Clinical Cancer Research

First-in-Human Phase I Study of Lumretuzumab, a Glycoengineered Humanized Anti-HER3 Monoclonal Antibody, in Patients with Metastatic or Advanced HER3-Positive Solid Tumors

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Abstract

Purpose: A first-in-human phase I study was conducted to characterize safety, efficacy, and pharmacokinetic (PK) and pharmacodynamic (PD) properties of lumretuzumab, a humanized and glycoengineered anti-HER3 monoclonal antibody, in patients with advanced cancer.

Experimental Design: Twenty-five patients with histologically confirmed HER3-expressing tumors received lumretuzumab (100, 200, 400, 800, 1,600, and 2,000 mg) every two weeks (q2w) in 3+3 dose-escalation phase. In addition, 22 patients were enrolled into an extension cohort at 2,000 mg q2w.

Results: There were no dose-limiting toxicities. Common adverse events (any grade) included diarrhea (22 patients, 46.8%), fatigue (21 patients, 44.7%), decreased appetite (15 patients, 31.9%), infusion-related reactions (13 patients, 27.7%), and constipation (10 patients, 21.3%). The peak concentration (C_{max}) and area under the concentration–time curve up to the last measurable concentration (AUC_{last}) of lumretuzumab increased more than dose proportionally from 100 mg up to 400 mg. Linear PK was observed with doses $\geq$400 mg q2w indicating target-mediated drug disposition saturation. Downregulation of HER3 membranous protein was observed in on-treatment tumor biopsies from 200 mg, and was maximal at and above 400 mg. An *ex vivo* assay demonstrated increased activation potential of peripheral NK lymphocytes with lumretuzumab compared with a non-glycoengineered anti-HER3 antibody. Ten patients (21.3%) had stable disease and remained on study at a median of 111 days (range, 80–225 days).

Conclusions: Lumretuzumab was well tolerated and showed evidence of clinical activity. Linear serum PK properties and plateauing of PD effects in serial tumor biopsies indicate optimal biologically active doses of lumretuzumab from 400 mg onwards.
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Introduction

Members of the human epidermal growth factor receptor (HER) family play a critical role in tumor growth, proliferation, and progression in numerous epithelial malignancies.

HER3 is a key dimerization partner of HER family members that activates several signal transduction pathways, particularly the PI3K–Akt pathway (1). Recent studies suggest that HER3 pathway activation is important in the development of resistance to EGFR- and HER2-targeted treatments (2–6). HER3 expression has been described as an adverse prognostic factor in many tumor types including breast, lung, ovarian, and colon cancers (7–11). In addition, autocrine loops involving the ligand heregulin and leading to HER3 activation have been described in head and neck, lung and ovarian cancer (8, 12–16).

Lumretuzumab (RG7116) is a humanized, glycoengineered immunoglobulin G1(IgG1) antibody, which selectively binds with high affinity to the extracellular domain of HER3. Prevention of heregulin binding to HER3 by lumretuzumab resulted in

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Translational Relevance

HER3 is implicated in tumor growth and maintenance in many solid tumor types and has been described as a potential escape mechanism of EGFR- and HER2-targeted therapies. Lumretuzumab is a glycoengineered monoclonal antibody directed against the extracellular domain of HER3, displacing its ligand and inhibiting heterodimerization and downstream signaling. In this first-in-human study in patients with HER3-positive carcinomas, pharmacokinetic (PK) and pharmacodynamic information from preclinical experiments was translated into the clinical setting to guide dose-finding and demonstrate target engagement. Downregulation of HER3 receptor in on-treatment tumor biopsies and linear serum PK indicated optimal biologic activity and dosing of lumretuzumab at doses at and above 400 mg. Lumretuzumab will be further developed in combination with other HER family receptor inhibitors and evaluated in molecularly targeted patient subsets.

almost complete inhibition of HER3 heterodimerization and phosphorylation, as well as inhibition of downstream Akt phosphorylation at concentrations of 1 nmol/L. Lumretuzumab inhibited tumor growth in cell line–based mouse models up to complete remission compared with controls. Furthermore, enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) potency of lumretuzumab compared with the non-glycoengineered parental antibody was demonstrated both *in vitro* and in orthotopic tumor xenografts (17).

This study evaluated the safety, efficacy, and pharmacokinetics (PK) of lumretuzumab. Potential biomarkers related to lumretuzumab were evaluated to determine the optimal biologic dose.

