Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing breast cancer: a phase 1 dose-escalation and dose-expansion study

Udai Banerji, Carla M L van Herpen, Cristina Saura, Fiona Thistlethwaite, Simon Lord, Victor Moreno, Iain R Macpherson, Valentina Boni, Christian Rolfo, Elisabeth G E de Vries, Sylvie Rottey, Jill Geenen, Ferry Eskens, Marta Gil-Martin, Ellen C Mommers, Norbert P Koper, Philippe Aftimos

Lancet Oncol 2019; 20: 1124–35

Published Online June 27, 2019 http://dx.doi.org/10.1016/S1470-2045(19)30328-6

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Institute of Cancer Research and The Royal Marsden, London, UK (Prof U Banerji MD); **Radboud University Medical Center, Nijmegen, Netherlands** (Prof C M L van Herpen MD); **Vall d'Hebrón University Hospital, Vall d'Hebrón Institute of Oncology, Barcelona, Spain** (C Saura MD); **The Christie NHS Foundation Trust and The University of Manchester, Manchester, UK** (F Thistlethwaite MD); **The Churchill Hospital, Oxford, UK** (S Lord MD); **START Madrid-FJD, Madrid, Spain** (V Moreno MD); **Beatson West of Scotland Cancer Center, Glasgow, UK** (I R Macpherson MD); **START-Madrid-CIOCC, Madrid, Spain** (V Boni MD); **University Hospital Antwerp, Edegem, Belgium** (Prof C Rolfo MD); **Greenebaum Comprehensive Cancer Center, Maryland University, Baltimore, MD, USA** (Prof C Rolfo); **University Medical Center Groningen, Groningen, Netherlands** (Prof E G E de Vries MD); **Ghent University Hospital, Ghent, Belgium** (Prof S Rottey MD); **The Netherlands Cancer Institute, Amsterdam, Netherlands** (J Geenen MD); **Erasmus Medical Center, Rotterdam, Netherlands** (F Eskens MD); **Institut Català d'Oncologia-IDIBELL, Barcelona, Spain** (M Gil-Martin MD); **Synthon Biopharmaceuticals, Nijmegen, Netherlands** (E C Mommers PhD, N P Koper PhD); **and Institut Jules Bordet-Université Libre**

Summary

Background Trastuzumab duocarmazine is a novel HER2-targeting antibody–drug conjugate comprised of trastuzumab covalently bound to a linker drug containing duocarmycin. Preclinical studies showed promising antitumour activity in various models. In this first-in-human study, we assessed the safety and activity of trastuzumab duocarmazine in patients with advanced solid tumours.

Methods We did a phase 1 dose-escalation and dose-expansion study. The dose-escalation cohort comprised patients aged 18 years or older enrolled from three academic hospitals in Belgium, the Netherlands, and the UK with locally advanced or metastatic solid tumours with variable HER2 status who were refractory to standard cancer treatment. A separate cohort of patients were enrolled to the dose-expansion phase from 15 hospitals in Belgium, the Netherlands, Spain, and the UK. Dose-expansion cohorts included patients aged 18 years or older with breast, gastric, urothelial, or endometrial cancer with at least HER2 immunohistochemistry 1+ expression and measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST). Trastuzumab duocarmazine was administered intravenously on day 1 of each 3-week cycle. In the dose-escalation phase, trastuzumab duocarmazine was given at doses of 0·3 mg/kg to 2·4 mg/kg (3+3 design) until disease progression or unacceptable toxicity. The primary endpoint of the dose-escalation phase was to assess safety and ascertain the recommended phase 2 dose, which would be the dose used in the dose-expansion phase. The primary endpoint of the dose-expansion phase was the proportion of patients achieving an objective response (complete response or partial response), as assessed by the investigator using RECIST version 1.1. This ongoing study is registered with ClinicalTrials.gov, number NCT02277717, and is fully recruited.

Findings Between Oct 30, 2014, and April 2, 2018, 39 patients were enrolled and treated in the dose-escalation phase and 146 patients were enrolled and treated in the dose-expansion phase. One dose-limiting toxic effect (death from pneumonitis) occurred at the highest administered dose (2·4 mg/kg) in the dose-escalation phase. One further death occurred in the dose-escalation phase (1·5 mg/kg cohort) due to disease progression, which was attributed to general physical health decline. Grade 3–4 treatment-related adverse events reported more than once in the dose-escalation phase were keratitis (n=3) and fatigue (n=2). Based on all available data, the recommended phase 2 dose was set at 1·2 mg/kg. In the dose-expansion phase, treatment-related serious adverse events were reported in 16 (11%) of 146 patients, most commonly infusion-related reactions (two [1%]) and dyspnoea (two [1%]). The most common treatment-related adverse events (grades 1–4) were fatigue (48 [33%] of 146 patients), conjunctivitis (45 [31%]), and dry eye (45 [31%]). Most patients (104 [71%] of 146) had at least one ocular adverse event, with grade 3 events reported in ten (7%) of 146 patients. No patients died from treatment-related adverse events and four patients died due to disease progression, which were attributed to hepatic failure (n=1), upper gastrointestinal haemorrhage (n=1), neurological decompensation (n=1), and renal failure (n=1). In the breast cancer dose-expansion cohorts, 16 (33%, 95% CI 20·4–48·4) of 48 assessable patients with HER2-positive breast cancer achieved an objective response (all partial responses) according to RECIST. Nine (28%, 95% CI 13·8–46·8) of 32 patients with HER2-low, hormone receptor-positive breast cancer and six (40%, 16·3–67·6) of 15 patients with HER2-low, hormone receptor-negative breast cancer achieved an objective response (all partial responses). Partial responses were also observed in one (6%, 95% CI 0·2–30·2) of 16 patients with gastric cancer, four (25%, 7·3–52·4) of 16 patients with urothelial cancer, and five (39%, 13·9–68·4) of 13 patients with endometrial cancer.

Interpretation Trastuzumab duocarmazine shows notable clinical activity in heavily pretreated patients with HER2-expressing metastatic cancer, including HER2-positive trastuzumab emtansine-resistant and HER2-low breast cancer, with a manageable safety profile. Further investigation of trastuzumab duocarmazine for HER2-positive breast cancer is ongoing and trials for HER2-low breast cancer and other HER2-expressing cancers are in preparation.

Funding Synthon Biopharmaceuticals.

de Bruxelles, Brussels, Belgium (P Aftimos MD)

Correspondence to:
Prof Udai Banerji,
Drug Development Unit,
Institute of Cancer Research and The Royal Marsden,
London SM2 5PT, UK
udai.banerji@icr.ac.uk

Introduction

HER2 is a transmembrane tyrosine kinase receptor protein that promotes cell proliferation and inhibits apoptosis. HER2 overexpression, amplification, or both, is seen frequently across different tumour types[1] and is associated with more aggressive disease and lower overall survival compared with cancers without HER2 overexpression.[2,3] During the past two decades, multiple drugs targeting HER2 have been developed for HER2-positive breast cancer, including (bispecific) antibodies, small molecules, vaccines, and antibody–drug conjugates. However, HER2-positive metastatic breast cancer is still incurable and eventual development of resistance to these treatments is almost inevitable.[4,5] Furthermore, trastuzumab did not improve the outcomes of patients with breast cancer expressing low amounts of HER2 (HER2-low), defined as HER2 immunohistochemistry (IHC) 1+ or IHC 2+ and in-situ hybridisation (ISH)-negative. Currently, no HER2-targeting drugs are licensed specifically for the treatment of any cancer with low expression of HER2. Therefore, new drugs that also target cancers with low HER2 expression will address an unmet need in several tumour types.

