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Phase I study of TrasGEX, a glyco-optimised anti-HER2 monoclonal antibody, in patients with HER2-positive solid tumours

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ABSTRACT

Purpose TrasGEX is a second-generation monoclonal antibody of trastuzumab, glyco-optimised to enhance antibody-dependent cellular cytotoxicity while fully retaining trastuzumab's antigen-binding properties to human epidermal growth factor receptor 2 (HER2). A phase I dose-escalation study was conducted to establish the optimal TrasGEX dose and regimen for phase II studies and to define the safety, pharmacokinetics (PK) and preliminary antitumour activity of TrasGEX.

Patients and methods A total of 37 patients with advanced HER2-positive carcinomas and progressive disease received TrasGEX intravenously every 3 weeks until disease progression in doses of 12–720 mg in a three-plus-three dose escalation design, including an expansion cohort at the highest dose.

Results No dose limiting toxicity was observed, and no maximum tolerated dose was reached. Drug-related adverse events were mainly infusion-related reactions occurring during the first infusion in 51% of patients; all but two were mild-to-moderate. Compared with trastuzumab, the PK parameters were dose dependent, with a mean terminal half-life ($t_{1/2}$) of 263±99 hours for the 720 mg dose. Clinical benefit in 15 out of 30 (50%) evaluable patients included one ongoing complete response, two partial remissions lasting 16 and 77 weeks and disease stabilisation (SD) in 12 patients lasting a median (range) of 17 (7–26) weeks; three of them had SD of 24, 25 and 26 weeks, respectively.

Conclusion TrasGEX was safe, well-tolerated and showed antitumour activity in 50% of evaluable patients, all with progressive disease at study entry. Infusions at an interval of 2–3 weeks should achieve clinically relevant trough levels for future studies (NCT01409343).

Key questions

What is already known about this subject?

- Trastuzumab is a mainstay in the treatment of human epidermal growth factor receptor 2 (HER2) overexpressing breast cancer, together with chemotherapy or as monotherapy.
- In addition to blocking HER2 and counteracting its oncogenic effect, trastuzumab mediates antibody-dependent cellular cytotoxicity (ADCC) primarily in patients with the favourable valine/valine FcγRIIIa allotype (20% of the population), less so in patients with the other two allotypes.
- Studies have shown that defucosylation of the constant fragment C domain of an antibody enhances ADCC to all three FcγRIIIa allotypes in vitro and in preclinical models.

What does this study adds?

- TrasGEX is an immunoglobulin G1 glycoengineered monoclonal antibody (mAb) of trastuzumab with the same binding properties to HER2 as trastuzumab but defucosylated to enhance ADCC.
- TrasGEX was safe and well tolerated by patients with solid tumours and progressive advanced disease, and pharmacokinetic characteristics were similar to those of trastuzumab.
- Preliminary antitumour activity was observed.
- Clinical efficacy was independent of the FcγRIIIa allotype.

How might this impact on clinical practice?

- TrasGEX and other glycoengineered mAbs, such as tomuzotuximab, may target a wider population replacing the parent antibody in combination therapies.

INTRODUCTION

Trastuzumab, an immunoglobulin G1 (IgG1) humanised monoclonal antibody (mAb) to HER2, is invaluable in the neoadjuvant, adjuvant and palliative treatment of breast cancer overexpressing HER2 in combination with chemotherapy or as monotherapy and is a standard option together with chemotherapy in the treatment of HER2-positive metastatic gastric cancer.[1–3] Trastuzumab acts by blocking HER2, a member of the epithelial growth factor receptor family involved in regulating cell growth, survival, differentiation and migration, thus counteracting the oncogenic effect of HER2 overexpression on cancer cells.[4 5]

Table 1 Demographic and baseline clinical characteristics of the study population