Materials and Methods

Study design

Study NCT01482377 was a phase Ia, open-label, non-randomized, dose-escalating, multicenter study investigating the safety, PK, pharmacodynamics (PD), and clinical activity of single-agent lumretuzumab in patients with metastatic or advanced HER3-positive carcinomas. The study was conducted in two parts: a dose-escalation phase following a standard "3+3" study design and an extension phase to further evaluate the highest dose level tested.

The average threshold concentration (C_{ave}) required to achieve 100% tumor growth inhibition in xenograft models was estimated to be 6.6 μg/mL (in-house data on file). Allometric scaling predicted the systemic exposures and the PK profile of lumretuzumab in humans based on monkey data. The simulated human systemic exposure showed that the estimated C_{ave} was expected to be reached during the first cycle of 100 mg i.v. administered q2w (in-house data on file), which was selected as the starting dose of the study.

Patients continued treatment until disease progression, unacceptable toxicity, or consent withdrawal.

Ethics

Local ethics committee approval was obtained and all patients provided written informed consent. The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki in six centers in Spain, The Netherlands, and Denmark.

Patients

Patients had a histologically confirmed diagnosis of an advanced or metastatic HER3-expressing carcinoma that was refractory to standard treatment or for which no standard therapy existed. Eligible patients were ≥18 years of age, had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2 and had adequate hematology, blood chemistry, and renal and liver function. Patients eligible for enrollment underwent a fresh (pretreatment) tumor biopsy that was used to assess the level of HER3 protein expression by immunohistochemistry (IHC) and central pathology review. Discernible HER3 membrane positivity in any neoplastic cell was considered diagnostically positive for HER3 protein expression.

Study drug administration

Patients received premedication 30 minutes before the start of the first infusion consisting of paracetamol [500–1,000 mg per os (p.o.)] and diphenhydramine (25–50 mg p.o. or i.v., or an alternative anti-histamine). Corticosteroids were allowed in case of ≥ grade 2 infusion-related reactions (IRRs). Lumretuzumab was administered as an i.v. infusion q2w starting at 50 mL/h, and escalating by 50 mL/h in 30-minute intervals to a maximum rate of 200 mL/h if well tolerated.

Tumor response and safety

Assessments of the metabolic response rate were based on (18F)-fluorodeoxyglucose-positron emission tomography (FDG-PET) and were carried out at baseline, after one and four cycles, respectively, in patients once PD-active doses had been achieved. FDG-PET acquisition procedures were standardized across the sites and images were sent for an independent central review. Metabolic response assessment was based on European Organization for Research and Treatment of Cancer (EORTC) criteria (18).

Tumor response assessment using RECIST 1.1 (19) was conducted at screening and every 8 weeks thereafter.

Safety assessments included physical (ECOG performance status, vital signs) and laboratory examinations and electrocardiogram (ECG). Adverse events (AEs) were defined according to the Common Terminology Criteria for AEs, version 4.0 (20). Lumretuzumab-specific anti-drug antibody (ADA) responses were assessed before each infusion and at the end of the study using a state-of-the-art, bridging-format immunoassay.

Definition of dose-limiting toxicity

A dose-limiting toxicity (DLT) was defined as an AE that occurred during the first two cycles of treatment (i.e., 28 days) with lumretuzumab that was considered to be study drug related and was either: grade 4 neutropenia [i.e., absolute neutrophil count (ANC) $< 0.5 \times 10^9$ cells/L for minimal duration of 7 days]; grade 3 and 4 febrile neutropenia; grade 4 thrombocytopenia; grade 3 thrombocytopenia associated with bleeding episodes; or grade ≥ 3 non-hematologic toxicity. IRRs were not considered DLTs.

Pharmacokinetic assessments

PK evaluation was conducted for all patients on day 1 of cycle 1 [blood samples taken before the infusion, at end of infusion (EOI), and at 2, 5, 24, 48, 100, 168, and 264 hours after EOI], day 1 of cycle 4 (blood samples taken before the infusion, at EOI, and at 2, 24, 48, 100, and 168 hours after EOI), day 1 of cycle 8 (blood samples taken before the infusion, at EOI, and at 24, 100, and 168 hours after EOI), and on day 1 for all other cycles (blood samples taken before the infusion and at EOI). PK parameters [area under the serum concentration–time curve (AUC), maximum-observed serum concentration (C_{max}) of binding competent lumretuzumab and half-life ($t_{1/2}$)] were computed by non-compartmental analysis (NCA; WinNonlin Version 6.2.0, Pharsight Corp.).