Antibody–drug conjugates are designed for selective delivery of potent cytotoxic drugs to tumour cells by linking the cytotoxins to monoclonal antibodies. Trastuzumab emtansine, a HER2-targeting antibody–drug conjugate that contains trastuzumab covalently linked to a microtubule inhibitor, significantly prolonged progression-free survival and overall survival with acceptable toxicity in patients with HER2-positive metastatic breast cancer.[6,7] Trastuzumab emtansine is currently recommended as second-line treatment for patients with breast cancer who have progressed after at least one line of trastuzumab-based treatment. Several new HER2-targeting antibody–drug conjugates with different linkers and payloads are currently in clinical development for multiple tumour types, with promising results.[8,9]

Trastuzumab duocarmazine (also known as SYD985) is a novel HER2-targeting antibody–drug conjugate comprising the monoclonal IgG1 antibody trastuzumab covalently bound to a linker drug containing duocarmycin, with a drug-to-antibody ratio of 2·8:1.[10–12] The linker drug contains a cleavable linker and the prodrug *seco*-duocarmycin–hydroxybenzamide–azaindole (*seco*-DUBA). After binding to HER2 and internalisation the linker is cleaved in the lysosome by proteases that release the active toxin (DUBA). The active toxin alkylates DNA, resulting in DNA damage in both dividing and non-dividing cells and ultimately cell death. Additionally, proteases such as cathepsin B can be active extracellularly through secretion by malignant cells.[13] Extracellular cleavage of the linker drug might, therefore, induce a bystander cell-killing effect that is not HER2-mediated.[14] Trastuzumab duocarmazine has shown encouraging preclinical antitumour activity in breast, ovarian, and other cancers with varying (low to high) HER2 expression and was more potent than trastuzumab emtansine.[12,15,16]

In this first-in-human study, we assessed the safety, pharmacokinetics, and preliminary antitumour activity of trastuzumab duocarmazine in patients with locally advanced or metastatic solid tumours. Here, we present data for the completed dose-escalation phase and safety and activity data for the fully recruited dose-expansion cohorts.

Research in context

Evidence before this study

We searched PubMed on Dec 20, 2018, for articles published in English, without restrictions on publication date, with the terms "metastatic breast cancer", "HER2", "antibody–drug conjugate", and "ADC" in several combinations. We retrieved several publications in which treatment options beyond the first two lines of standard HER2-targeting treatment for metastatic breast cancer—ie, taxane plus trastuzumab plus pertuzumab followed by trastuzumab emtansine—consisted of various combinations of trastuzumab plus chemotherapy or the oral combination of lapatinib with capecitabine. These treatments generally resulted in a median progression-free survival of about 4 months. No HER2-targeting drugs are currently licensed specifically for the treatment of breast or any other cancer with low HER2 levels. Antibody–drug conjugates can be regarded as targeted chemotherapy using a single agent and might provide a novel, potentially more effective, treatment option for these patients.

Added value of this study

To our knowledge, our study is the first to report the clinical profile of trastuzumab duocarmazine and its safety and activity in HER2-expressing metastatic breast and other cancers, including triple-negative breast cancer.

Implications of all the available evidence

Trastuzumab duocarmazine warrants further investigation and might be a novel treatment option for patients with HER2-positive breast cancer who have been previously treated with trastuzumab plus pertuzumab or trastuzumab emtansine, or both. Our results suggest this HER2-targeting antibody–drug conjugate should be investigated for patients with low levels of HER2-expression in multiple tumour types.

Methods

Study design and participants

We did a phase 1 dose-escalation and dose-expansion study. Eligible patients were aged 18 years or older, with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, an estimated life expectancy of at least 12 weeks, and adequate organ function (see appendix pp 11–13 for full eligibility criteria). For the dose-escalation phase, patients were recruited from three academic hospitals in Belgium, the Netherlands, and the UK. Patients were eligible if they had locally advanced or metastatic solid tumours refractory to standard treatment, regardless of HER2 status. For the dose-expansion phase, patients with HER2-expressing breast, gastric (including adenocarcinomas of the gastro-oesophageal junction), urothelial, or endometrial cancer were recruited from 15 hospitals in Belgium, the Netherlands, Spain, and the UK (appendix p 82). Eligible patients had to have at least one measurable tumour lesion as defined by Response Evaluation Criteria for Solid Tumours (RECIST) version 1.1, and centrally assessed tumour HER2 expression should have been at least IHC 1+.

See Online for appendix

Key exclusion criteria for both study phases were anthracycline treatment within the previous 3 months or any other cancer treatment within the previous 4 weeks; a history of infusion-related reaction, hypersensitivity to trastuzumab or trastuzumab emtansine, or both of these; left-ventricular ejection fraction (LVEF) less than 55%; severe uncontrolled systemic disease; and symptomatic brain metastases or treatment for brain metastases within 4 weeks. Further details can be found in the protocol (appendix pp 8–80).

The study protocol, amendments, and informed consent forms were reviewed and approved by local authorities and independent ethics committees at each study site. All patients provided written informed consent before any protocol-related activities started. The study was done in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines.

Procedures

In the dose-escalation phase, trastuzumab duocarmazine (Synthon Biopharmaceuticals, Nijmegen, Netherlands) was administered intravenously on day 1 of each 3-week cycle with a first-in-human starting dose of 0·3 mg/kg. The first infusion was given over 1 h and, if well tolerated, subsequent infusions could be given over 30 min. We used a standard 3+3 dose-escalation design; doses were initially doubled for subsequent dose cohorts if no dose-limiting toxic effect was recorded in the first treatment cycle. The definition of dose-limiting toxicity is provided in the appendix (p 1). If a dose-limiting toxic effect was reported in one patient during the first cycle, at least three additional patients were to be treated at that dose level. Intrapatient dose escalation was not permitted, but dose reductions and delays were allowed by protocol (appendix pp 41–43). Patients were treated until disease progression or unacceptable toxicity. The highest dose level at which no more than one of six patients had a dose-limiting toxic effect was determined to be the maximum tolerated dose. The recommended phase 2 dose was determined based on all available safety, pharmacokinetic, and activity data. This dose was used in the dose-expansion phase.

Patients in both study phases were assessed for toxicity at least once per week in the first two cycles and once during subsequent cycles, according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03. Vital signs, haematology, blood chemistry, 12-lead electrocardiograms (ECGs), cardiac biomarkers, and urinalysis were assessed at each visit. Weight, physical, and ophthalmological examinations, and LVEF assessments were done at each or every other cycle. Blood samples were obtained at each visit for pharmacokinetic assessment and before each infusion for immunogenicity analyses (measurement of antibodies against trastuzumab duocarmazine).

After completion of the dose-escalation phase, several prophylactic measures were introduced, as per an approved protocol amendment (prepared and approved before the start of the dose-expansion phase in 2016), to assess the effect of trastuzumab duocarmazine on ocular toxicity. Lubricating eye drops were to be prescribed to all patients enrolled in the dose-expansion phase. Vasoconstrictive phenylephrine and anti-inflammatory dexamethasone eye drops were to be administered 1 h before the start of the infusion, and the anti-inflammatory dexamethasone eye drops were to be continued up to 2 days after infusion in the HER2-low breast cancer cohorts and in the non-breast cancer cohorts. In the HER2-positive breast cancer expansion cohort, patients were randomly allocated to either 1·2 mg/kg every 3 weeks continuously, 1·2 mg/kg every 3 weeks for four cycles followed by 0·9 mg/kg every 3 weeks, or 1·2 mg/kg every 3 weeks for four cycles followed by 1·2 mg/kg every 6 weeks to investigate the effect of different dosing regimens on ocular toxicity. Randomisation was done in a 1:1:1 ratio using a permutated block design with a block size of three. Allocation of subsequent patients was controlled centrally and communicated by the clinical research organisation (INC Research, Amsterdam, Netherlands) to the investigators, who were to adjust the dosing schedule accordingly (dosing was open-label).