Number of patients	37
Age in years, median (range)	61 (39–92)
Gender, n (%)	
Male	9 (24.3)
Female	28 (75.7)
Ethnic origin, n (%)	
Caucasian	37 (100)
Eastern Cooperative Oncology Group performance status, n (%)	
0	24 (64.9)
1	9 (24.3)
2	4 (10.8)
Time from diagnosis in months, median (range)*	26.1 (1–228)
Primary tumour site, n (%)	
Breast	15 (40.5)
Stomach	5 (3.5)
Colon/rectum	4 (10.7)
Ovary	3 (8)
Pancreas	2 (5.4)
Oesophagogastric junction	2 (5.4)
Lung	2 (5.4)
Distal oesophagus	1 (2.7)
Bladder	1 (2.7)
Parotid gland	1 (2.7)
Adenocarcinoma, primary site unknown	1 (2.7)
HER2 expression, n (%)	
Immunohistochemistry†	34 (91.9)
1+	13 (35.1)
2+	6 (16.2)
3+	15 (40.5)
Fluorescence in situ hybridisation (FISH)‡	25 (67.6)
Negative	18 (48,6)
Amplified	7 (18.9)
FcγRIIIa status, n (%)§	36 (97.3)
FF	14 (37.8)
FV	16 (43.2)
VV	6 (16.2)
Prior chemotherapy regimens, n (%)¶	35 (94.6)
1	2 (5.4)
2	0 (0)
≥3	33 (89.2)
Any prior antibody therapy, n (%)**	20 (54.1)
Trastuzumab	12 (32.4)
Bevacizumab	11 (29.7)
Cetuximab	1 (2.7)
Panitumumab	2 (5.4)

*(Date of first dose of study drug – date of initial diagnosis of disease +1)/30.
†IHC was performed locally, at each study centre. Three IHC results missing.
‡FISH (PathVysion Kit II, Abbott Molecular, Wiesbaden, Germany) was performed centrally (Department of Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany). Adequate tumour samples were not available in 12 cases.
¶Two patients did not receive chemotherapy.
§One patient refused consent to FcγRIIIa genotyping.
**Four patients received more than one antibody.
F, phenylalanine; HER2, human epidermal growth factor receptor 2; V, valine.

Additionally, antibody-dependent cellular cytotoxicity (ADCC) plays a key role in the mechanism of action of trastuzumab. ADCC is dependent on interactions between the constant fragment C (Fc) portion of an antibody and Fc gamma receptors (FcγR) on immune effector cells of innate immunity, particularly natural killer (NK) cells expressing FcγRIIIa[6 7] and is affected by FcγRIIIa 158 valine (V)/phenylalanine (F) genomic polymorphism.[8 9] Clinical benefit was observed in 82% of patients with breast cancer receiving trastuzumab plus taxane for metastatic disease that had the favourable genotype FcγRIIIa V/V compared with 40% in patients with the V/F or F/F genotype.[10]

IgG antibody binding to the FcγRIIIa on NK cells is affected by the presence of fucose-linked oligosaccharides in the Fc domain. Afucosylated IgG mAbs show improved binding to all FcγRIIIa genotypes and enhanced ADCC compared with their fucosylated counterpart.[11 12] Preclinical studies showed enhanced ADCC and better antitumour activity in in vivo models of HER2-amplified breast cancer with afucosylated trastuzumab compared with conventional trastuzumab.[13 14] TrasGEX is a second-generation antibody of trastuzumab produced in the GlycoExpress system using human glycoengineered production cell lines to give it a fully human glycosylation pattern. In contrast to trastuzumab that is produced on Chinese hamster ovary (CHO) cells, the N-glycans of TrasGEX contain low amounts of fucose that results in a higher binding affinity of TrasGEX to FcγRIIIa on NK cells and enhanced ADCC activity while fully retaining its binding properties and down-modulation of HER2 (data on file; Glycotope GmbH, Berlin, Germany). Importantly, TrasGEX mediates ADCC of both high and low HER2 expressing breast cancer cell lines for all donor FcγRIIIa allotypes that is strongly enhanced compared with trastuzumab suggesting that TrasGEX may reach a wider patient population than trastuzumab (online supplementary figure S1).

The present study in patients with advanced carcinomas and progressive disease was undertaken to establish the recommended dose of TrasGEX for phase II trials, as

well as to investigate the safety, tolerability, pharmacokinetics (PK) and preliminary clinical activity of the drug.