Pharmacodynamic assessments

HER3 expression was assessed by using a prototype IHC assay in fresh biopsies from primary tumor or metastases (assay developed by Ventana Inc., and performed by Source Bioscience Ltd.). Skin biopsies were collected during screening and on day 14 of cycle 1. Collection of tumor biopsies at day 14 of cycle 1 was initiated after demonstrating PD activity in skin, i.e., membrane HER3 downregulation at day 14 of cycle 1 in skin biopsies. Changes in the coexpression of HER receptors (EGFR and HER2), cMet, and HER3 pathway activation [phosphorylated HER3 (pHER3), phosphorylated Akt (pAkt)], tumor proliferation status (Ki67), and immune effector cell infiltration (T cells, natural killer (NK) cells and macrophages] were also assessed in serial biopsies using validated IHC methods. Endpoints were assessed semiquantitatively using an immunoreactive score (IRS) according to: IRS = staining intensity (SI) × percent tumor cells stained (PS), where SI = 1 × "+" score + 2 × "++" score + 3 × "+++" score)/100 and PS = ("+" score + "++" score + "+++" score)/100.

Blood was obtained pre-infusion for all treatment cycles for (i) immunophenotyping of lymphocytes and (ii) ADCC function assessed by an *ex vivo* NK activation assay (21) where isolated peripheral lymphocytes were incubated with target cells expressing HER3 and lumretuzumab or wild-type anti-HER3 monoclonal antibody (mAb). NK cell activation as measured by CD107a expression assay, which measures the number of NK cells that are activated on exposure to antibody and in the presence of T47D target cells expressing HER3, was quantified by flow cytometry (Covance Inc.).

Heregulin mRNA expression was determined as a potential predictive biomarker for lumretuzumab using a quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay developed by Roche Molecular Systems Inc. Heregulin mRNA expression was reported as delta cycle threshold (ΔC_t) where $\Delta C_t = C_t(\text{Reference}) - C_t(\text{Heregulin})$.

HER3, pHER3, and pAkt levels were determined in protein extracts from matched fresh-frozen tumor biopsies taken during screening and on day 14 of cycle 1, using an exploratory Luminex assay methodology (NMI).

Statistical analysis

All patients who received at least one dose of study medication were included in the safety and efficacy populations. Descriptive statistics were used for demographics, safety, and antitumor activity. Summary statistics of the PK and PD parameters were presented using arithmetic mean, median, SD, coefficient of variation (CV%), max and min.

Results

Patients

Patient demographics and baseline characteristics are presented in Table 1. In the dose-escalation phase, 25 patients were enrolled into 6 dose groups, i.e., 100 ($n = 3$), 200 ($n = 3$), 400 ($n = 3$), 800 ($n = 7$), 1,600 ($n = 5$), and 2,000 mg ($n = 4$). In the extension phase, 22 patients received 2,000 mg. Forty-three patients (91.5%) discontinued the study due to progressive disease, 2 patients (4.3%) were withdrawn due to an AE (grades 2 and 3 IRR both in the 800-mg cohort) and 2 patients (4.3%) withdrew consent.

Safety

No DLTs were reported and the MTD was not reached up to the highest dose tested (i.e., 2,000 mg). A total of 379 AEs were reported in 46 of 47 patients (97.9%; Table 2). Most AEs (90.2%) were grades 1 or 2 in intensity. A total of 37 grade 3/4 AEs were reported for 25 patients (53.2%). Only 2 events in 2 patients (4.3%) were of grade 4: Blood bilirubin increased (unrelated), caused by bile duct stenosis of a liver metastasis leading to study drug interruption and considered unresolved at study discontinuation; and platelet count decreased (related) leading to study drug interruption and considered resolved without sequelae. There was 1 patient (2.1%) with a grade 5 event [general physical health deterioration leading to death (unrelated)]. The most frequent AEs included diarrhea [22 patients (46.8%)], fatigue [21 patients (44.7%)], decreased appetite [15 patients (31.9%)], and IRR [13 patients (27.7%)]. Of the 26 SAEs, only one (IRR) was considered study drug related.