HER2 tumour expression was assessed by IHC and ISH using archival or fresh tissue according to the American Society of Clinical Oncology and College of American Pathologists guidelines for breast and gastric cancer (appendix pp 47–49). Fresh tissue was obtained just before the start of study treatment if no archival tissue was available. HER2-positive disease was defined as IHC 3+ or ISH-positive. In the dose-escalation phase, tissue analysis was done by the local site laboratory, whereas in

the dose-expansion phase, tissue analysis was done centrally using HER2 IHC and dual ISH assays (Ventana; F Hoffmann-La Roche, Welwyn Garden City, UK). Patients with HER2 IHC 3+ or ISH-positive breast cancer were enrolled into the HER2-positive breast cancer cohort; patients with IHC 2+ or 1+ and ISH-negative breast cancer were enrolled in either the HER2-low hormone receptor-positive cohort or the HER2-low hormone receptor-negative cohort so that we could assess these populations separately, in view of these patients' substantially different biology, natural history, and treatment recommendations. Non-breast cancer cohorts included patients with HER2-low and HER2-positive tumours. Validated ELISA-based methods were used to measure the plasma concentration of total antibody (irrespective of the amount of conjugated toxins—ie, drug:antibody ratio ≥0) and conjugated antibody (antibodies that have at least one conjugated toxin—ie, drug-to-antibody ratio ≥1). A validated liquid chromatography–tandem mass spectrometry method was used for quantification of DUBA (free toxin) in plasma (appendix p 21).

Tumour response was assessed by the investigator at baseline and every 6 weeks during treatment according to RECIST version 1.1, using CT, PET-CT, or MRI.

Outcomes

The primary endpoint of the dose-escalation phase was to assess safety and to ascertain the maximum tolerated dose and recommended phase 2 dose for trastuzumab duocarmazine. The primary endpoint of the dose-expansion phase was the proportion of patients who achieved an objective response (defined as either a complete response or a partial response, by RECIST 1.1). Secondary endpoints were safety, pharmacokinetics, immunogenicity, quality of life (data not reported here), and anti-tumour activity. Anti-tumour activity endpoints were best percentage change in target lesion measurements, progression-free survival (defined as the time from first day of treatment to tumour progression or death from any cause), clinical benefit (the proportion of patients with a complete response, partial response, or stable disease for ≥6 months; data not reported here), duration of response (time from first observation of response to disease progression; data not reported here), and overall survival (time from treatment initiation to death from any cause; data not reported here).

Statistical analysis

We estimated that up to 24 patients (three to six patients per dose level) would need to be enrolled in the dose-escalation phase to ascertain the recommended phase 2 dose. In the dose-expansion phase, we initially estimated that up to 128 patients would be enrolled in six cohorts—ie, 48 patients in the HER2-positive breast cancer cohort and 16 in each of the other five cohorts. A Simon's two-stage design was applied to all cohorts except for the HER2-positive breast cancer cohort. The null hypothesis that the true response was 5% or less was to be tested against the one-sided alternative of a response of 20%. In case the null hypothesis could be rejected—ie, if two or more responders were found in the initial 16 enrolled patients in a cohort—a maximum of 14 additional patients could be enrolled in that cohort for a total of 30 patients. This design had a type I error of 5% and a power of 80% when the true response was 20%.

Safety and most of the activity endpoints were assessed in the safety population, which was defined as all patients who received at least one dose of study treatment. However, for the tumour response analysis, we excluded patients without measurable disease at baseline or without a post-baseline RECIST assessment. The population for pharmacokinetic analyses included all patients for whom at least one pharmacokinetic variable could be calculated. Descriptive statistics were used to summarise patients' demographics, baseline characteristics, and safety data. Activity proportions were summarised with exact binomial 95% CIs. Progression-free survival was analysed using Kaplan-Meier quartile estimates and two-sided 95% CIs; data were censored either at the date of the last RECIST assessment when no documented date of progression (according to RECIST version 1.1) or death was available or when death or progression occurred after two or more consecutive missed assessments. Actual blood sampling times relative to the time of dose were used to ascertain pharmacokinetic variables. Values lower than the limit of quantification were imputed as zero. Statistical analyses were done with SAS version 9.4. Pharmacokinetic analyses were done with Phoenix WinNonlin version 8.1.

This study is registered with ClinicalTrials.gov, number NCT02277717.

Role of the funding source

The funder contributed to study design, data collection, data analysis, data interpretation, and writing of the report. UB, CMLvH, ECM, NPK, and PA had full access to all the study data. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Between Oct 30, 2014, and April 2, 2018, 39 patients were enrolled and treated in the dose-escalation phase and 146 patients were enrolled and treated in the dose-expansion phase (figure 1). In the dose-expansion phase, 50 patients had HER2-positive metastatic breast cancer, 32 had HER2-low hormone receptor-positive metastatic breast cancer, 17 had HER2-low hormone receptor-negative metastatic breast cancer, 17 had gastric cancer, 16 had urothelial cancer, and 14 had endometrial cancer. At data cutoff (July 5, 2018), three patients with HER2-positive breast cancer, two patients with endometrial cancer, and one patient with urothelial cancer were still on treatment.

Median follow-up for all patients was 5·0 months (IQR 2·9–7·6) until the final safety assessment

