Tumor specimens, including baseline (either archival or biopsy performed during screening) and on-treatment biopsies, were used to assess LY6E expression using an immunohistochemistry (IHC)-based assay, as well as by quantitative real-time-polymerase chain reaction (qRT-PCR). Selected patients who had consented for the procedure underwent a progression biopsy at the end of treatment. IHC for LY6E was performed using a mouse monoclonal IgG1 antibody (clone 15A5) (Genentech, Inc.) with automated staining on the BenchMark® XT system (Ventana Medical Systems, Tucson, AZ) as previously described (1). Tissues with no detectable signal in >50% of tumor cells were given an IHC score of 0. Tumors with >50% of cells staining for LY6E with the majority of cells staining weakly, moderately, or strongly, were given an IHC score of 1+, 2+, or 3+, respectively. The percentage of cells with LY6E staining was also recorded. H-scores (range: 0–300) were calculated as the summation of the percentage of cells at each staining intensity level (0, 1, 2, and 3) multiplied by the respective staining intensity level.

For LY6E qRT-PCR, mRNA was isolated from four formalin-fixed paraffin-embedded (FFPE) tumor sections (whole slide scrapes where tumor content ≥50%, macro-dissected tumor epithelium where tumor content <50%) using Roche HighPure FFPET RNA isolation kit (Roche Diagnostics Corporation, Indianapolis, IN) according to manufacturer's instructions. Fifty ng total RNA was tested using a Roche LY6E-specific Cobas qRT-PCR assay. Relative expression of LY6E was expressed as ∂C_p. Exploratory whole transcriptome RNA sequencing analysis was also performed on total RNA isolated from samples collected at baseline and at time of study discontinuation, if available.

Statistical methods

This study was intended to obtain descriptive preliminary safety, PK, and activity information in the treated populations, and as such, sample sizes did not reflect explicit power and type I error considerations. Patients who withdrew from the study prior to completing the DLT assessment window (cycle 1, days 1–21) for reasons other than DLT were considered non-evaluable for DLT and MTD assessments. All analyses were based on the safety-evaluable population, and presented according to the assigned dose level. All statistical analyses were carried out in SAS 9.2 and R 3.1.1. software.

RESULTS

Patient characteristics

Between April 2014 and July 2017, a total of 69 patients with solid tumors including breast, NSCLC, ovarian, and pancreatic cancers were enrolled at 5 centers in the USA, 68 of whom received ≥1 dose of DLYE5953A and were therefore safety evaluable. Demographics for patients with HER2-negative MBC and NSCLC dosed at the RP2D are provided (Table 1); disease characteristics by dose level are provided for patients who participated in the dose escalation stage in Supplementary Table S3. Overall, the median patient age was 58 (range 33–82 years). For NSCLC, 52% of the patients were female and 48% were male,

whereas in MBC, 97% were female and 3% were male. At the RP2D (*n*=29 MBC and *n*=25 NSCLC), 32% of NSCLC patients had squamous cell carcinomas while 68% were non-squamous; 28% of MBC patients had TNBC and 72% had HR positive/HER2-negative MBC. Of the patients with HER2-negative MBC and NSCLC who were treated at 2.4 mg/kg with DLYE5953A and whose tumors were evaluable for LY6E (*n*=46/54, 85%), 63% were from the primary tumor and 35% were from a metastatic lesion; one sample (2%) was of unknown origin. A higher percentage of patients with MBC (26/29; 90%) treated at the RP2D of 2.4 mg/kg had been previously treated with a taxane than NSCLC patients (16/25; 64%). In this same subset of patients, only 2 patients (7%) with MBC had been previously treated with a checkpoint inhibitor, whereas all but 3 of NSCLC patients (88%) had received prior immune checkpoint blockade. Prior platinum-based therapy included 31% for MBC patients (9/29) and 100% (25/25) for NSCLC patients. Analyses of tumor tissue samples by IHC showed high (2/3+) LY6E expression in 74% of HER2-negative MBC patients and 76% of NSCLC patients enrolled in this study.

Study drug exposure

Safety evaluable patients (*n*=68) received a median of 4 cycles (range 1–27) of DLYE5953A at doses ranging from 0.2 mg/kg to 2.4 mg/kg IV Q3W, including 10/68 (15%) patients who remained on study treatment for over 6 months. Twenty patients received DLYE5953A in dose escalation cohorts at 0.2 (*n*=3), 0.4 (*n*=3), 0.8 (*n*=3), 1.6 (*n*=4), and 2.4 (*n*=7) mg/kg (Supplementary Figure S1). Fifty-five patients received DLYE5953A at the RP2D of 2.4 mg/kg, including patients with MBC (*n*=29; *n*=23 from expansion and *n*=6 from escalation), NSCLC (*n*=25 from expansion), and one patient in dose escalation with ovarian cancer. Time on treatment is presented for all MBC and NSCLC patients treated at 2.4 mg/kg (Figure 1) and for patients treated at doses below the RP2D of 2.4 mg/kg or with indications other than NSCLC or MBC in Supplementary Figure S2. Patients with MBC who were treated at the RP2D of DLYE5953A (*n*=29) were on study treatment for a median of 4 cycles (1–20) or 82 (22–471) days, whereas NSCLC patients (*n*=25) remained on study for a median of 4 cycles (1–27) or 87 (10–611) days (Figure 1).