Patients and methods

Study population

This multicentre phase I study was conducted in five institutions in Austria, Italy, Switzerland and Germany between September 2011 and November 2013. Thirty-seven patients were enrolled in the study. The eligible population consisted of male or female subjects 18 years or older with locally advanced and/or metastatic HER-2-positive cancer and progressive disease for whom no antitumour therapy of proven benefit was available at study enrolment. HER2 expression (table 1) was assessed by immunohistochemistry (IHC score at least 1+) and by fluorescence in situ hybridisation (FISH). Measurable or evaluable disease as defined by Response Evaluation Criteria in Solid Tumours (RECIST 1.1)[15] was desirable, but mandatory only for subjects enrolled in the expansion cohort. Measurable disease at baseline was documented and further evaluated by CT or MRI every 8±1 week. Further eligibility criteria included completion of anticancer chemotherapy, radiotherapy, immunotherapy or investigational agents at least 4 weeks prior to start of study treatment; recovery from all prior therapy toxicities to National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE) grade I or lower[16]; life expectancy of ≥3 months; Eastern Cooperative Oncology Group performance status 0 or 1; and adequate haematology, blood chemistry and liver and renal function. Exclusion criteria included newly diagnosed brain metastases, metastases that had been documented to be stable for less than 3 months, or metastases for which systemic corticosteroids was required; anticancer chemotherapy, radiotherapy, immunotherapy or investigational agents administered within 4 weeks of the first dose of TrasGEX; exposure to trastuzumab within 7 weeks of the first dose of TrasGEX; major surgery within 4 weeks of the first dose of TrasGEX; concurrent steroid therapy; history of active autoimmune disorders requiring systemic immunosuppressive therapy; history of allergic reactions attributed to compounds of similar chemical or biologic composition as TrasGEX; history of myocardial infarction within 12 months of the administration of the first dose of TrasGEX; history of congestive heart failure class II to IV (New York Heart Association classification) within 12 months of the administration of the first dose of TrasGEX; left ventricular ejection fraction <50%; and active infection or serious intercurrent disease.

Study design and dosing

Patients were sequentially enrolled in a three-plus-three dose escalation design to receive TrasGEX intravenously in 90 min, every 3 weeks, in doses of 12, 60, 120, 240, 480 and 720 mg (six patients). Following a preliminary PK evaluation that showed similar results to those of trastuzumab, dose escalation was stopped at 720 mg TrasGEX, and 16 additional patients were enrolled in a 720 mg expansion cohort. The duration of infusion was extended to 4 hours, and premedication was introduced in the course of the study to minimise IRR. Treatment was continued until clinical or radiological disease progression, occurrence of intolerable toxicity or withdrawal of consent.

Safety and dose-limiting toxicity (DLT)

Toxicities were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE version 4.0).[16] A DLT was defined as any haematological or non-haematological toxicity ≥grade III (except for grade III IRR), occurring during the first cycle of TrasGEX and considered to be related to the study drug. Three evaluable patients were entered at each dose level. If a DLT occurred, the cohort was to be expanded to six patients before escalating to the next dose level; an MTD was reached if more than two patients experienced a DLT at any given dose in the first cycle.

PK analysis

Blood for PK analysis was collected before and at the end of the first infusion, 1, 4 and 24 hours after the end of the first infusion and on days 8 and 15. Thereafter, samples were collected before the second to fifth infusion. TrasGEX serum levels (Glycotope GmbH, Berlin, Germany) were measured with an electrochemiluminescence assay using Meso Scale Discovery (MSD) technology (Rockville, Maryland, USA) and based on the specific recognition of HER2 by TrasGEX. Briefly, Multi-Array 96-Well plates (MSD) were coated with human extracellular domain of HER2 (Sino Biological, Beijing, China). After washing and blocking steps, calibration, control and study samples diluted in phosphate-buffered saline/1% bovine serum albumin fraction V/0.05% Tween/0.1% serum was added. After incubation and washing TrasGEX bound to the plate was detected using a sulfo-Tag labelled goat antihuman antibody. After washing, wells were filled with read buffer, and chemiluminescence was measured at a SectorImager SI2400 (MSD). Thereby, the bound sulfo-Tag emits light on electrochemical stimulation initiated at the electrode surface of the Multi-Array microplate, and the measured light signal is proportional to the concentration of TrasGEX in the sample. The lower limit of quantification of the assay was 0.135 µg/mL. PK parameters were derived from the individual patient serum concentration time profiles after first infusion using non-compartmental methods (FUNCALC 3, Prolytic GmbH, Frankfurt, Germany). The maximum (Cmax) and minimum (Cmin) serum concentration after administration were directly taken from analytical data. Dose linearity and proportionality of the PK parameters (present for R^2 >0.8), Cmax, Cmin, area under the plasma concentration time curve from time 0 to infinity ($AUC_{0-\infty}$) and area under the plasma concentration time curve from time 0 to the last measured concentration ($AUC_{0-tlast}$) were investigated over the dose range, based on the individual values by linear regression

analysis. A trough level ≥20 µg/mL of the drug was set as target for the study, based on published literature that defines 10–20 µg/mL as the minimally effective concentration for anti-proliferative effects and ADCC.[17–19] Accumulation of TrasGEX was assessed by dividing the trough concentrations after the second and subsequent doses by the trough concentration after the first dose.