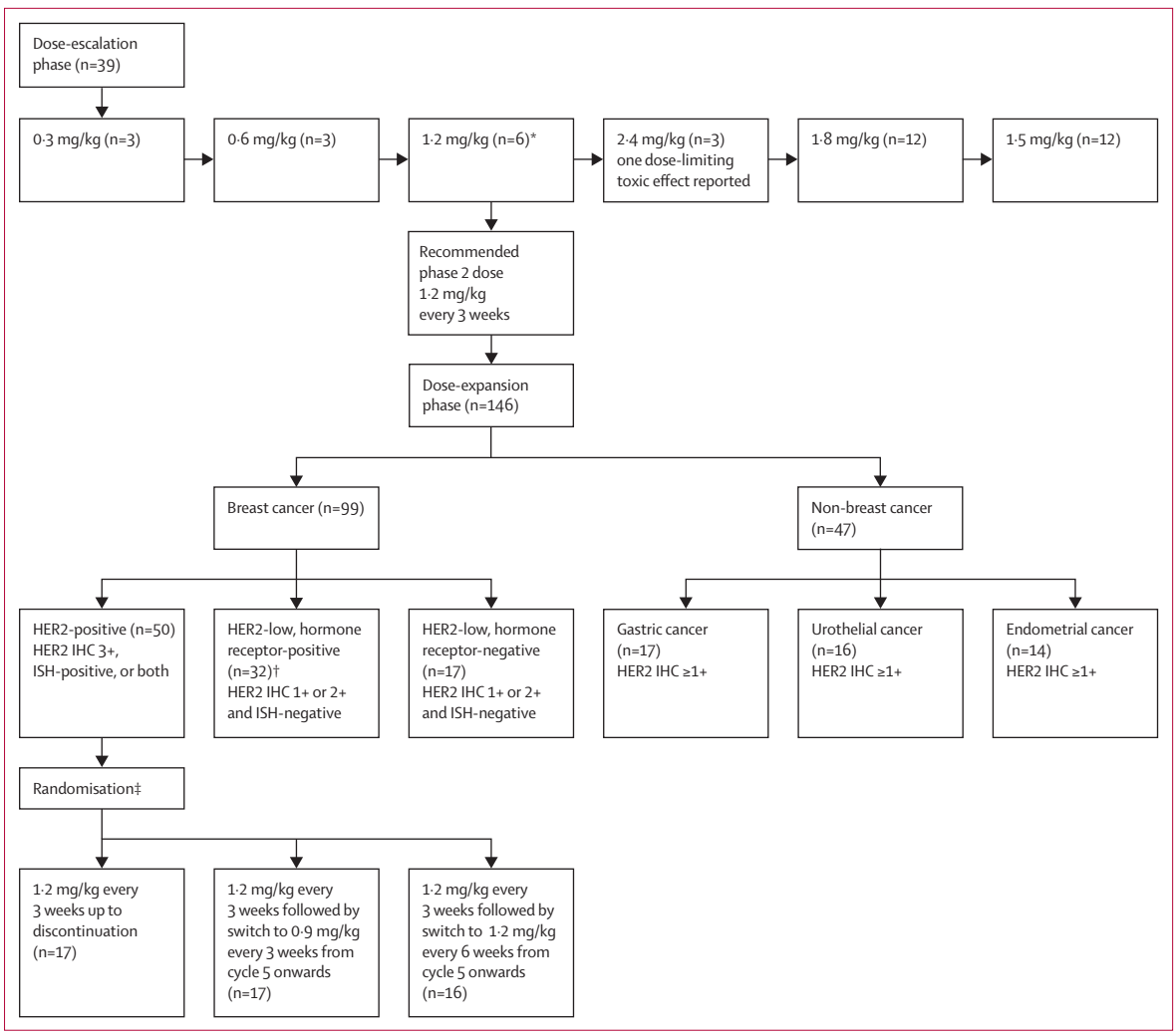

Figure 1: **Trial profile**
IHC=immunohistochemistry. ISH=in-situ hybridisation. *Three patients were added to the 1·2 mg/kg cohort at the same time that enrolment started for the 1·5 mg/kg cohort. †Cohort was expanded from 16 to 32 patients when two of the initial 16 patients had a partial response (Simon two-stage design). ‡Patients with HER2-positive metastatic breast cancer were randomly allocated to one of three different treatment subgroups to investigate the effect of different dosing regimens on ocular toxicity.

(July 5, 2018). Patients' demographics and baseline characteristics are provided in table 1 and the appendix (p 2). Patients were heavily pretreated with anticancer drugs, with a mean of 5·2 (SD 3·3) previous lines of treatment. In the HER2-positive metastatic breast cancer expansion cohort, 40 (80%) of 50 patients had received previous trastuzumab emtansine.

In the dose-escalation phase, initial doses of trastuzumab duocarmazine were doubled from 0·3 mg/kg up to 2·4 mg/kg because no dose-limiting toxic effects occurred in the first treatment cycle. One of three patients dosed with 2·4 mg/kg trastuzumab duocarmazine developed pneumonitis in cycle 2, which was considered possibly related to the study drug, and after the third infusion this patient died. Although the event did not occur during the first cycle, it was considered a dose-limiting toxic effect. Because of the seriousness of this toxic effect and because promising activity was already seen at the 1·2 mg/kg dose level, the decision was made not to enrol three additional patients at the 2·4 mg/kg dose but to assess lower doses in more detail. Therefore, the protocol-defined maximum tolerated dose of trastuzumab duocarmazine has not been defined. No dose-limiting toxic effects occurred at doses of 1·8 mg/kg (n=12), 1·5 mg/kg (n=12), or 1·2 mg/kg (n=6). The overall median duration of trastuzumab duocarmazine exposure was 3·5 months (IQR 1·4–5·4) and was longest at the 1·2 mg/kg dose (8·1 months [IQR 5·9–9·8] (appendix p 5).

Treatment-related adverse events are shown in table 2 and divided per dose level in the dose-escalation phase in the appendix (p 3). In the dose-escalation phase, grade 3

	Dose-escalation cohort (n=39)	Dose-expansion cohorts (n=146)	HER2-positive metastatic breast cancer (n=50)	HER2-low, hormone receptor-positive metastatic breast cancer (n=32)	HER2-low, hormone receptor-negative metastatic breast cancer (n=17)	Non-breast cancer expansion cohorts (n=47)*
Demographics						
Age (years)	55 (47–63)	57 (49–65)	54 (47–63)	53 (47–61)	53 (45–62)	64 (54–71)
Sex						
Female	30 (77%)	120 (82%)	50 (100%)	32 (100%)	17 (100%)	21 (45%)
Male	9 (23%)	26 (18%)	0	0	0	26 (55%)
Ethnic origin						
White	38 (97%)	140 (96%)	47 (94%)	32 (100%)	17 (100%)	44 (94%)
Other	1 (3%)	6 (4%)	3 (6%)	0	0	3 (6%)
Clinical characteristics						
ECOG performance status						
0	22 (56%)	69 (47%)	26 (52%)	19 (59%)	5 (29%)	19 (40%)
1	17 (45%)	77 (53%)	24 (48%)	13 (41%)	12 (71%)	28 (60%)
Time since initial diagnosis (months)	67 (33–143)	53 (27–100)	78 (47–107)	94 (55–136)	43 (23–84)	25 (16–45)
Cancer type						
Breast	26 (67%)	99 (68%)	50 (100%)	32 (100%)	17 (100%)	0
Gastric†	6 (15%)	17 (12%)	0	0	0	17 (36%)
Colorectal	3 (8%)	0	0	0	0	0
Urothelial	0	16 (11%)	0	0	0	16 (34%)
Endometrial	1 (3%)	14 (10%)	0	0	0	14 (30%)
Other	3 (8%)	0	0	0	0	0
Number of metastatic sites	3 (2–4)	3 (2–4)	3 (2–4)	3 (2–3)	3 (2–4)	3 (2–4)
Known brain metastases	3 (8%)	8 (5%)	5 (10%)	0	0	3 (6%)
HER2 expression						
Immunohistochemistry 3+	15 (39%)	57 (39%)	41 (82%)	0	0	16 (34%)
Immunohistochemistry 2+	12 (31%)	37 (25%)	3 (6%)	10 (31%)	7 (41%)	17 (36%)
ISH-positive	2 (5%)	6 (4%)	3 (6%)	0	0	3 (6%)
ISH-negative	8 (20%)	27 (18%)	0	10 (31%)	7 (41%)	10 (21%)
ISH-equivocal	1 (3%)	3 (2%)	0	0	0	3 (6%)
ISH-unassessable	1 (3%)	1 (1%)	0	0	0	1 (2%)
Immunohistochemistry 1+	6 (15%)	51 (35%)	5 (10%)‡	22 (69%)	10 (59%)	14 (30%)
Immunohistochemistry 0	4 (10%)	1 (1%)	1 (2%)‡	0	0	0
Missing	2 (5%)	0	0	0	0	0
Previous treatment						
Systemic treatments	6 (2–8)	4 (3–7)	6 (4–8)	7 (5–9)	4 (3–5)	2 (2–3)
1–3	13 (33%)	50 (34%)	5 (10%)	3 (9%)	6 (35%)	36 (77%)
4–6	9 (23%)	55 (38%)	24 (48%)	11 (34%)	9 (53%)	11 (23%)
>6	17 (44%)	41 (28%)	21 (42%)	18 (56%)	2 (12%)	0
HER2-targeting treatment§	20 (51%)	62 (42%)	47 (94%)	6 (19%)	1 (6%)	8 (17%)
Trastuzumab	20 (51%)	61 (42%)	46 (92%)	6 (19%)	1 (6%)	8 (17%)
Trastuzumab emtansine	16 (41%)	43 (29%)	40 (80%)	3 (9%)	0	0
Lapatinib	9 (23%)	26 (18%)	23 (46%)	2 (6%)	1 (6%)	0
Pertuzumab	2 (5%)	17 (12%)	15 (30%)	2 (6%)	0	0
CDK4 or CDK6 inhibitors	2 (5%)	5 (3%)	0	5 (16%)	0	0
PD-1 or PD-L1 inhibitors	0	14 (10%)	1 (2%)	2 (6%)	1 (6%)	10 (21%)

Data are median (IQR) or n (%). ECOG=Eastern Cooperative Oncology Group. ISH=in-situ hybridisation. *Baseline characteristics by cancer type are in the appendix (p 2).†Gastric cancer including adenocarcinoma of the gastro-oesophageal junction. ‡Tumour tissue was HER2 ISH-positive for these patients. §In the dose-expansion part of the study, patients with breast cancer were allocated to a cohort based on centrally assessed HER2 status on the most recent available tumour tissue, which in some patients deviated from previous locally assessed HER2 status.