Safety

No dose-limiting toxicities were reported in the study. MTD was not reached and RP2D for DLYE5953A was determined to be 2.4 mg/kg Q3W. The rationale for stopping dose escalation at 2.4 mg/kg was based on the totality of safety data, including tolerability beyond cycle 1 at 2.4 mg/kg Q3W dosing. Two out of 6 dose escalation patients who were treated beyond cycle 1 with 2.4 mg/kg Q3W required dose reductions for adverse events, suggesting higher doses of DLYE5953A were unlikely to be tolerated. Overall, 6/68 patients (9%) discontinued treatment with DLYE5953A due to an AE, of which 5 were considered related to study drug, all at doses of either 1.6 mg/kg or 2.4 mg/kg. The only AE that led to study drug discontinuation in more than one patient was peripheral neuropathy (*n*=1 grade 3, *n*=2 grade 2). All other AEs that led to treatment discontinuation were reported in one patient each: neutropenia with port infection (grade 3); abdominal pain and vomiting (both grade 3); and pleural effusion (grade 5). Dose modifications (interruptions, delays, or reductions) were limited to patients initially treated at the 1.6 mg/kg or 2.4 mg/kg dose levels. Five patients had a total of 7 AEs leading to dose reduction, including 2 patients with grade 3 events.

Seven patients had a total of 11 AEs leading to dose interruption, all of which were signs and symptoms of infusion related reactions (IRRs) of grade 1 (3 AEs), grade 2 (7 AEs), and grade 3 (1 AE).

The most common treatment-related AEs, defined as occurring in ≥20% (17/68) patients at the RP2D were alopecia (53%), fatigue (46%), nausea (38%), peripheral neuropathy (32%; includes peripheral neuropathy, peripheral sensory neuropathy, peripheral motor neuropathy, paresthesia, hypoesthesia, neuralgia, and muscular weakness), chills (28%) decreased appetite (28%) and diarrhea (21%) (Table 2). Grade ≥3 related AEs (Supplementary Table S4) occurred in 17/68 (25%) of patients, with neutropenia being the most common (7/58; 10%), including one patient with a serious AE of neutropenia and port infection leading to DLYE5953A discontinuation (noted above). However, no dose reductions occurred due to neutropenia, which was managed with dose delays and growth factor administration. Twelve patients (12/68, 18%) received growth factor support. All AEs that occurred at ≥10% frequency regardless of attribution and grade ≥3 AEs regardless of attribution are summarized in Supplementary Tables S5 and S6, respectively. Summaries of all and related adverse events by dose for patients who participated in dose escalation are provided in Supplementary Tables S7 and S8, respectively.

Overall, 18 of 68 (27%) patients experienced serious AEs (SAEs). Five of these patients experienced SAEs that were assessed as related to DLYE5953A (Supplementary Table S9) (*n*=1 nausea and pain in extremity; *n*=1 peripheral neuropathy; *n*=1 device related port infection and neutropenia; *n*=1 abdominal pain, constipation, and vomiting; *n*=1 supraventricular tachycardia and ileus). Four deaths were reported, none considered related to DLYE5953A. Two patients in the dose escalation stage died due to disease progression. The other two patients died from AEs, including one patient with ovarian cancer with suspected bacterial peritonitis and pleural effusion, and another with triple negative breast cancer with lung, bone, and brain metastases who had respiratory failure.

Infusion-related reactions—Overall, grade ≥2 AEs related to IRRs were reported in 15 (22%) patients with a single AE being a grade ≥3 AE (grade 3 hypertension). Chills were the most frequently reported IRR symptoms and were managed with meperidine as needed. Starting at the 2.4 mg/kg dose level, patients were recommended to receive prophylactic therapy with diphenhydramine and acetaminophen. If an IRR was experienced despite this prophylactic regimen, the patient was generally pretreated with steroids prior to subsequent DLYE5953A infusions. At 2.4 mg/kg dosing, one of the 4 patients who did not receive pre-medications had a grade ≥2 IRR-related AE, and 8 of 51 patients who were reported to have received pre-medications had such an event. No patients discontinued or were dose reduced due to IRRs; however, DLYE5953A dosing was interrupted in 7 patients due to IRRs (median time protocol-defined infusion time was exceeded: 45 minutes; range: 0–140 min).

Alopecia—Thirty-seven of 68 (54%) patients developed alopecia, which included grade 1 (*n*=20) and grade 2 (*n*=17) events. Hair loss in some patients involved eyelashes and eyebrows. All but 2 patients who experienced alopecia concurrently experienced ocular AEs; hair loss preceded the AE related to eye disorder in all cases. Two patients experienced

ocular AEs but did not experience alopecia: one patient presented with vitreous floaters, and the other, dry eyes.