Altogether 229 AEs in 42 patients were considered as study drug related. IRR [13 patients (27.7%)], fatigue [12 patients (25.5%)], and diarrhea [11 patients (23.4%)] were the most common related AEs. Seven related grade 3/4 AEs occurred in 6 patients (12.8%): Platelet count decreased (grade 4), neutropenia (grade 3), diarrhea (grade 3), GGT increased (grade 3), hypomagnesemia (grade 3), and hypophosphatemia (grade 3). No dose-dependent

Table 1. Baseline patient demographics and characteristics

Characteristic	Total ($N = 47$)
Sex, *n* (%)	
Male	25 (53.2)
Female	22 (46.8)
Age (y), median (range)	61 (28–76)
ECOG score, *n* (%)	
0	16 (34.0)
1	27 (57.4)
2	4 (8.5)
Prior chemotherapy, *n* (%)	46 (97.9)
Median number (range)	2 (1–6)
Prior EGFR-targeted therapy, *n* (%)	17 (36.2)
Prior surgery, *n* (%)	29 (61.7)
Prior radiotherapy	20 (42.6)
Tumor type, *n* (%)	
Colorectal	22 (46.8)
Head and neck	4 (8.5)
Bladder	4 (8.5)
Non–small cell lung cancer	3 (6.4)
Breast	3 (6.4)
Other[a]	11 (23.4)

[a]Other primary tumors included one patient with ovarian cancer, gastric cancer, uterine cancer, esophageal cancer, small cell lung cancer, pancreatic cancer, renal cell cancer, and two patients with cancer of unknown primary origin and anal cancer, respectively.

Table 2. Summary of AEs of any grade and of grade ≥3 AEs

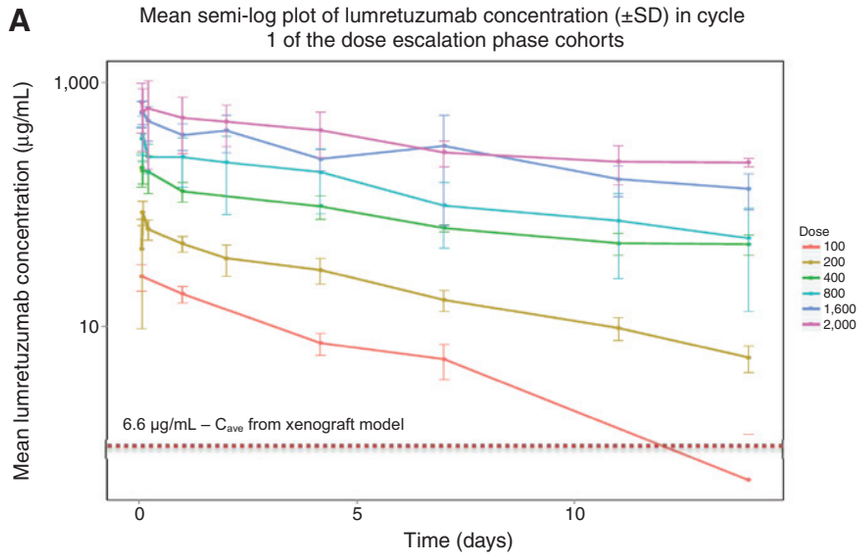
	No. of patients having an AE (%)			
	All AEs		Study drug-related AEs	
AE	All grades	Grade ≥3	All grades	Grade ≥3
Diarrhea	22 (46.8)	3 (6.4)	11 (23.4)	1 (2.1)
Fatigue	21 (44.7)	5 (10.6)	12 (25.5)	0
Decreased appetite	15 (31.9)	1 (2.1)	8 (17.0)	0
IRR	13 (27.7)	1 (2.1)	13 (27.7)	1 (2.1)
Constipation	10 (21.3)	0	9 (19.1)	0
Nausea	9 (19.1)	0	5 (10.6)	0
Pyrexia	9 (19.1)	1 (2.1)	7 (14.9)	0
Stomatitis	7 (14.9)	0	2 (4.3)	0
Vomiting	7 (14.9)	0	5 (10.6)	0
Edema peripheral	7 (14.9)	0	7 (14.9)	0
Dyspnea	6 (12.9)	1 (2.1)	6 (12.8)	0
Dry skin	6 (12.8)	0	6 (12.8)	0
Rash	6 (12.8)	0	5 (10.6)	0
Anemia	6 (12.8)	2 (4.3)	4 (8.5)	0
Abdominal pain	5 (10.6)	0	0	0
Abdominal pain upper	5 (10.6)	0	5 (10.6)	0
Dry mouth	5 (10.6)	0	1 (2.1)	0
Cough	5 (10.6)	0	5 (10.6)	0
Hypomagnesaemia	4 (8.5)	1 (2.1)	4 (8.5)	1 (2.1)
Dizziness	4 (8.5)	0	4 (8.5)	0
Urinary tract infection	4 (8.5)	0	4 (8.5)	0
Back pain	4 (8.5)	1 (2.1)	3 (6.4)	0
Insomnia	4 (8.5)	0	4 (8.5)	0
Dysphagia	3 (6.4)	1 (2.1)	2 (4.3)	0
Asthenia	3 (6.4)	0	3 (6.4)	0
Mucosal inflammation	3 (6.4)	0	3 (6.4)	0
Pain	3 (6.4)	1 (2.1)	3 (6.4)	0
Hypokalemia	3 (6.4)	2 (4.3)	1 (2.1)	0
Dysgeusia	3 (6.4)	0	3 (6.4)	0
Peripheral sensory neuropathy	3 (6.4)	0	1 (2.1)	0
Myalgia	3 (6.4)	0	3 (6.4)	0
Aspartate aminotransferase increased	3 (6.4)	1 (2.1)	2 (4.3)	0
Gamma glutamyl transferase increased	3 (6.4)	2 (4.3)	2 (4.3)	1 (2.1)