Immunogenicity

Samples were screened for antidrug antibodies (ADAs) before the first to the fifth infusion and 28 days after the last infusion of TrasGEX (Glycotope GmbH, Berlin, Germany) with an electrochemiluminescence assay in the bridging format using MSD technology as described above. Briefly, samples (positive controls, negative controls and study samples) were diluted 1:50 in dilution buffer and treated with 300 mM acetic acid to dissociate complexes of ADA and TrasGEX. Then the samples were incubated with biotinylated TrasGEX-Fab fragment and sulfo-Tag labelled TrasGEX-Fab fragment in solution (so called master mix). Subsequently the solution was neutralised with 1.5 M TRIS base and transferred to blocked 96-well plates. After incubation and washing wells are filled with read buffer and chemiluminescence is measured; the measured light signal is proportional to the concentration of ADA against TrasGEX in the sample. For the confirmatory assay, the samples were tested additionally in the presence of TrasGEX added to the master mix, and procedures were carried out as described above. Finally, ADA titration was to be performed on samples confirmed as positive.

Cytokines interleukin (IL)-6, IL-8, interferon-gamma and tumour necrosis factor-alpha serum levels were analysed during the first 24 hours of the first infusion as specified for PK analysis, and before and at the end of the second to fifth infusion.

Tumour assessment

Tumour response in patients with measurable disease was evaluated according to RECIST 1.1 guidelines.[15] Baseline imaging was assessed within 4 weeks before the first TrasGEX dose, and then every 8 weeks until withdrawal from study. Imaging included CT and/or MRI of target lesions. Disease stabilisation (SD) needed imaging confirmation after 8 weeks, complete response (CR) and partial remission (PR) at least 4 weeks after the criteria for response were first met and using the same imaging tests. The objective response rate (ORR) was calculated as the proportion of patients with CR or PR, and the disease control rate (DCR) as the proportion of patients with CR, PR and SD ≥24 weeks, as well as all SD.

Statistical analysis

Descriptive statistics were used on the intent-to-treat population, which included all patients who received at least 1 dose of the study drug, to summarise patient demographics and baseline characteristics, treatment administration, safety parameters and PK variables (SAS V.9.3). The distribution of IRR was analysed in contingency tables according to premedication. Cytokine levels in relation to IRR were analysed using the Mann-Whitney U test. Antitumour responses were evaluated in the efficacy evaluable population (EEP), which included all patients who received at least two cycles of TrasGEX and had at least one disease assessment following the initiation of treatment.

Table 2 Extent of exposure to TrasGEX, number of infusions administered in 3-weekly cycles and number (%) of patients with infusion related reactions (IRR)

Dose (mg)	12	60	120	240	480	720*	Total
No. of patients	3	3	3	3	3	22	37
Extent of exposure in days, median (range)†	1 (1–170)	86 (20–150)	42 (1–150)	71 (43–148)	66 (43–561)	44.5 (20–234)	45 (1–561)
Number of infusions administered, median (range)†	1 (1–9)	5 (2–8)	3 (1–3)	4 (3–8)	4 (3–27)	3 (2–12)	3 (1–27)
IRR at first infusion, no. patients (%)‡§¶							
Grade I	1 (33)	2 (67)	0	0	0	5 (23)	8 (22)
Grade II	2 (67)	1 (33)	1 (33)	1 (33)	1 (33)	3 (14)	9 (24)
Grade III	0	0	1 (33)	0	0	0	1 (3)
Total	3 (100)	3 (100)	2 (67)	1 (33)	1 (33)	8 (37)	18 (49)

*Includes six patients in the 720 mg cohort and 16 patients in the 720 mg expansion cohort.
†Calculated until study closure (November 2013). Two patients continued treatment under named patient: one received two additional infusions before progressing; the other is in CR and received the drug until February 2017.
‡Percentage is calculated using the number of patients in the column heading as denominator.
§Most frequent symptoms of IRR included fever (10 patients, 56%), chills (9 patients, 50%), feeling cold (3 patients, 17%), nausea (4 patients, 22%), vomiting (3 patients, 17%) and hypertension (2 patients, 11%). The same patient may have contributed to two or more symptoms.
¶Only 13 IRRs in seven patients (five of them in one patient) were observed in the subsequent infusions: three in grade I, nine in grade II and one in grade III (the drug was withdrawn).