Peripheral neuropathy—Peripheral neuropathy is an AE commonly reported with tubulin inhibitors such as MMAE (9). Nineteen of 55 (35%) patients at the RP2D experienced peripheral neuropathy that was considered related to DLYE5953A, presenting more often in patients with MBC (13/29; 45%) than in patients with NSCLC (6/25; 24%). Peripheral motor neuropathy was only reported in MBC patients (8/29, 28%). Most events of peripheral neuropathy were grade 1, although 4 MBC patients experienced grade 2 (n=2) or grade 3 (n=2) events and 1 NSCLC patient experienced one grade 3 event. Median time to onset for patients who experienced related grade ≥ 2 peripheral neuropathy was 160 days. Three patients, all treated at the RP2D of 2.4 mg/kg, discontinued DLY5953A due to peripheral neuropathy. Peripheral neuropathy recurred after initial resolution in 9/13 (69%) of patients with MBC treated at the RP2D; none of the patients with NSCLC experienced recurrent events of peripheral neuropathy. Every patient who experienced recurrent events of peripheral neuropathy also had a history of peripheral neuropathy prior to study enrollment. Time of initial onset for peripheral neuropathy while on study ranged from as early as study day 3 in a patient with MBC to as late as study day 169 in a patient with NSCLC. The majority of peripheral neuropathy AEs was reported as resolved or resolving at the end of the reporting period for the study, which ended 30 days after a patient's last dose.

Pharmacokinetics and Immunogenicity

All 68 patients who received at least one dose of DLYE5953A were evaluable for PK analyses. Systemic exposure of DLYE5953A increased with increasing dose (Supplementary Figure S3). Total antibody $AUC_{0\text{-}inf}$ was dose proportional at doses ≥0.8 mg/kg (Table 3). At 2.4 mg/kg of DLYE5953A, clearance was 10.7± 3.6 mL/day/kg and the half-life was 7.21±2.35 days. The C_{max} of unconjugated MMAE at the 2.4 mg/kg dose of DLYE5953A was 4.77 ± 2.3 ng/mL. There was no difference in PK between MBC and NSCLC patients.

The post-baseline incidence of ADAs was 25% (16 treatment-emergent ADAs out of 64 post-baseline evaluable patients). Formation of ADAs appeared to be related to increased clearance of DLYE5953A total antibody in two patients; however, no apparent effect of ADAs on the PK was observed in the other 14 patients with treatment-emergent ADAs. Partial responses were observed in the presence of ADAs in 5 patients including one patient for whom ADAs appeared to have increased DLYE5953A total antibody clearance.

Clinical Activity

All of the 68 safety evaluable patients were evaluable for efficacy, including seven patients with no post-baseline radiographic tumor response assessment (5 at the RP2D of 2.4 mg/kg and 2 in dose escalation) who were considered non-responders. Thirty-seven of 68 (54%) patients had stable disease as their best response (Supplementary Figure S4). The confirmed overall objective response rate was 12%. All responders (8/68 patients) had PR and all had received the RP2D dose of 2.4 mg/kg (Figure 2). This included 2 patients with MBC in the 2.4 mg/kg dose escalation cohort, 1 patient in the MBC expansion cohort (MBC: 3/29;

10%), and 5 patients in the NSCLC expansion cohort (5/25; 20%). Six of the eight patients with confirmed partial response had tumors with LY6E IHC scores of 2+ or 3+.

One of the patients who experienced a PR was a 64-year old female diagnosed with NSCLC with an LY6E IHC score of 3+ (Figure 3A). This patient had progressive disease after successive treatments with cisplatin/pemetrexed (neo-adjuvant setting), carboplatin/pemetrexed, and nivolumab. She was treated with DLYE5953A at 2.4 mg/kg and experienced a PR at cycle 2 that was confirmed at cycle 4 at −46%. She was discontinued from study after cycle 6 due to growth of a new lesion. Another patient diagnosed with TNBC experienced nearly complete amelioration of skin lesions (Figure 3B). This patient was a 46-year old female with an LY6E IHC score of 3+. She had previously received cyclophosphamide/adriamycin/taxol (adjuvant setting), cisplatin, and eribulin/pembrolizumab. She was treated with DLYE5953A at 2.4 mg/kg and achieved a best radiographic response of −14% at the end of cycle 2. Her cycle 4 scans indicated regrowth of target lesions (increase of 13% from cycle 2) and she withdrew from study following cycle 5 to undergo a mastectomy.

Biomarkers

NK cells—There was a dose dependent decline in peripheral NK cells (Supplementary Figure S5), but no evidence of decline in T cells or B cells compared to pre-treatment baseline levels with DLYE5953A treatment (data not shown). For most patients, NK levels remained within the normal range. No unusual/opportunistic infections were reported in patients who experienced a decline in peripheral NK cells.

Progression biopsies—Three patients, all with MBC, provided post-progression biopsies (Supplementary Figure S6), including the patient featured in Figure 3B (patient 2 in Supplementary Figure S6). IHC scores for LY6E changed minimally over treatment with DLYE5953A and H-score did not decline >15% for any of these 3 patients. However, the multi-drug resistant pump, ABCB1, was expressed in all 3 post-progression tumor biopsies and was increased compared to pre-treatment levels in the 2 patients with available pre-treatment tumor RNA samples. ABCC2 was likewise increased in the post-progression biopsy in the 2 patients with pre/post RNA samples.