NOTE: Only AEs reported by >5% of the patients are shown.

Table 3. Lumretuzumab serum PK parameters at cycle 1 following administration of ascending doses of lumretuzumab

Dose (mg)	Descriptive statistic	C_{max} (μg/mL)	AUC_{last} (day × μg/mL)	V_d (mL)	Total CL (mL/day)	$t_{1/2}$ (day)
100	*N*	3	3	3	3	3
	Mean	26.0	91.3	3,640	1,040	2.40
	CV%	24.9	24.6	49.5	18.3	42.5
200	*N*	3	3	3	3	3
	Mean	86.6	314	3,740	586	4.50
	CV%	22.7	17.2	9.36	20.3	12.0
400	*N*	3	3	3	3	3
	Mean	215	1,090	3,590	222	11.5
	CV%	28.6	16.8	20.6	24.1	28.5
800	*N*	7	7	7	7	7
	Mean	439	1,830	3,780	383	8.90
	CV%	23.4	48.1	45.3	38.7	37.4
1,600	*N*	5	5	4	4	4
	Mean	658	3,590	4,980	329	10.4
	CV%	18.5	31.1	44.8	16.6	34.1
2,000	*N*	4	4	4	4	4
	Mean	699	4,300	4,340	292	12.0
	CV%	42.4	34.6	18.6	51.1	38.7
2,000 Extension	*N*	22	22	22	22	22
	Mean	970	4,530	4,390	264	11.7
	CV%	52.2	17.9	24	29.7	38.3

Abbreviations: AUC_{last}, area under the concentration—time curve up to the last measurable concentration; C_{max}, maximum-observed serum concentration; $t_{1/2}$, half life; total CL, total clearance; V_d, volume of distribution



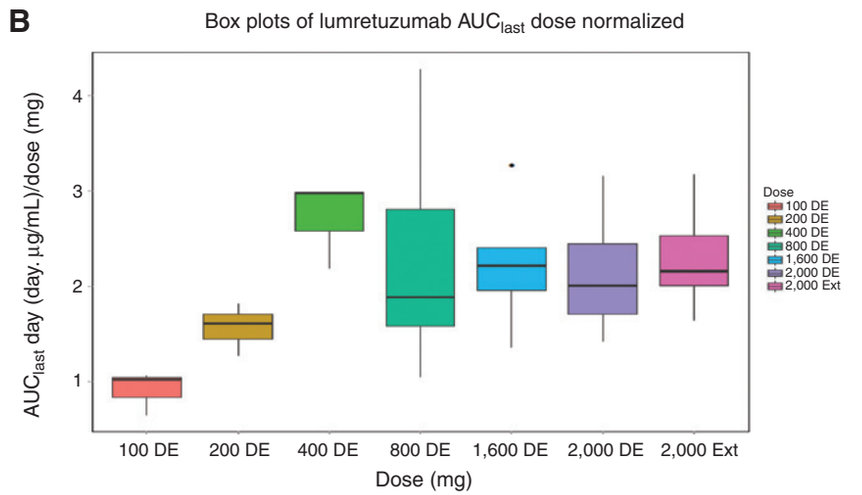


Figure 1.
A, mean (±SD) serum lumretuzumab profiles following single ascending doses from 100 mg up to 2,000 mg for cycle 1. B, AUC_{last}/dose normalized versus dose (100–2,000 mg) box plot. Black lines indicate the median, and boxes indicate the interquartile range.

toxicities were seen with lumretuzumab treatment with the exception of grade ≥3 diarrhea, which was only reported for patients from the extension cohort at 2,000 mg.