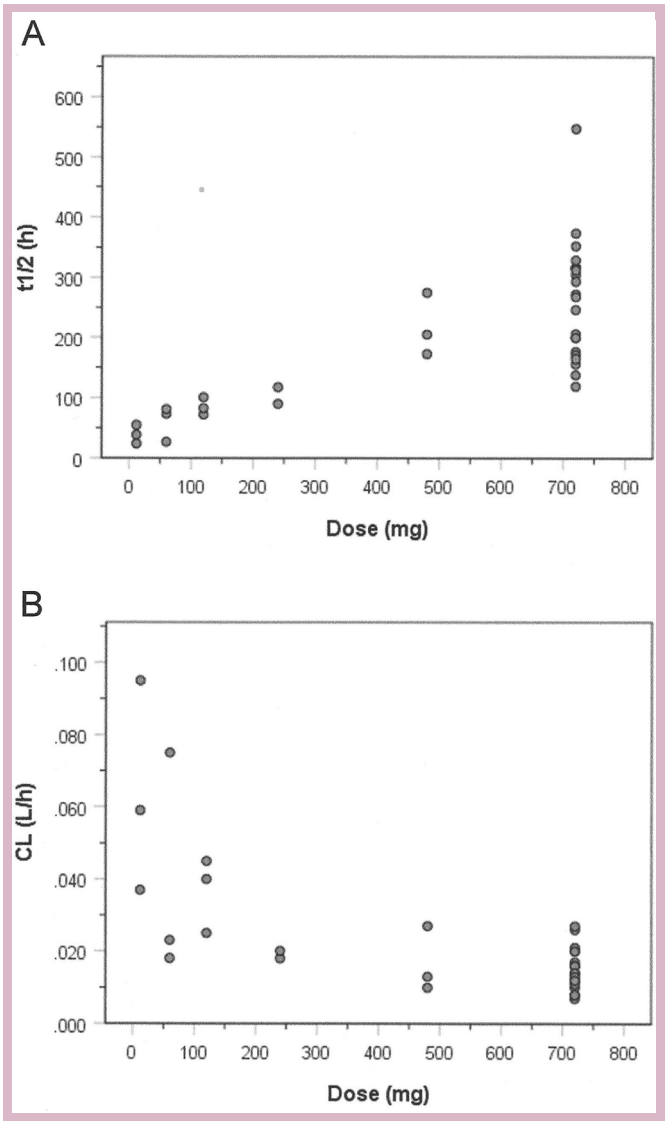

Figure 1 Scatterplot of (A) terminal half-life ($t_{1/2}$) and (B) clearance (CL) by dose cohort calculated for the first TrasGEX infusion.

RESULTS

Patient characteristics

Clinical characteristics of the 37 patients enrolled in the study (NCT01409343) are contained in table 1. On entering the study, all patients had progressive metastatic disease and had exhausted available standard treatment procedures. Twelve patients (32.4%) had previously received trastuzumab.

Drug exposure, safety and tolerability

Reason for study termination was disease progression (30 patients), adverse events (five patients) and withdrawal of informed consent (one patient); one patient is still in CR.

Drug exposure and number of infusions administered are listed in table 2. Of the 37 patients enrolled in this study, 35 (94.6%) experienced at least one treatment-emergent adverse event (TEAE). The majority of drug-related TEAE were IRRs occurring in 19 of 37 (51%) patients at first TrasGEX infusion in all but one case (table 2). Most IRRs were mild-to-moderate and resolved quickly after a pause of the infusion and, in some cases, symptomatic medication. Initially, no premedication was given, but paracetamol and corticosteroids were introduced from the 60 and 240 mg dose onwards, respectively, to minimise IRRs. Only 9 of the 27 patients (33%) that received the final premedication had an IRR at the first infusion, compared with 9 out of 10 patients (90%) that did not receive it (Fisher's exact test, two tailed p=0.003). Premedication was restricted to the first infusion or the one following an IRR in the previous infusion to limit the negative effect of steroids on ADCC.[20] Infusion duration was extended from 90 min to 4 hours after an IRR grade III occurred in a patient receiving 120 mg TrasGEX; the drug was withdrawn. The total of drug withdrawals due to IRRs was 2 of 37 (5.4%) patients. Additionally, three patients experienced mild-to-moderate drug-related TEAEs: one case of interstitial lung disease (grade I) and two cases of elevated troponin T (grades I and II, respectively) that were not associated with cardiac symptoms or changes in ECG. No DLTs were observed in the course of the study, and the MTD was not reached. The majority of TEAEs other than IRRs were mild to moderate, consisted mostly of gastrointestinal or general disorders (abdominal pain, nausea, fever and cough, each reported in seven (18.9%) patients) and were unrelated to the study drug. Fifteen (41%) patients experienced a serious TEAE, only two of them drug related (grade III IRR). No significant changes in ECG or left ventricle ejection fraction were observed during the study.