DISCUSSION

Results from this phase I study demonstrate that DLYE5953A, an anti-LY6E ADC, has an acceptable safety profile when administered by IV infusion Q3W to patients with solid tumors, including MBC and NSCLC. No DLTs were identified for DLYE5953A in dose escalation cohorts up to 2.4 mg/kg Q3W, which was the maximum administered dose. While a protocol-defined MTD was not reached, the RP2D for DLYE5953A was identified to be 2.4 mg/kg Q3W, which has been selected for several other ADCs delivering MMAE with similar drug-antibody ratios (10,11). DLYE5953A at the RP2D had an acceptable safety and tolerability profile with manageable AEs. The overall response rate was 12% with all responses occurring at the RP2D (5/25 [20%] in NSCLC and 3/29 [10%] in MBC). The safety profile of DLYE5953A was in line with previous reports for ADCs using MMAE as the cytotoxic agent (12).

However, notable differences in the safety profile of DLY5953A include rates of alopecia and IRRs. Alopecia was reported in 54% of all patients, a rate ~2–3 fold higher than other vc-MMAE-containing ADCs (12–14). Alopecia may be a target-mediated effect given the expression of LY6E in skin (15) and hair follicles (16), although limitations of a small phase I trial and a lack of a comparator should be acknowledged. DLYE5953A treatment also caused IRRs in some patients, all of which were non-serious and clinically manageable. IRRs may also be a target-mediated effect since LY6E is expressed on several different populations of immune cells, including NK cells, and to a lesser extent, T- and B-cells (1,2,4,17). Reductions in peripheral blood NK cells in some patients treated with DLYE5953A at 2.4 mg/kg further suggests that targeting LY6E may modulate immune function.

Interestingly, the frequency of some AEs was higher in MBC patients treated at the RP2D in comparison to NSCLC patients, despite a similar overall time on treatment between the two indications. Particularly notable was the higher frequency of peripheral neuropathy reported in MBC patients at 2.4 mg/kg compared to NSCLC patients, possibly explained by their higher prior exposure to taxanes (18). Overall, however, the toxicity profile in this phase I study of DLYE5953A appears to be mainly driven by the MMAE component, and exploration of other cytotoxic payloads conjugated to anti-LY6E antibodies may yield an ADC with a more favorable therapeutic index.

PK of DLYE5953A was linear at ≥0.8 mg/kg. The PK of total antibody and unconjugated MMAE appear to be comparable to other vcMMAE ADCs that have been evaluated clinically (6,12,13). Minimal accumulation of total antibody for Q3W dosing regimen was expected based on the terminal $t_{1/2}$ of ~7 days at 2.4 mg/kg. The post-baseline incidence of ADAs was 25%. Formation of ADAs appeared to be related to increased clearance of DLYE5953A total antibody in two patients; however no apparent effect of ADAs on the PK was observed in the other 14 patients with treatment-emergent ADAs. Partial responses were observed in the presence of ADAs in 5 patients including one patient for whom ADAs appeared to have increased DLYE5953A total antibody clearance.

Anti-tumor activity was observed in patients whose tumor LY6E IHC scores were 2+ or 3+ in HER2-negative MBC patients and NSCLC patients, all at the RP2D. While the majority of patients treated with DLYE5953A had IHC scores of 2 or 3, objective anti-tumor activity within this patient population varied widely (from +70% to −72%). In patients with NSCLC, there was no correlation between prior cancer immunotherapy and overall response.

Possible mechanisms that might explain acquired resistance to DLYE5953A include a loss of target expression (i.e., LY6E) by the tumor cells, failure of LY6E to be internalized, resistance to MMAE-induced apoptosis, and/or upregulation of multi-drug resistance pumps (MDRs). In the three patients who provided both pre-treatment and progression tumor biopsies, there was no indication that LY6E expression declined appreciably. However, the multi-drug resistant pump ABCB1 (or PgP), which is a known efflux pump for MMAE (19), was increased in the two samples for which pre- and post-treatment RNA samples were available. In addition, ABCC2 (or MRP2), also a known drug-resistant pump expressed highly in liver (20), was expressed in all the post-progression biopsies and was increased

in the two paired samples. These findings suggest that induction of MDRs may partially contribute to acquired resistance to DLYE5953A (21).

In conclusion, DLYE5953A demonstrated anti-tumor activity in patients with locally advanced or metastatic HER2-negative MBC and NSCLC, a population with relatively poor prognosis. Patients with evaluable tumor tissue and an objective response to treatment had LY6E IHC scores of 2+ or 3+. These findings provide clinical rationale for developing LY6E directed therapies for patients with malignancies that overexpress LY6E, in particular HER2-negative MBC and NSCLC. While the safety profile of DLYE5953A was acceptable, use of a cytotoxic agent other than MMAE may improve the therapeutic index of an ADC targeted against LY6E.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Statement of Translational Relevance

Chemotherapy remains an important standard-of-care treatment for many cancers. Delivery of cytotoxic agents using antibody-drug conjugates (ADCs) has the potential to achieve a wider therapeutic index since the toxin is preferentially targeted to malignant cells by way of tumor associated cell-surface antigens. DLYE5953A is an ADC designed to deliver the cytotoxic agent, monomethyl auristatin E, an anti-mitotic agent and tubulin binder, by targeting the LY6E cell surface antigen that is highly expressed on several solid tumors. This phase I study characterized the safety, pharmacokinetics, and activity of DLYE5953A 2.4 mg/kg Q3W in HER2-negative MBC and NSCLC patients. Preliminary activity was demonstrated in patients with tumors having moderate to high LY6E expression, although not all patients with high LY6E-expressing tumors responded. Analyses of paired biopsies suggested that acquired resistance to DLYE5953A may be due to upregulation of multi-drug resistance pumps and warrants further exploration.