In the present study, IRRs seem to be independent of the dose administered. Only 1 patient (2.1%) from the 800-mg cohort experienced a grade 3 IRR. Two patients (4.3%) from the 800-mg cohort were withdrawn from the study due to re-occurrence of IRR after re-challenge with lumretuzumab, but no increase in the intensity of the IRR signs and symptoms was noted. Levels of IgE, tryptase, and coagulation parameters remained within the normal range. Retrospective analyses revealed that these two patients were ADA-positive before the onset of the event, without decreases of complement factors C3 and C4.

Three patients (6.4%) died due to disease progression during the study.

Treatment-induced lumretuzumab-specific ADAs were seen in 4 patients (8.5%) who developed ADAs after the first dose with lumretuzumab. However, there was no impact of ADAs on drug exposure.

Pharmacokinetics

The C_{max} and area under the concentration–time curve up to the last measurable concentration (AUC_{last}) of lumretuzumab increased more than dose proportionally from 100 up to 400 mg, accompanied by a decline in clearance over the same dose range, indicating that elimination of lumretuzumab across this dose range is predominantly target mediated (Table 3, Fig. 1A and B). PK approached linearity at the higher doses studied (400–2,000 mg; Table 3). Mean $t_{1/2}$ estimates increased with increasing doses from 100 up to 400 mg, from ≥400 mg mean $t_{1/2}$ values were within similar ranges (Table 3). Both C_{max} and minimum-observed serum concentration (C_{min}) reached steady state after cycle 3 (Supplementary Fig. S1). Similar PK estimates were observed

following administration of 2,000 mg of lumretuzumab for the extension cohort during cycle 1.

Following administration of 100 mg lumretuzumab serum levels were above the C_{ave} that was associated with maximal efficacy in mouse xenograft models ($C_{ave} = 6.6$ μg/mL; in-house data on file) for more than 1 week (Fig. 1A). At dose levels >200 mg, the mean C_{min} was at least 3.5-fold higher than the C_{ave} of 6.6 μg/mL.

PK analysis of the target saturation (as described in detail in Meneses-Lorente and colleagues; ref. 22) indicated that ≥95% target saturation over the entire dosing interval was achieved from ≥400 mg.

Pharmacodynamics

HER3 membrane expression was used as the primary PD marker of lumretuzumab activity. Baseline expression levels of pHER3 and pAkt were too low in the majority of the clinical samples for PD assessments. HER3 membrane expression was decreased in all evaluable skin biopsy samples collected at day 14 of cycle 1 when compared with expression in paired pretreatment screening samples ($n = 46$, Supplementary Table S1). After observing PD activity in skin biopsy samples of patients dosed with 100 mg of lumretuzumab the subsequent collection of tumor biopsies at day 14 of cycle 1 from the 200-mg cohort onwards was initiated.

Downregulation of HER3 membrane expression was observed for cycle 1 day 14 tumor biopsies compared with expression in paired pretreatment screening samples at all doses of lumretuzumab and in 35 of 38 (92%) of patients (Supplementary Table S1 and Fig. 2).

There was no apparent relationship in the extent of HER3 downregulation with dose or cancer type, nor significant changes in other IHC markers, aside from EGFR membrane expression (ΔIRS EGFR expression for patients with progressive disease 0.1995, for patients with stable disease −0.3543, $P < 0.01$).

Primary archival biopsy samples, while not mandatory, were available for 34 of 47 (72%) of patients in the study. HER3, EGFR, and cMET expressions were significantly higher in the fresh baseline sample compared with the archival sample (Supplementary Table S2).