Pharmacokinetics

PK parameters are contained in online supplementary table S1. Mean $t_{1/2}$ for doses 12–720 mg TrasGEX ranged from 39 to 310 hours, with a mean (SD) of 263 (99) hours for the 720 mg dose. Higher TrasGEX doses led to higher $t_{1/2}$ with the clearance (CL) decreasing with increasing doses (figure 1). The dose dependency of $t_{1/2}$ and CL indicate non-linear PK properties of TrasGEX in the dose range studied. Mean volume of distribution (Vz) ranged from 0.03 L/kg to 0.09 L/kg, approximating the human serum volume of 3–5 L. A dose linear increase for Cmax, $AUC_{0-tlast}$ and $AUC_{0-\infty}$ is present over the whole TrasGEX dose range. However, with the three parameters normalised by dose, Cmax is constant over the whole dose range, but $AUC_{0-tlast}$ and $AUC_{0-\infty}$ first increase with dose and become constant at doses ≥480 mg (online supplementary figure S2), suggesting that above the 480 mg dose, the PK properties of TrasGEX may become linear. TrasGEX mean (SD) trough level (Cmin) by the end of the first cycle was 33 (15) µg/mL for dose 720 mg. A trough level of ≥20 µg/mL was reached in 17 out of 22 patients (77.3%) already after one infusion of 720 mg TrasGEX. A measurable accumulation of TrasGEX was found over the investigated dose range of 60–720 mg. The accumulation ratios for Cmin over 3–5 infusions could be calculated for 11 patients and were in the range of 1.18–2.48. The low number of available data does not allow a reliable conclusion whether steady state was attained .

Table 3 Summary of clinical characteristics of patients with a clinical benefit

Tumour response	Primary tumour site	Disease status at start of treatment	Dose TrasGEX (mg)	Duration of response (weeks)*	HER2 expression		FcγRIIIa	Prior treatment with trastuzumab
					IHC	FISH		
CR	Parotid	Parapharyngeal LN metastasis	720	139, ongoing	3+	Amplification	FF	No
PR	Sigma/rectum	Liver, adrenal cortex, retroperitoneal and lung metastases	480	81	3+	Amplification	FV	No
PR	Breast	Chest wall and mediastinal metastases	240	16	3+	NA	FF	Yes
SD	Breast	Lung and bone metastases	12	26	3+	Amplification	FF	Yes
SD	Breast	Liver and bone metastases	60	18	1+	Negative	FF	No
SD	Breast	Lung and bone metastases	60	24	2+	Negative	FF	No
SD	Bladder	Preportal, paraortic and iliac LN metastases	240	12	1+	Negative	FV	No
SD	Breast	Lung metastases	480	7	3+	NA	FV	Yes
SD	Breast	Liver and bone metastases	720	17	3+	Amplification	FV	Yes
SD	Breast	Liver and bone metastases	720	25	3+	NA	VV	Yes
SD	Lung	Lung tumour and paratracheal LN metastasis	720	18	2+	Negative	FF	No
SD	Ovary	Multiple mediastinal, abdominal and pelvic LN metastases and multiple peritoneal nodules	720	17	1+	Negative	VV	No
SD	Stomach	Multiple liver metastases	720	14	2+	Amplification	FV	No
SD	Stomach	Ileocecal mass, bladder, rectal and pelvic peritoneal metastases	720	14	1+	NA	VV	No
SD	Lung	Primary tumour and multiple lung metastases	720	16	3+	NA	FF	No

*Duration of CR and PR is defined as the time at which criteria for CR or PR are first met until the first date that progressive disease is objectively documented. Duration of SD is defined as the time from the start of treatment until the criteria for progression are met.
CR, complete remission; FISH, fluorescence in situ hybridisation; IHC, immunohistochemistry; LN, lymph node; NA, tumour sample not available; PR, partial remission; SD, stable disease.

Immunogenicity

Cytokine levels were significantly elevated in relation to basal levels after the first infusion of TrasGEX, approximating basal levels after 24 hours (online supplementary figure S3) and ranked significantly higher in patients that experienced an IRR than in those with no IRR (online supplementary figure S4). No significant differences in cytokine levels were observed in the follow-up infusions. No ADAs were detected at the tested time points.