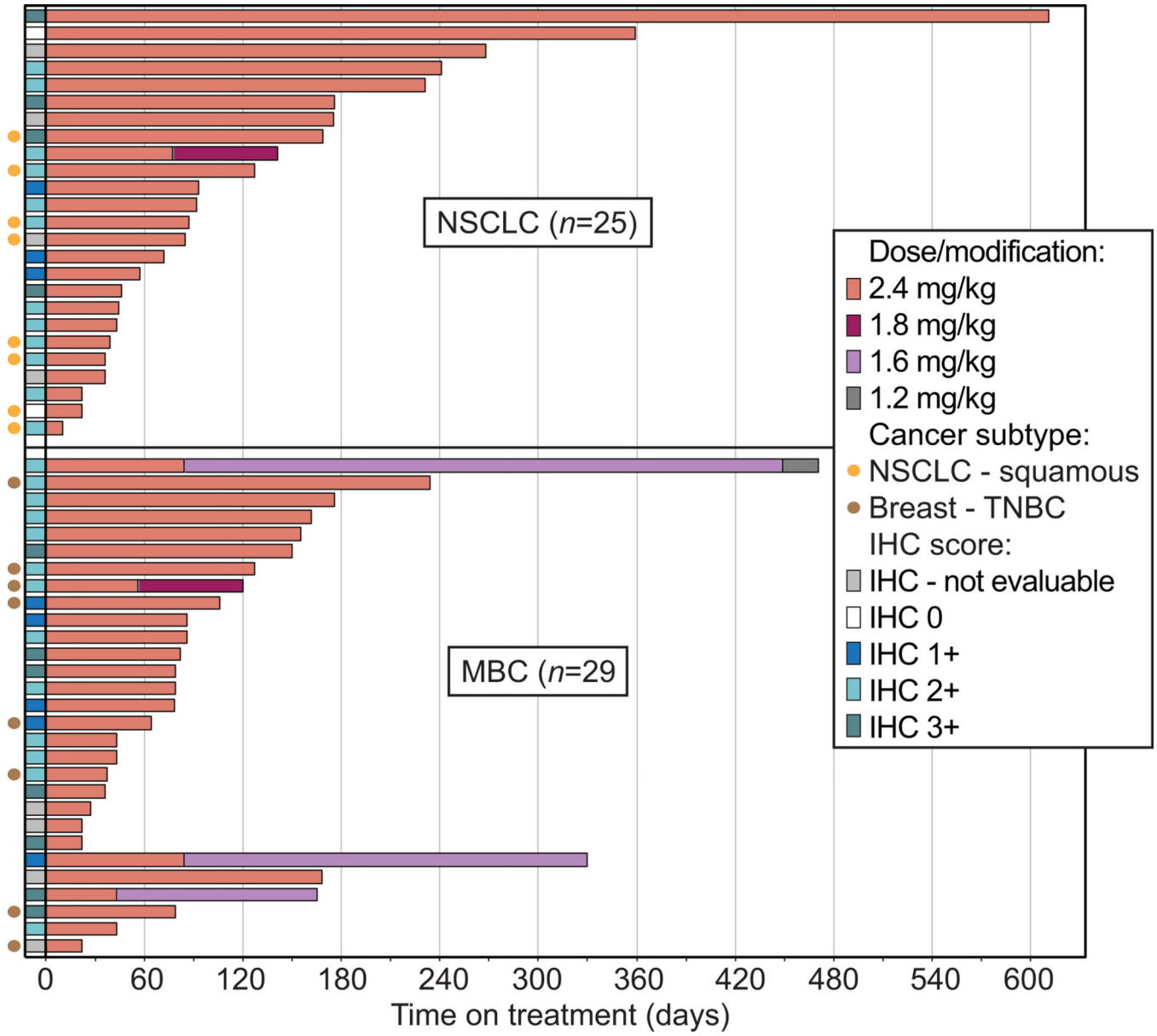

Figure 1.

Time on DLYE5953A treatment at RP2D of 2.4 mg/kg Q3W for NSCLC patients (non-squamous histology, unless noted otherwise) and MBC patients (HER2-negative/HR+, unless noted otherwise). MBC patients (n=6) dosed at 2.4 mg/kg DLYE5953A during dose escalation are also included as the 6 lanes at the bottom of the MBC plot. All patients have discontinued DLYE5953A treatment.