Table 1: **Baseline characteristics**

or grade 4 treatment-related adverse events occurred in 13 (33%) of 39 patients (some patients had more than one event) and events reported more than once were keratitis (n=3) and fatigue (n=2). The most commonly recorded treatment-related adverse events of any grade in the dose-escalation phase were conjunctivitis (12 [31%] of 39 patients), fatigue (11 [28%]), and dry skin (ten [26%]). A reversible decrease in LVEF was reported as an adverse

	Dose-escalation cohort (n=39)*			Dose-expansion cohorts (n=146)		
	Grades 1–2	Grade 3	Grade 4	Grades 1–2	Grade 3	Grade 4
Fatigue	9 (23%)	2 (5%)	0	43 (29%)	5 (3%)	0
Conjunctivitis	11 (28%)	1 (3%)	0	41 (28%)	4 (3%)	0
Dry eye	6 (15%)	0	0	44 (30%)	1 (1%)	0
Lacrimation increased	8 (21%)	0	0	29 (20%)	0	0
Dry skin	10 (26%)	0	0	26 (18%)	0	0
Decreased appetite	6 (15%)	1 (3%)	0	27 (18%)	2 (1%)	0
Keratitis	3 (8%)	3 (8%)	0	25 (17%)	3 (2%)	0
Alopecia	8 (21%)	0	0	26 (18%)	0	0
Nausea	6 (15%)	0	0	27 (18%)	0	0
Stomatitis	8 (21%)	1 (3%)	0	24 (16%)	0	0
Skin hyperpigmentation	5 (13%)	0	0	23 (16%)	0	0
Neutropenia	3 (8%)	1 (3%)	0	14 (10%)	9 (6%)	0
Vomiting	5 (13%)	0	0	17 (12%)	0	0
Anaemia	6 (15%)	1 (3%)	0	13 (9%)	2 (1%)	0
Pyrexia	9 (23%)	0	0	9 (6%)	0	0
Dysgeusia	7 (18%)	0	0	11 (8%)	0	0
Infusion-related reaction	3 (8%)	0	0	13 (9%)	2 (1%)	0
Vision blurred	0	0	0	15 (10%)	1 (1%)	0
LVEF decreased	1 (3%)	1 (3%)	0	10 (7%)	1 (1%)	0
Diarrhoea	1 (3%)	0	0	9 (6%)	1 (1%)	0
Thrombocytopenia	2 (5%)	0	0	7 (5%)	1 (1%)	0
Aspartate aminotransferase increased	1 (3%)	0	0	7 (5%)	1 (1%)	0
Lymphopenia	0	0	0	7 (5%)	2 (1%)	0
Dyspnoea	4 (10%)	0	0	2 (1%)	2 (1%)	0
Asthenia	0	1 (3%)	0	7 (5%)	0	0
Mouth ulceration	1 (3%)	0	0	5 (3%)	1 (1%)	0
Pericardial effusion	0	1 (3%)	0	2 (1%)	2 (1%)	1 (1%)
γ-glutamyltransferase increased	0	0	0	4 (3%)	0	2 (1%)
Rash maculopapular	1 (3%)	0	0	4 (3%)	1 (1%)	0
Blood alkaline phosphatase increased	1 (3%)	0	0	3 (2%)	1 (1%)	0
Pneumonitis	3 (8%)	0	0	0	0	1 (1%)
Aminotransferase enzymes (not otherwise specified) increased	1 (3%)	0	0	3 (2%)	1 (1%)	0
White blood cell count decreased	0	0	0	4 (3%)	1 (1%)	0
Episcleritis	0	1 (3%)	0	4 (3%)	0	0
Lymphocyte count decreased	0	0	0	1 (1%)	3 (2%)	0
Platelet count decreased	0	0	1 (3%)	1 (1%)	2 (1%)	0
Palmar-plantar erythrodysaesthesia syndrome	0	0	0	3 (2%)	1 (1%)	0
Neutrophil count decreased	0	1 (3%)	0	2 (1%)	0	0
Hepatic enzymes increased	0	0	0	1 (1%)	1 (1%)	0
Injection-site reaction	0	1 (3%)	0	1 (1%)	0	0
Pleural effusion	0	0	0	1 (1%)	1 (1%)	0
Pancytopenia	0	0	0	0	1 (1%)	0
Corneal toxicity not otherwise specified	0	0	0	0	1 (1%)	0
Retinal haemorrhage	0	0	0	0	1 (1%)	0

(Table 2 continues on next page)

	Dose-escalation cohort (n=39)*			Dose-expansion cohorts (n=146)		
	Grades 1–2	Grade 3	Grade 4	Grades 1–2	Grade 3	Grade 4
(Continued from previous page)						
Ventricular dysfunction	0	0	0	0	1 (1%)	0
Haemoptysis	0	0	0	0	1 (1%)	0
Conjunctivitis bacterial	0	0	0	0	1 (1%)	0
Pain in extremity	0	0	0	0	1 (1%)	0
Delirium	0	0	0	0	0	1 (1%)
Haematuria	0	0	0	0	1 (1%)	0

Data are presented as n (%). Included in this table are maximum grade adverse events by preferred term deemed related to study drug. Grade 1 and 2 events are presented if they were recorded in at least 10% of all patients, and all grade 3 and 4 events are presented. One patient in the dose-escalation cohort (2·4 mg/kg) died from pneumonitis that was deemed a dose-limiting toxicity; one further patient died (1·5 mg/kg) due to disease progression attributed to general health decline. No patients in the dose-expansion cohorts died from adverse events related to treatment; four patients died due to disease progression, which were attributed to hepatic failure (n=1), upper gastrointestinal haemorrhage (n=1), neurological decompensation (n=1), and renal failure (n=1). LVEF=left-ventricular ejection fraction. *Adverse events related to treatment by dose level are in the appendix (p 3).

Table 2: **Adverse events considered related to trastuzumab duocarmazine**

event for two (5%) of 39 patients, and for three (8%) patients, an absolute worst decrease in LVEF from baseline of at least 10% to a value below 50% was measured during treatment. One further patient in the 1·5 mg/kg cohort died in the dose-escalation phase (attributed to general physical health deterioration), which was related to disease progression. 11 (28%) of 39 patients in the dose-escalation cohorts discontinued the study because of treatment-related toxicity (three patients each in the 1·2 mg/kg and 1·5 mg/kg cohorts, four patients in the 1·8 mg/kg cohort, and one in the 2·4 mg/kg cohort). Discontinuations were most commonly attributable to ocular adverse events after between five and ten cycles (n=5) or pneumonitis after between two and six cycles (n=4). Ocular toxicity improved after treatment discontinuation and was reported as recovered at the cutoff date for four patients. All four events of pneumonitis occurred at doses of 1·5 mg/kg or higher and three events without respiratory symptoms (of which one was grade 1 and two were grade 2) resolved within 1 month after study discontinuation (the fourth event was the fatal event described). Overall, doses up to 1·8 mg/kg were tolerated well without a clear dose-related occurrence of adverse events (appendix p 3), although ocular toxicity seemed to occur earlier at higher doses and increased with augmented exposure (data not shown). Based on these data, in combination with the observed treatment duration and activity data, the recommended phase 2 dose of trastuzumab duocarmazine was set at 1·2 mg/kg, because the 1·5 mg/kg and 1·8 mg/kg doses seemed not to improve the benefit:risk ratio for patients. The 1·2 mg/kg dose was used in the dose-expansion cohorts.