Clinical antitumour activity

All patients had progressive disease at study entry. Tumour response was evaluated in 30 patients (EEP);

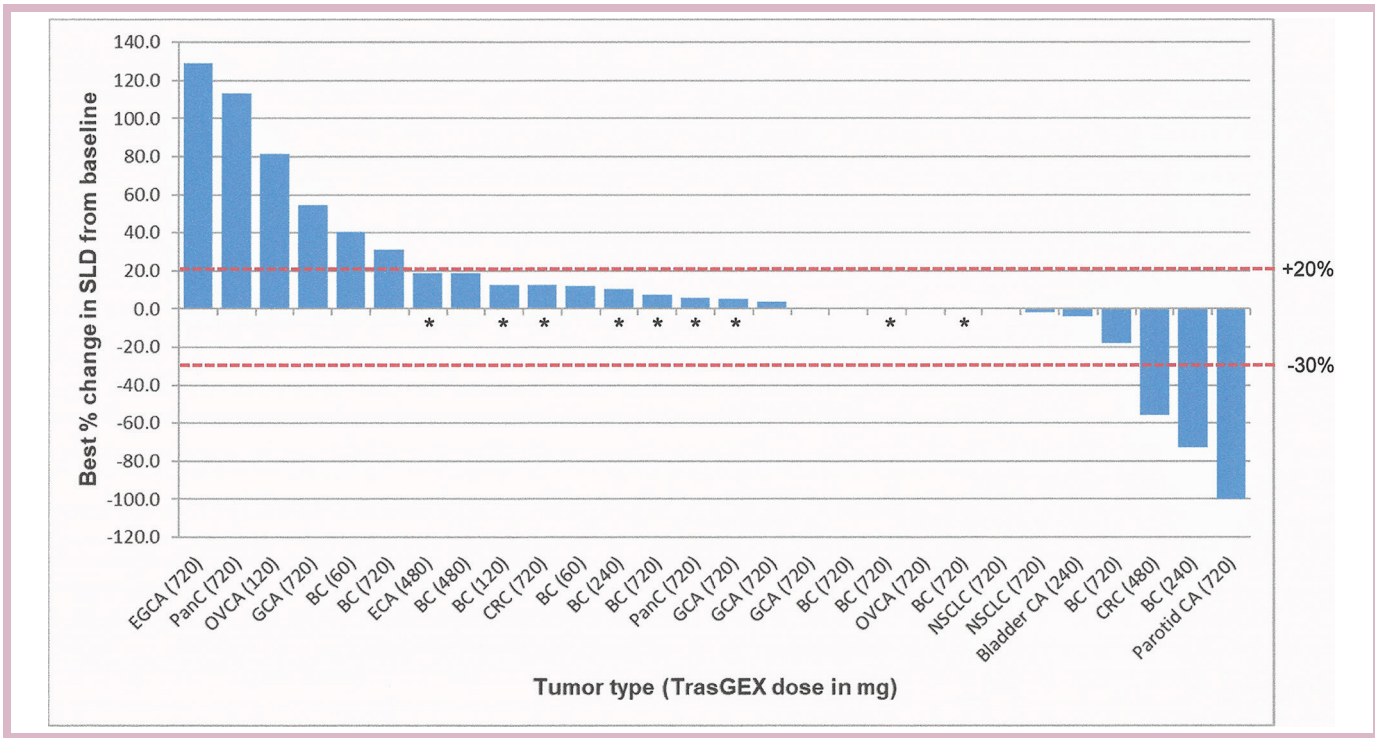

Figure 2 Waterfall plots of the best per cent change from baseline in sum of longest diameters (SLD) for target lesions. Baseline is defined as the last non-missing value before the first dose of TrasGEX. Twenty-eight patients in the total population (n= 37) had valid baseline and postbaseline values. Tumour assessment was not performed in nine patients because of absence of target lesions (n= 2), or early withdrawal due to clinical deterioration (n= 3), adverse event (n=3) or withdrawal of informed consent (n= 1). The red dotted lines indicate the cut off for partial response (–30%) and progressive disease (+20%). Bars marked with an asterisk denote nine patients with stable target lesions but progressive disease because of progression of non-target lesions or appearance of new lesions. BC, breast cancer; bladder CA, urinary bladder cancer; CRC, colorectal cancer; EGCA, esophagogastric junction cancer; GCA, gastric cancer; NSCLC, non-small cell lung cancer; OVCA, ovarian cancer; PanC, pancreatic cancer; parotid CA, parotid gland cancer.

seven patients were non-evaluable because of premature withdrawal (six patients) or withdrawal of informed consent (one patient). Clinical benefit included one CR, two PRs and 12 SDs observed in 15/30 patients and distributed across all doses (table 3 and figure 2). The DCR was 50% (95% CI 31.3% to 68.7%) considering all SD, and 20% (95% CI 5.7% to 34.3%) considering only the three SD ≥24 weeks; the ORR was 10% (95% CI 2.1% to 26.5%).