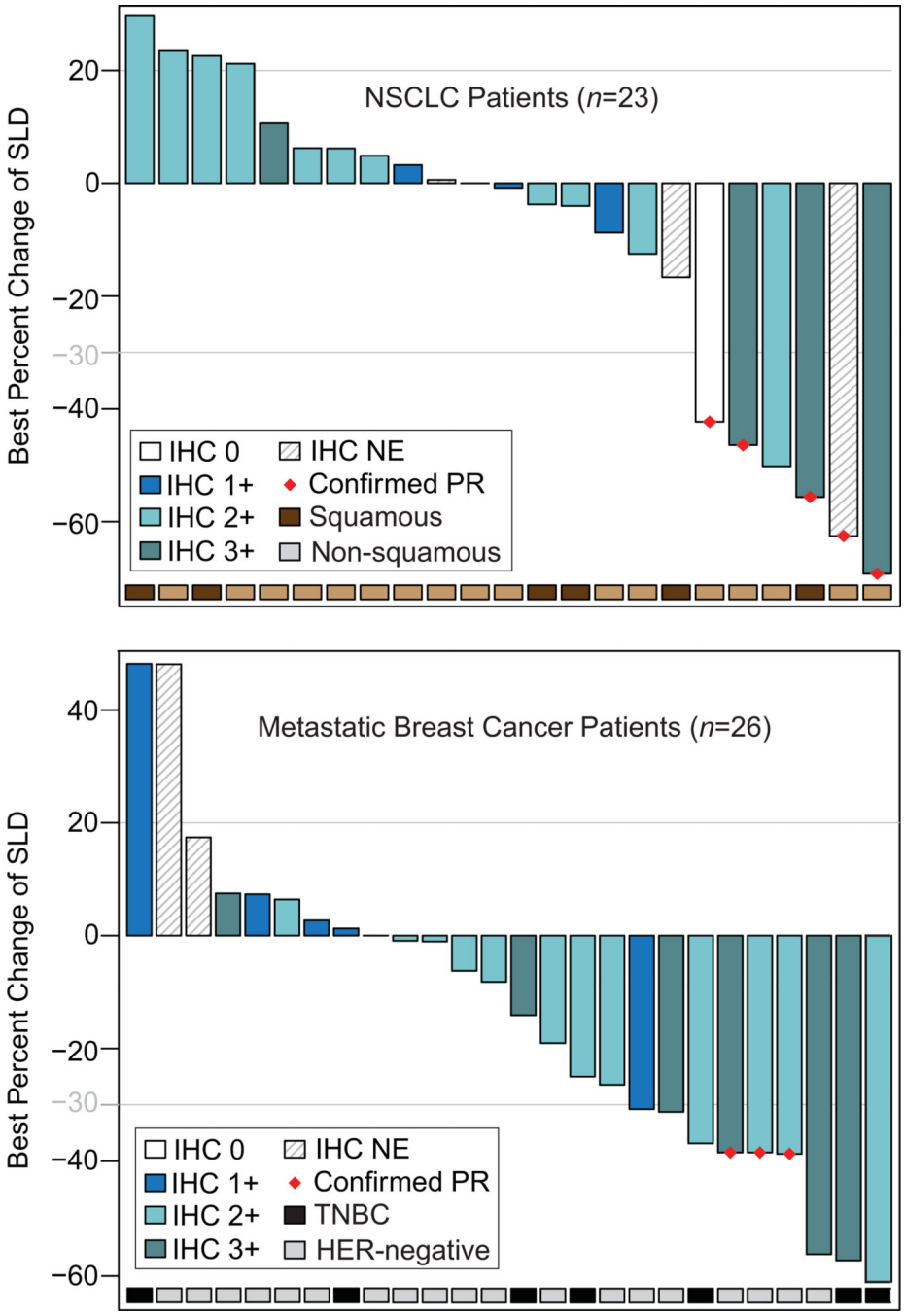

Figure 2.

Best response to DLYE5953A treatment at the RP2D of 2.4 mg/kg Q3W for NSCLC and MBC patients. Patients who did not undergo a post-treatment CT scan (n=5) were non-evaluable for best response. MBC patients (n=6) dosed at 2.4 mg/kg DLYE5953A during dose escalation are also included.