In the dose-expansion cohorts, the mean treatment duration was longest in the cohort with HER2-positive breast cancer (mean 7·1 [SD 4·6] months). Mean treatment durations were similar in the cohorts with HER2-low hormone receptor-positive breast cancer (mean 3·7 [SD 2·3] months), HER2-low hormone receptor-negative breast cancer (3·3 [2·2] months), gastric cancer (3·2 [2·1] months), urothelial cancer (3·5 [2·2] months), and endometrial cancer (4·0 [2·8] months).

The most common treatment-related adverse events of any grade in the dose-expansion cohorts were fatigue (48 [33%] of 146 patients), conjunctivitis (45 [31%]), and dry eye (45 [31%]; table 2). Grade 3 or 4 treatment-related adverse events occurred in 51 (35%) of 146 patients and the most common of these were neutropenia (nine [6%]), fatigue (five [4%]), and conjunctivitis (four [3%]). 104 (71%) of 146 patients had one or more ocular adverse events, with grade 3 events in ten (7%) of 146 patients. Occurrence of ocular toxic effects and their severity generally increased with prolonged exposure (data not shown), with a median time to grade 3 events of 7·6 months (IQR 4·3–8·9). Reduced dosing after four infusions of trastuzumab duocarmazine in patients with HER2-positive breast cancer—ie, either a decrease to 0·9 mg/kg every 3 weeks or to 1·2 mg/kg every 6 weeks—or use of prophylactic eye drops, or both of these, did not greatly improve tolerability, although several patients seemed to benefit (ie, toxicity remained stable or improved) from dose delays or dose reduction and could continue with trastuzumab duocarmazine treatment beyond 1 year (figure 2). Most ocular events improved or recovered with treatments such as eye drops or ointments, although recovery sometimes took several months. A decrease in LVEF was reported as an adverse event for 11 (8%) of 146 patients, of which eight were reported as resolved before data cutoff. In eight (5%) of 146 patients, an absolute worst decrease in LVEF from baseline of at least 10% to a value below 50% was measured during treatment. Treatment-related serious adverse events were reported in 16 (11%) of 146 patients, most commonly infusion-related reactions (two [1%]) and dyspnoea (two [1%]). Four patients died in the dose-expansion phase, one each from hepatic failure, upper gastrointestinal haemorrhage, neurological decompensation, and renal failure. These deaths were all related to disease progression and were not judged to be related to

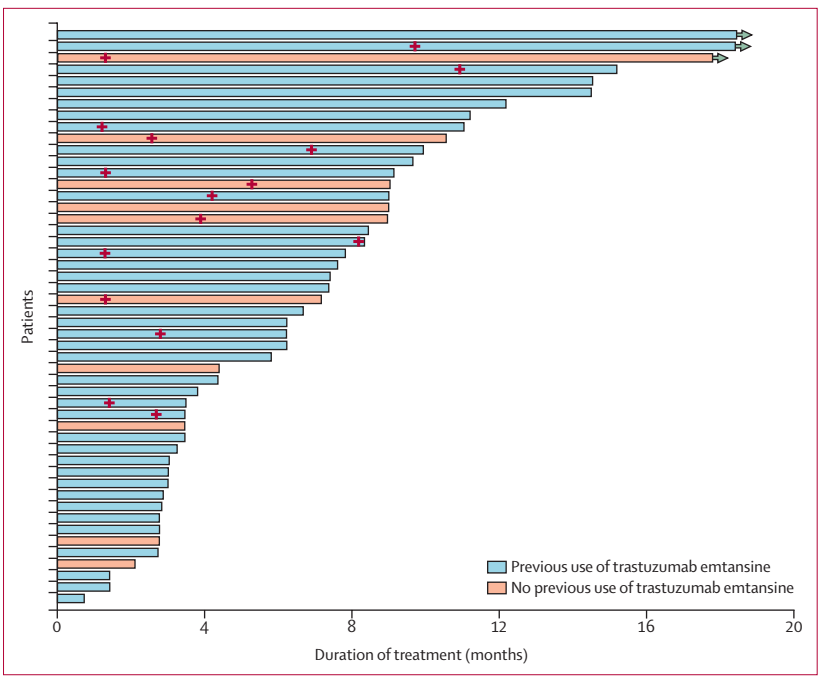

Figure 2: **Duration of treatment for HER2-positive breast cancer expansion cohort**
Cross indicates the time of first partial response. Arrows indicate patients still on treatment at the cutoff date. All patients were treated with 1·2 mg/kg and were randomised to a lower dose (0·9 mg/kg) or longer interval (every 6 weeks) from cycle 5 (about 15 weeks) if dosing was not delayed.

treatment. Overall, 62 (43%) of 146 patients had at least one treatment-related adverse event leading to one or more dose delays or dose reductions, and 27 (19%) of 146 patients discontinued the study because of treatment-related toxic effects, of which 15 (10%) were attributable to ocular toxicity. Dyspnoea, decreased LVEF, and decreased appetite were each reported for two (1%) patients as having contributed to treatment discontinuation; other reasons were reported as single events. Most of these events were reported as resolved at data cutoff.

Total antibody, conjugated antibody, and DUBA (free toxin) pharmacokinetics after intravenous infusion of trastuzumab duocarmazine followed a monophasic log-linear decline and were time independent. Concentrations were generally close to or below the limit of quantification at 3 weeks after treatment at all dose levels, with minimal accumulation across consecutive cycles (appendix p 4). Pharmacokinetics of all analytes were dose-proportional within the dose range of 1·2 mg/kg to 2·4 mg/kg. Dose levels below 1·2 mg/kg had faster elimination of total and conjugated antibody, which is indicative of target-mediated drug disposition. Elimination half-life was 2–3 days for conjugated antibody at doses of 1·2 mg/kg or higher. Free toxin exposure was generally 3000 times lower compared with conjugated antibody (on a molar basis) with peak concentrations in the pg/mL range. Pharmacokinetic variables for conjugated antibody and DUBA (free toxin) are presented in the appendix (p 5). No patients were found to have antibodies against trastuzumab duocarmazine at any timepoint.

In the dose-escalation phase, five of 39 patients were not assessable because of non-measurable disease (n=3) or a missing post-baseline RECIST assessment (n=2). Of the 34 assessable patients, 11 (32%, 95% CI 17·4–50·5) had a partial response, of whom ten (six confirmed) had breast cancer and one (unconfirmed) had gastric cancer. Responses were seen in both HER2-positive and HER2-low tumours and all occurred at doses of 1·2 mg/kg or higher (figure 3A). Two patients (one with breast and one with other [duodenal] cancer) depicted in figure 3A had a partial response in target lesions but had progressive disease in non-target lesions at the same assessment.

In the dose-expansion phase, six of 146 patients were not assessable because of a missing post-baseline RECIST assessment (n=5) or non-measurable disease at baseline (n=1). In addition, for five of 146 patients not all target lesions were assessed post baseline: these patients are included in the objective response analysis but are omitted from figure 3 on best percentage change in tumour size. In the three breast cancer expansion cohorts, 67 (71%) of 95 assessable patients showed a reduction in target lesions and 31 (33%) had a partial response, of which 23 were confirmed responses. An objective response (all partial responses) was achieved by 16 (33%, 95% CI 20·4–48·4) of 48 patients with HER2-positive breast cancer (figure 3B), nine (28%, 13·8–46·8) of 32 patients with HER2-low hormone receptor-positive breast cancer (figure 3C), and six (40%, 16·3–67·6) of 15 patients with HER2-low hormone receptor-negative breast cancer (figure 3D). Median progression-free survival was 7·6 months (95% CI 4·2–10·9) in patients with HER2-positive breast cancer, 4·1 months (2·4–5·4) in patients with HER2-low hormone receptor-positive breast cancer, and 4·9 months (1·2–not estimable [NE]) in patients with HER2-low hormone receptor-negative breast cancer.