A patient with a HER2 3+ salivary duct carcinoma and FcγRIIIa allotype FF entered the study 16 months after primary surgery and adjuvant radiotherapy presenting a parapharyngeal lymph node metastasis 27 mm in longest diameter. The lesion decreased in size and finally disappeared after 178 days in treatment with 720 mg TrasGEX; the patient received TrasGEX until February 2017 and continues in CR to the present, 53 months from start of treatment. A patient with HER2 3+ colorectal cancer and allotype FV, diagnosed 9 years before entering the study and repeatedly treated with surgery, chemotherapy and mAb therapy, achieved a PR (56% tumour reduction) after 56 days treatment with 480 mg TrasGEX that lasted 81 weeks before progressing.[21] A second PR was observed in a patient with HER2 3+ breast cancer and an FF allotype diagnosed 5.5 years before study entry and treated with various chemotherapy regimens, bevacizumab and trastuzumab. The patient had received trastuzumab as maintenance therapy after neoadjuvant chemotherapy and radical mastectomy and again with second-line and with fourth-line chemotherapy, followed by two additional lines of chemotherapy before entering the study. The patient had a 73% tumour reduction after 62 days in treatment with 240 mg TrasGEX that lasted 113 days. Twelve patients (40%) had SD (median 17.2 weeks, range 7–26 weeks), three of them had SD ≥24 weeks (median 25 weeks, range 24–26 weeks). Five out of seven patients with breast cancer (71%), one PR and four SD, had been previously exposed to trastuzumab (table 3). One patient with an SD of 26 weeks received trastuzumab with second-line and third-line chemotherapy prior to entering the study after a failed fourth chemotherapy course. Another patient with an SD of 25 weeks received trastuzumab with second-line chemotherapy and again shortly with third-line chemotherapy, followed by fourth-line chemotherapy before entering the study. The other two patients received numerous lines of chemotherapy that included trastuzumab in three (SD 7 weeks) and four (SD 17 weeks) of them. The mean duration of SD did not differ significantly in relation to HER2 expression levels or FcγRIIIa allotype. Similarly, in the efficacy, population percentage

of patients with clinical benefit did not differ in relation to FcγRIIIa allotype (FF: 6 out of 11, 55%; FV: 6 out of 13, 46%; VV: 3 out of 5, 60%; Pearson χ^2, p=0.846) or HER2 expression (HER2 1+: 4 out of 8, 50%; HER2 2+: 3 out of 5, 60%; HER2 3+: 8 out of 15, 53%; Pearson χ^2, p=0.519).

DISCUSSION

TrasGEX was safe and well tolerated after repeated administration. The MTD was not reached after a maximum dose of 720 mg, approximately 50% higher than the standard 3 weekly 6 mg/kg maintenance dose of trastuzumab. Drug-related AE were for the most part mild to moderate IRRs confined to the first infusion, and no ADA responses were observed. As described for trastuzumab,[22–24] TrasGEX exhibited non-linear PK properties up to a dose level of 480 mg. Above that dose level PK properties seem to become linear suggesting a saturation of the antigen sink. The target trough level of 20 µg/mL was amply reached at the highest dose tested. Intervals of 2 or 3 weeks appear feasible for further studies, the choice depending on the clinical indication.

Engagement of Fcγ receptors on effector cells is a dominant component of the in vivo activity of antibodies against tumours and results in potent ADCC activity at lower antigen density.[25] TrasGEX fully retains the antitumour activity mediated by trastuzumab but is glycoengineered for enhanced ADCC. In a pilot study of neoadjuvant trastuzumab in breast cancer, patients with pathological complete or partial remission of the primary tumour had a higher tumour infiltration of leukocytes and a higher capability to mediate in vitro ADCC.[26] An association between clinical response to trastuzumab and FcγRIIIa-158V/V phenotype has been noted in some studies but not in others.[10 27 28] We found no differences in distribution of clinical efficacy among FcγRIIIa allotypes; more so, two of the three best responders had an FcγRIIIa F/F allotype, the most unresponsive to ADCC (table 3). The clinical benefit of trastuzumab is associated with high HER2 tumour expression, and clinical trials include patients with HER2 2+ or 3+ tumours, with best results observed for HER2 3+ tumours.[29–31] Even though preclinical data support a role of ADCC potentially killing also tumour cells with lower HER2 expression, in this study, we observed clinical responses only in HER2 3+ patients, so additional data are required to identify the patient population more likely to benefit from a glycoengineered antibody such as TrasGEX.

In conclusion, TrasGEX was safe and well tolerated, and antitumour activity was observed in 50% of the evaluable patients irrespective of the FcγRIIIa allotype. Infusions at an interval of 2–3 weeks should achieve clinically relevant trough levels for future studies.

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