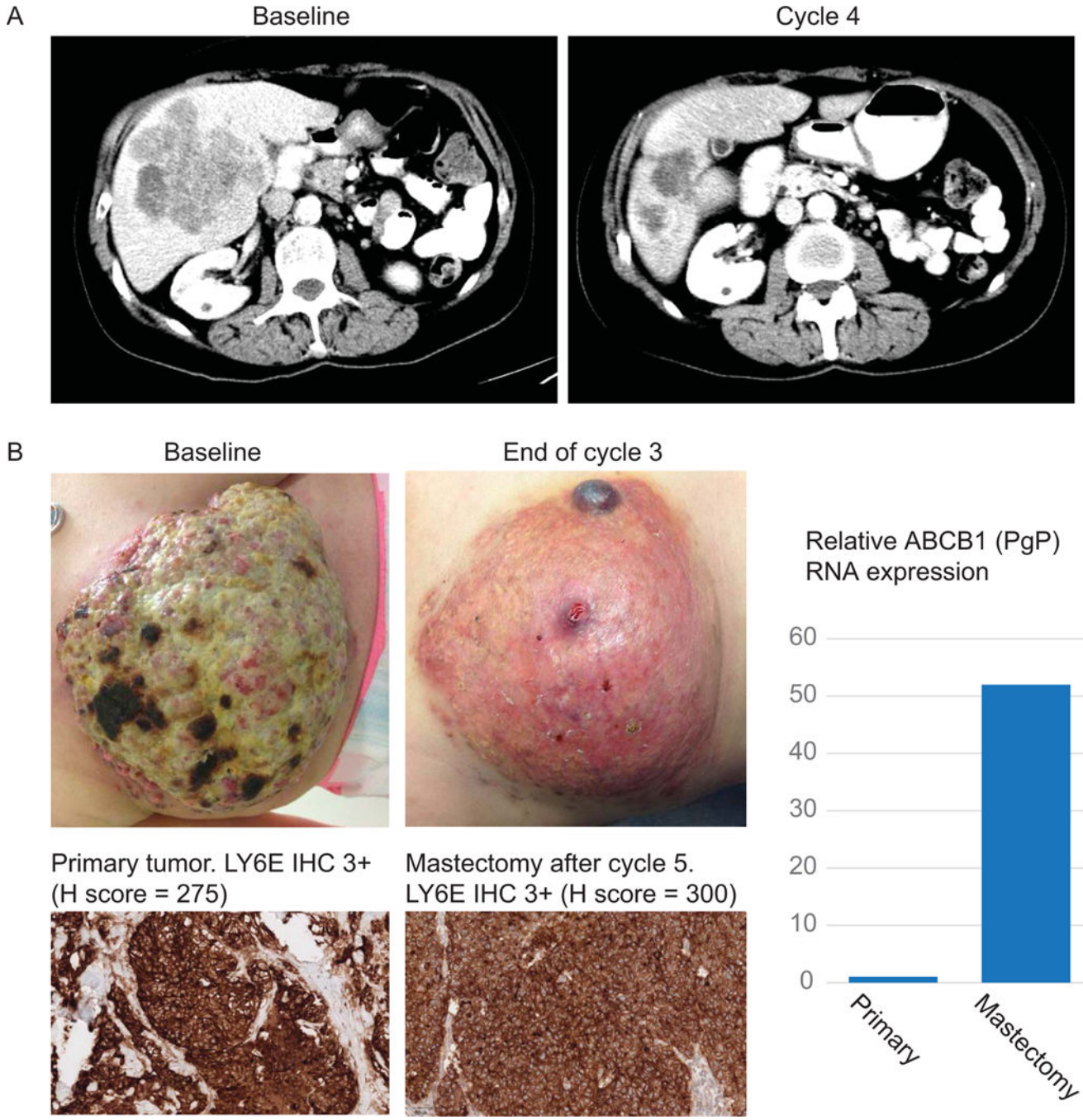

Figure 3.

Patient vignettes. (**A**) A 64-year old female diagnosed with NSCLC in 2013 with LY6E IHC score of 3+ had received prior cisplatin/pemetrexed (neo-adjuvant), carboplatin/pemetrexed, and nivolumab. This patient received DLYE5953A at the R2PD and demonstrated a PR after cycle 2 that was confirmed with subsequent imaging (~46% best response). This patient was discontinued from study due to growth of a new lesion following cycle 6. (**B**) A 38-year old female diagnosed with TNBC in 2014 with a LY6E IHC score of 3+ had received cyclophosphamide/doxorubicin/paclitaxel (adjuvant), cisplatin, and eribulin/pembrolizumab.

This patient received 5 cycles of DLYE5953A at the RP2D and achieved a best response of SD at −14% at cycle 2. Her C4 scans indicated regrowth of target lesions (increase of 13% from nadir at C2). She withdrew from study following cycle 5 to undergo a mastectomy.

Table 1.

Baseline and disease characteristics of MBC and NSCLC patients treated at the RP2D of 2.4 mg/kg and all patients on treatment (0.2 – 2.4 mg/kg)

Variable	2.4 mg/kg MBC (*n*=29)	2.4 mg/kg NSCLC (*n*=25)	All patients (*N*=68)
Age (years) median	54	63	58
(range)	(33–71)	(45–82)	(33–82)
Gender, n (%)			
Male	1 (3)	12 (48)	13 (19)
Female	28 (97)	13 (52)	55 (81)
ECOG status, n (%)			
0	17 (59)	4 (16)	23 (34)
1	12 (41)	21 (84)	45 (66)
Median time (months) between initial diagnosis and metastatic disease *	31.5	0.2	8
(range)	(0–256.3)	(0–35.2)	(0–256.3)
NSCLC histology			
Squamous	-	8 (32)	8 (32)
Non-squamous	-	17 (68)	17 (68)
Breast cancer histology			
HR+, HER2−	21 (72)	-	24 (69)
TNBC	8 (28)	-	11 (31)
Median prior lines of hormone therapy in HR+, HER2- MBC (adv/met) #	3	-	-
(range)	(0–4)	-	-
Cytotoxic regimen (adv/met)			
Prior platinum (≥ 1)	9 (31)	25 (100)	43 (63)
Prior taxane (≥ 1)	26 (90)	16 (64)	53 (78)
Prior immunotherapy (≥ 1)	2 (7)	22 (88)	24 (35)

ECOG = Eastern Cooperative Oncology Group; NSCLC = non-small cell lung cancer.

* One patient in the MBC group had no metastatic date available and one patient in dose escalation had no initial diagnosis date available.

Represents the median number of lines of hormonal therapy in the advanced/metastatic setting for the 21 patients with HR+, HER2- patients treated at 2.4 mg/kg. See Supplement Table 1 for demographic data for remaining patients who participated in the dose escalation.

Table 2.