No significant changes in peripheral blood immune cell populations were observed pre- and post-lumretuzumab infusion. CD56 NKp46 NK populations were marginally increased at cycle 2 compared with baseline, although this response was not sustained at cycle 3 (Supplementary Table S3). In addition, there was a trend toward increased CD16 (FcγRIIIA) mean equivalent soluble fluorescent intensity (MESF) of the $CD16^+$ population within the NK population [$CD3^-$/$CD56^+$]) at cycle 2 and 3 compared with pre-infusion day 1 of cycle 1.

Data from an *ex vivo* NK activation assay demonstrated an increased activation potential of NK lymphocytes by glycoengineered anti-HER3 mAb (lumretuzumab) compared with wild-type anti-HER3 mAb, but there was no change in peripheral NK reactivity or tolerance to *ex vivo* activation after systemic treatment with lumretuzumab (Supplementary Table S4).

Similarly, no significant changes in immune cell infiltration were observed in tumor samples obtained pre-dose compared with day 14 of cycle 1 (Supplementary Table S5).

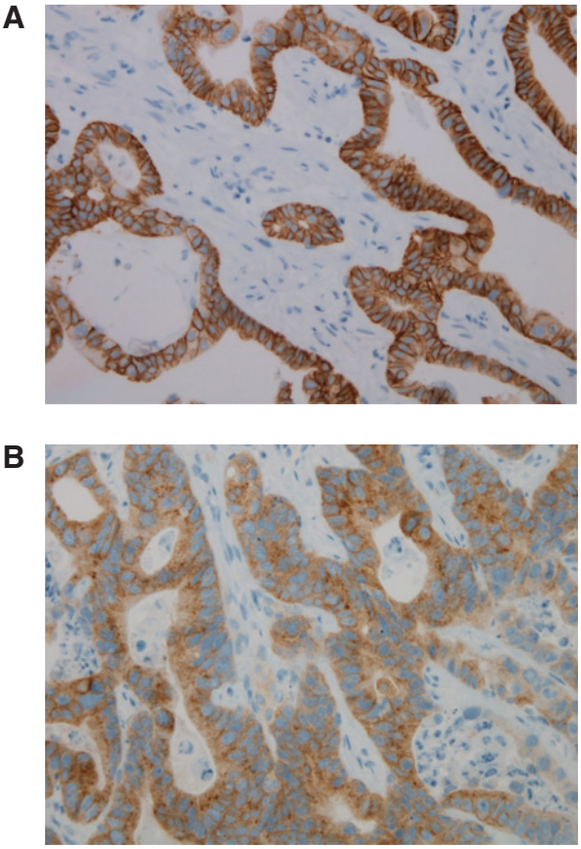


Figure 2.
HER3 expression in a liver lesion biopsy from a patient with colon cancer sampled prior and 14 days after treatment with 400 mg lumretuzumab. (×20 objective, ×10 eye piece). Assay platform Ventana Benchmark XT with primary antibody HER3 MAb clone 7.3.8. The images show intense staining of tumor cell membranes and low cytoplasmic staining in the pre-treatment biopsy (A) with loss of membrane staining and gain of cytoplasmic staining in the posttreatment sample (B). The objective IRS for membrane HER3 was 2.6 pre-dose (A) and 0.0 post-dose (B). Cytoplasmic intensity was 1.20 pre-dose (A) and 2.70 post-dose (B).

Antitumor activity

Of the 47 enrolled patients, 10 (21.3%) had stable disease and 29 (61.7%) had progressive disease (Supplementary Table S6). Five patients (10.6%) had clinical progression and for 3 patients (6.4%) no on-treatment response assessment was available. No complete or partial responses were seen. Patients with stable disease remained on study at a median of 111 days (range, 80–225 days).

A triple-negative breast cancer patient from the 1,600-mg cohort who received four prior chemotherapy regimens for metastatic disease but had not been treated with any HER-targeted therapy showed almost complete absence of vital tumor cells in a biopsy of a skin metastasis at day 14 after start of treatment. This patient showed a dramatic shrinkage of tumor lesions, including supraclavicular adenopathies and subcutaneous metastases in the anterior thorax and pleural effusion which was virtually absent after 8 weeks of treatment (Fig. 3A and B). A mixed lytic/blastic bone lesion was defined as