In the non-breast cancer expansion cohorts, 25 (57%) of 45 assessable patients had a reduction in target lesions (appendix pp 6, 7). An objective response (all partial responses) was achieved by one (6%, 95% CI 0·2–30·2) of 16 patients with gastric cancer, four (25%, 7·3–52·4) of 16 patients with urothelial cancer, and five (39%, 13·9–68·4) of 13 patients with endometrial cancer. Median progression-free survival was 3·2 months (95% CI 1·6–5·3) in patients with gastric cancer, 4·0 months (1·3–NE) in patients with urothelial cancer, and 4·3 months (2·4–9·9) in patients with endometrial cancer.

Of 50 patients with HER2-positive breast cancer in the dose-expansion phase, 28 (56%) received trastuzumab duocarmazine for longer than 6 months and seven (14%) were treated for longer than 1 year (figure 2). Six of the seven patients with HER2-positive breast cancer treated for longer than 1 year had previously received trastuzumab emtansine.

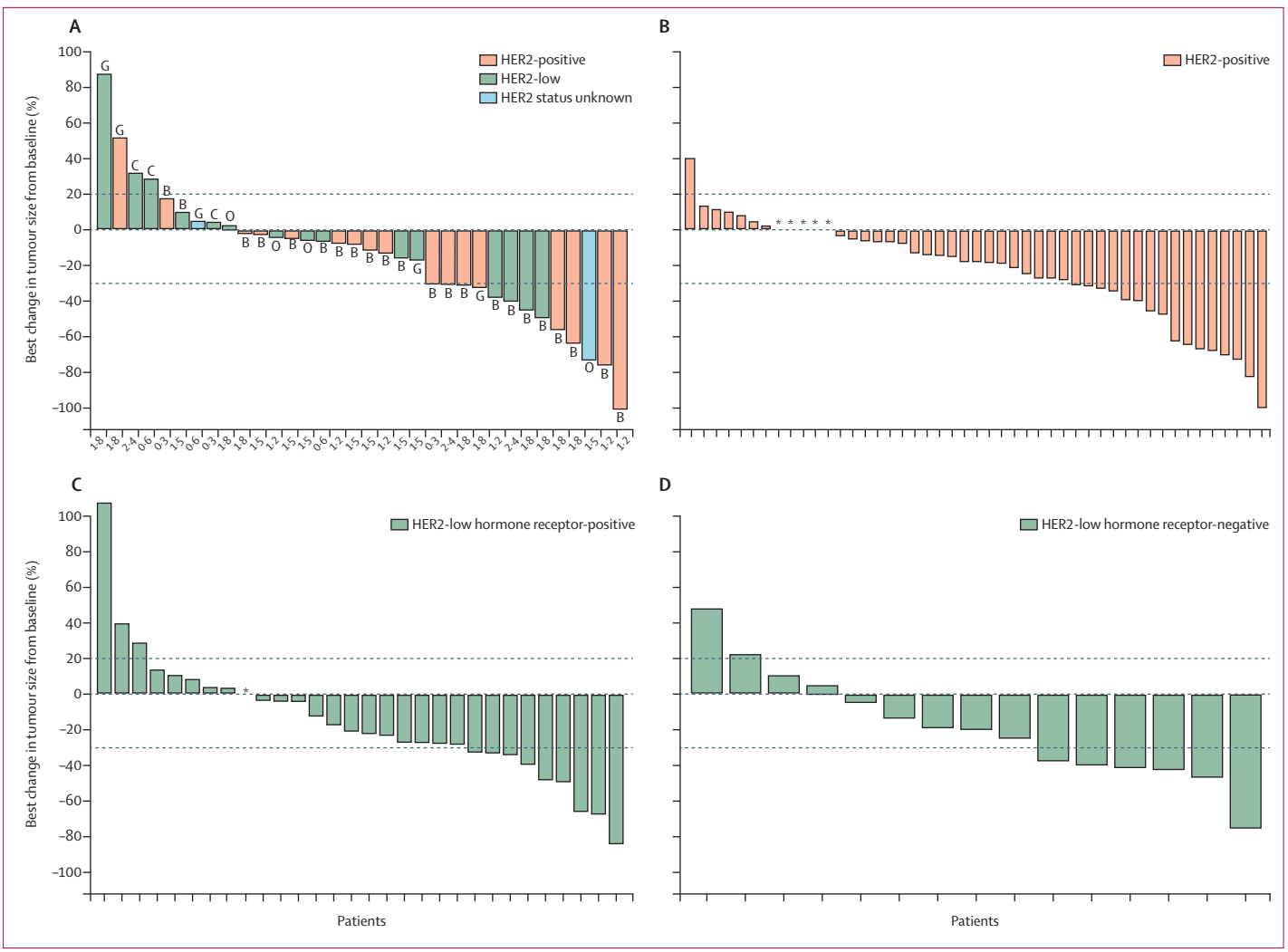

Figure 3: **Best percentage change in tumour size from baseline in target lesions for assessable patients**
(A) Dose-escalation phase, by cancer type, HER2 expression, and dose (in mg/kg). (B) Dose-expansion phase, HER2-positive breast cancer cohort. (C) Dose-expansion phase, HER2-low hormone receptor-positive breast cancer cohort. (D) Dose-expansion phase, HER2-low hormone receptor-negative breast cancer cohort. Dotted lines reflect 20% increase or 30% reduction in tumour size. Post-baseline target lesion assessment was incomplete for seven patients: three patients in (B), two patients in (C), and two patients in (D). HER2-low=low expression of HER2. G=gastric cancer including adenocarcinoma of the gastro-oesophageal junction. C=colorectal cancer. B=breast cancer. O=other cancer. *Best percentage change was 0%.

Discussion

To our knowledge, our phase 1 study in heavily pretreated patients with locally advanced or metastatic solid tumours is the first to report a novel antibody–drug conjugate with a DNA-alkylating duocarmycin payload. Trastuzumab duocarmazine showed a manageable safety profile with few grade 3 or 4 adverse events. The recommended phase 2 dose was set at 1·2 mg/kg. Responses were noted across all tumour types, not only in HER2-positive tumours but also in tumours expressing lower levels of HER2.

The side-effect profile of trastuzumab duocarmazine has both similarities and differences with other HER2-targeting antibody–drug conjugates. The most common side-effects seen with trastuzumab duocarmazine were attributable to ocular toxicity. Although such toxic effects have been described with other antibody–drug conjugates, they are less typical in HER2-targeting antibody–drug conjugates, and the pathophysiology of these events is not yet well understood.[16] Planned dose reductions, decreasing the frequency of administration, or the use of prophylactic eye drops did not substantially change long-term tolerability of trastuzumab duocarmazine overall, but several patients were able to continue with study drug beyond 1 year and most ocular events were reported as recovered or improving at data cutoff. However, in view of the relative scarcity of data, additional observations—particularly over a prolonged treatment period—are necessary for drawing more definitive conclusions. Grade 3–4 thrombocytopenia was recorded in fewer than 1% of patients after treatment with trastuzumab duocarmazine, and grade 3–4 neutropenia

was noted in 6% of patients—frequencies that are lower than with other HER2-targeting antibody–drug conjugates.[7,17] This finding could be of importance when investigating future combination strategies. Pneumonitis was reported as a dose-limiting toxic effect at the 2·4 mg/kg dose, but risk was diminished at the recommended phase 2 dose of 1·2 mg/kg. This type of adverse event has also been reported for both trastuzumab emtansine[18] and trastuzumab deruxtecan—a HER2-targeting topoisomerase antibody–drug conjugate that is in development.[17,19] However, the underlying mechanism or risk factor is not yet clear.