Adverse events related to DLYE5953A in MBC patients (2.4 mg/kg), NSCLC patients (2.4 mg/kg), and all patients (0.2–to 2.4 mg/kg), and occurring in ≥10% patients overall

MedDRA Term	2.4 mg/kg MBC (*n*=29)[a]	2.4 mg/kg NSCLC (*n*=25)	0.2–2.4 mg/kg All Patients (*N*=68)
Patients with ≥1 adverse event	29 (100)	23 (92)	63 (93)
Alopecia	20 (69)	13 (52)	36 (53)
Fatigue	15 (52)	10 (40)	31 (46)
Nausea	12 (41)	8 (32)	26 (38)
Peripheral neuropathy[b]	13 (45)	6 (24)	22 (32)
Peripheral sensory neuropathy	12 (48)	3 (12)	16 (24)
Peripheral motor neuropathy	8 (28)	0	8 (12)
Parasthesia	1 (3)	2 (8)	4 (6)
Chills	8 (28)	5 (20)	19 (28)
Decreased appetite	8 (28)	7 (28)	19 (28)
Diarrhea	8 (28)	4 (16)	14 (21)
Constipation	9 (31)	1 (4)	12 (18)
Aspartate aminotransferase increased	6 (21)	5 (20)	11 (16)
Back pain	6 (21)	2 (8)	10 (15)
Alanine aminotransferase increased	6 (21)	3 (12)	9 (13)
Anemia	7 (24)	1 (4)	8 (12)
Dry mouth	4 (14)	1 (4)	8 (12)
Mucosal inflammation	6 (21)	1 (4)	9 (13)
Myalgia	5 (18)	2 (8)	9 (13)
Neutropenia	4 (14)	5 (20)	9 (13)
Pruritus	8 (28)	1 (4)	9 (13)
Vomiting	4 (14)	3 (12)	8 (12)
Arthralgia	5 (17)	0	7 (10)
Dry eye	4 (14)	3 (12)	7 (10)
Headache	5 (17)	0	7 (10)
Pyrexia	4 (14)	2 (8)	7 (10)

MBC = metastatic breast cancer; NSCLC = non-small cell lung cancer.

[a] Six of the MBC patients were studied in the 2.4 mg/kg dose escalation cohort and 23 were studied in the dose expansion cohort at the RP2D of 2.4 mg/kg. See Supplement Table 3 for all common AEs occurring in MBC and NSCLC patient treated at the RP2D of 2.4 mg/kg. Supplement Table 4 provides a summary of AEs in patients who participated in the dose escalation, including one patient with ovarian cancer treated at 2.4 mg/kg.

[b] Peripheral neuropathy includes the following terms: peripheral neuropathy, peripheral sensory neuropathy, peripheral motor neuropathy, neuralgia, hypoesthesia, paresthesia, and muscular weakness. Terms with incidence n=1 in the total patient population are not included in the Table 2. Some patients experienced more than one peripheral neuropathy event.

Table 3.

Selected mean (SD) pharmacokinetic parameters for total antibody and unconjugated MMAE after DLYE5953A treatment at cycle 1

TOTAL ANTIBODY	No. of patients[a]	C_{max} (ug/mL)	$AUC_{0\text{-}inf}$ (day*ug/mL)	$t_{1/2}$ (day)	V_{ss} (mL/kg)	CL (mL/day/kg)
Cohorts: 0.2 mg/kg	3	3.45 (1.14)	13.6 (6.2)	7.05 (1.61)	126 (39)	16.6 (6.1)
0.4 mg/kg	3	7.74 (0.62)	29.8 (3.6)	8.38 (1.89)	109 (13.9)	13.6 (1.8)
0.8 mg/kg	3	16.1 (4.6)	89.7 (37.1)	13.3 (1.2)	129 (41)	10.1 (4.0)
1.6 mg/kg	4	36.9 (8.9)	141 (37)	4.97 (1.94)	71.6 (27.3)	12.0 (2.5)
2.4 mg/kg	7	44.1 (11.5)	219 (69)	7.60 (2.709)	90.5 (20.4)	11.9 (3.3)
2.4 mg/kg MBC	22	47.0 (9.7)	258 (77)	7.02 (2.31)	88.0 (24.4)	10.0 (2.7)
2.4 mg/kg NSCLC	24	46.4 (7.6)	253 (101)	7.27 (2.38)	87.4 (20.9)	10.9 (4.4)
UNCONJUGATED MMAE	**No. of patients[a]**	**C_{max} (ng/mL)**	**$AUC_{0\text{-}inf}$ (day*ng/mL)**	**t_{max}(day)**		
Cohorts: 0.2 mg/kg	3	0. 461 (0.057)	3.18 (0.61)	1.64 (0.57)		
0.4 mg/kg	3	0.728 (0. 161)	7.13 (NA)	1.65 (0.57)		
0.8 mg/kg	3	1.69 (1.05)	13.4 (11.7)	2.01 (0.05)		
1.6 mg/kg	4	6.17 (3.85)	66.6 (35.6)	5.43 (6.95)		
2.4 mg/kg	7	6.07 (2.26)	53.1 (27)	3.01 (2.62)		
2.4 mg/kg MBC	14	4.08 (2.08)	34.9 (18.2)	3.10 (3.46)		
2.4 mg/kg NSCLC	10	4.85 (2.44)	36.5 (16.8)	2.71 (2.23)		

[a]Number of patients with at least one pharmacokinetic parameter estimated

$AUC_{0\text{-}inf}$ = area under the concentration−time curve from time zero extrapolated to infinite time; CL = clearance; C_{max} = maximum observed plasma concentration; V_{ss} = volume of distribution at steady state; $t_{1/2}$ = terminal half-life; t_{max} = time to reach maximum concentration.