The pharmacokinetic profile in combination with the DNA-alkylating mode of action of trastuzumab duocarmazine supports a dosing schedule of once every 3 weeks. Systemically free toxin levels were substantially lower compared with other antibody–drug conjugates such as trastuzumab emtansine[20] and trastuzumab deruxtecan.[17] Amounts of antibody–drug conjugate achieved in patients are consistent with amounts achieved in mice, which showed significant xenograft growth delay with trastuzumab duocarmazine.[12]

Our study has some limitations. First, we enrolled patients who had completed several late-line treatment options for metastatic disease. However, pertuzumab was not yet commonly prescribed for patients with HER2-positive breast cancer in Europe when the study started because of pending reimbursement discussions after approval of the drug in 2013, so fewer than half of the patients enrolled were pretreated with pertuzumab. Second, tumour assessments were not assessed centrally. Resulting estimates should be viewed with this limitation in mind but are nonetheless very encouraging in a phase 1 setting.

Trastuzumab duocarmazine showed meaningful single-agent clinical activity in three areas of unmet need. First, relevant clinical activity was noted in patients with HER2-positive metastatic breast cancer, which is especially important because trastuzumab emtansine therapy is set to move to adjuvant treatment paradigms after the results of the KATHERINE study.[21] Thus, the need for novel treatment options is increased for HER2-positive breast cancer in patients with metastatic disease after progression on trastuzumab emtansine. The TULIP randomised phase 3 study (NCT03262935) comparing trastuzumab duocarmazine with standard-of-care chemotherapy combinations in patients with HER2-positive breast cancer is ongoing. Second, activity of single-agent trastuzumab duocarmazine was also seen in patients with HER2-low (IHC 1+ or IHC 2+ ISH-negative) hormone receptor-negative disease, for whom no HER2-targeted drugs and antibody–drug conjugates are currently approved. These triple-negative breast cancers are a highly diverse group of cancers[22] for which several antibody–drug conjugates targeting different antigens are in development. For example, the anti-TROP2 antibody–drug conjugate sacituzumab govitecan has shown encouraging activity in a phase 1 study.[23] Moreover, the prolonged progression-free survival reported with atezolizumab in combination with nab-paclitaxel in a selective group of triple-negative patients is promising.[24] Nevertheless, there is still a high unmet need to improve outcomes in these patients, and trastuzumab duocarmazine could potentially be of benefit in this setting. Third, trastuzumab duocarmazine showed some activity in non-breast HER2-expressing metastatic cancers, which have few treatment options and poor prognoses (eg, urothelial and endometrial cancers). Several responses were noted in these patients, most of whom had HER2-low tumours. Although HER2 expression data are variable between studies,[1,25] further investigation of HER2-targeting drugs in this subset of patients would be worthwhile.

In conclusion, this phase 1 study of trastuzumab duocarmazine has shown important and relevant clinical activity and a manageable safety profile in heavily pretreated patients with HER2-expressing metastatic cancer, including HER2-positive trastuzumab emtansine-resistant and HER2-low breast cancer. Further investigation of trastuzumab duocarmazine for HER2-positive breast cancer is ongoing in the phase 3 TULIP study to assess the efficacy and safety of trastuzumab duocarmazine compared with clinician's choice in patients with locally advanced or metastatic breast cancer who progressed during or after at least two previous HER2-targeting treatment regimens or during or after trastuzumab emtansine. Additional studies to further investigate the encouraging signal from our phase 1 trial are in preparation.

Contributors

UB, CMLvH, ECM, NPK, and PA had the idea for and designed the study and contributed to data analysis and data interpretation. UB, CMLvH, CS, FT, SL, VM, IRM, VB, CR, EGEdV, SR, JG, FE, MG-M, and PA treated patients and collected data. All authors contributed to writing and review of the report and approved the final version.

Declaration of interests

UB reports grants from Chugai, Onyx Pharmaceuticals, BTG International, Verastem, and AstraZeneca, outside of the submitted work; and personal fees from Lilly, Phoenix Solutions, Novartis, Astex Pharmaceuticals, Karus Therapeutics, Vernalis, and Astellas, outside of the submitted work. CMLvH reports grants from Bristol-Myers Squibb, MSD, Regeneron, AstraZeneca, Merck, Ipsen, Novartis, Sanofi, and Bayer, outside of the submitted work. CS reports grants from AstraZeneca, Roche, Genentech, Macrogenics, Novartis, Pfizer, Piqur Therapeutics, Puma, and Synthon, during the conduct of the study; and personal fees from AstraZeneca, Celgene, Daiichi Sankyo, Eisai, Roche, Genomic Health, Novartis, Pfizer, Pierre Fabre, Piqur Therapeutics, Puma, and Synthon, outside of the submitted work. IRM reports personal fees from Roche Products UK, Pierre Fabre, and Daiichi Sankyo, outside of the submitted work. CR reports personal fees from MSD, Novartis, Guardant Health, and Mylan, outside of the submitted work; grants from Novartis and Sanofi, outside of the submitted work; and non-financial support from Oncompass, OncoDNA, International Association for the Study of Lung Cancer, International Society of Liquid Biopsy, European Society for Medical Oncology, American Society of Clinical Oncology, and Oncology Latin America Association, outside of the submitted work. EGEdV reports grants from Synthon Biopharmaceuticals, during the conduct of the study; grants from Amgen, Genentech, Roche, Chugai Pharma,

CytomX Therapeutics, Nordic Nanovector, G1 Therapeutics, AstraZeneca, Radius Health, and Bayer, outside of the submitted work; and consulting or advisory fees from the National Surgical Adjuvant Breast and Bowel Project, Daiichi Sankyo, Pfizer, Sanofi, Synthon Biopharmaceuticals, and Merck, outside of the submitted work. ECM and NPK are employees of Synthon Biopharmaceuticals. NPK has a pending clinical application patent for the product investigated in this report (SYD985 [trastuzumab duocarmazine]). PA reports personal fees from Boehringer Ingelheim, Macrogenics, Synthon, Amgen, Novartis, Roche, and Novartis, outside of the submitted work; and non-financial support from MSD, Roche, Pfizer, and Amgen, outside of the submitted work. FT, SL, VM, VB, SR, JG, FE, and MG-M declare no competing interests.

Data sharing

Synthon Biopharmaceuticals BV will share patient-level and study-level data after de-identification for trastuzumab duocarmazine when approved in both the EU and the USA. These data will be shared with qualified, non-commercial, scientific and medical researchers, on the researcher's request. Requests for data sharing can be made to NPK, including a detailed proposal for data meta-analysis, which must be approved by Synthon. When Synthon has agreements related to joint research, development, or commercialisation, or when the product has been out-licensed, the responsibility for disclosure might be dependent on these agreements. Under these circumstances, Synthon will endeavour to gain agreement with its contractual parties to share data in response to approved research requests.

Acknowledgments

This study was sponsored by Synthon Biopharmaceuticals. We thank the patients and their families for participating in this study and all site personnel for their contributions. UB acknowledges infrastructural support from Cancer Research UK, The Experimental Cancer Medicine Centre, and Biomedical Centre Initiatives awarded to The Institute of Cancer Research and The Royal Marsden Hospital NHS Foundation Trust. UB is a recipient of a National Institute of Health Research award (ref RP-2016-07-028).

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