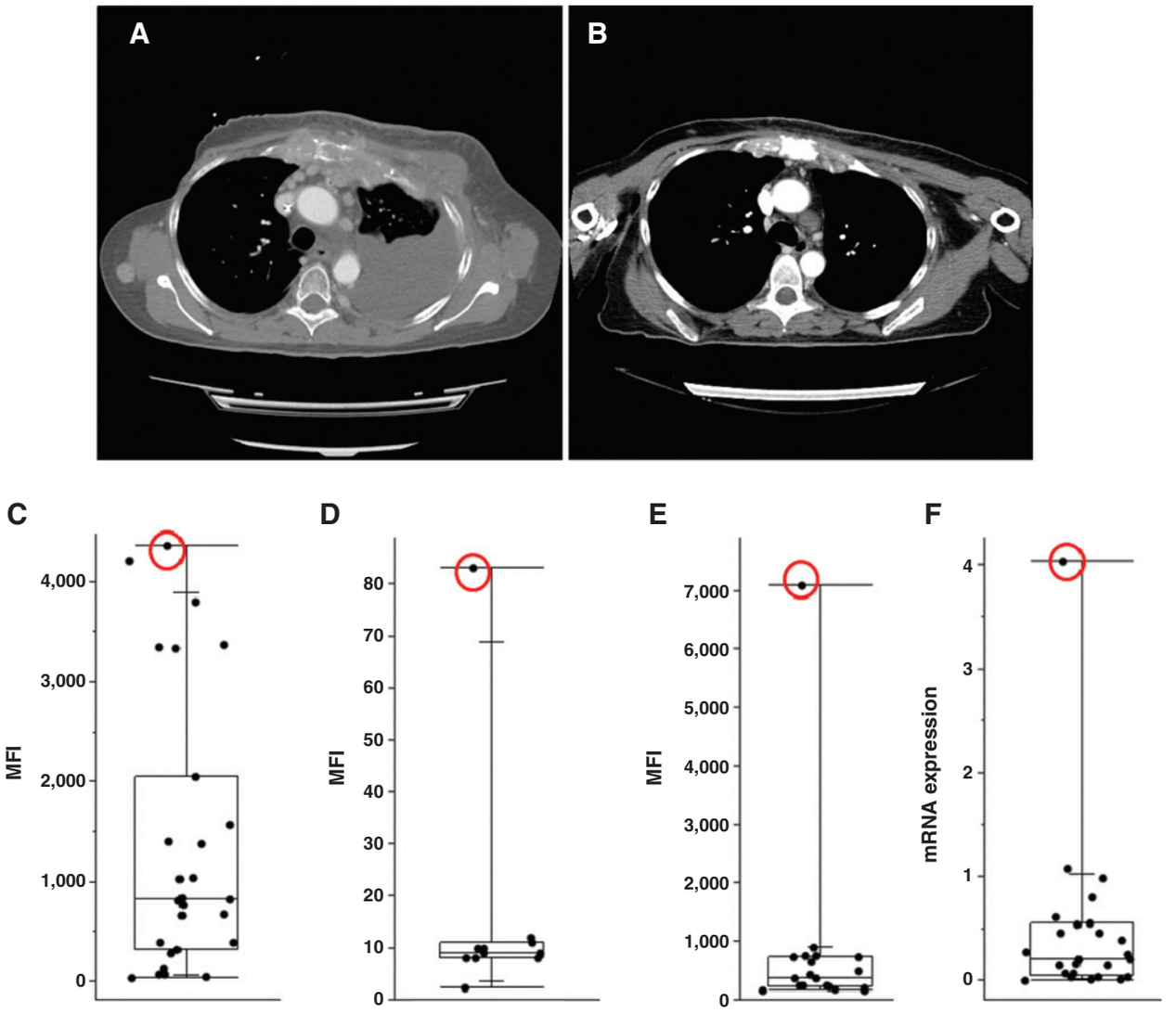


Figure 3.
CT scan of a triple-negative breast cancer patient in the 1,600-mg cohort at screening (A) and at day 14 of cycle 4 (B). Non-target lesions (anterior thorax soft tissue, subcutaneous metastases and mediastinal adenopathies) show substantial decrease in size. In addition, the pleural effusion on the right side almost vanished without drainage. Protein levels of HER3 ($N = 27$; C), pHER3 ($N = 11$; D), and pAKT ($N = 20$; E) in fresh-frozen tumor biopsies determined by Luminex immunoassay and heregulin mRNA ($N = 25$; F) in formalin-fixed paraffin-embedded tumor biopsies determined by qRT-PCR. Biopsy samples were obtained before dosing with lumretuzumab. The triple-negative breast cancer patient from the 1,600-mg cohort is identified by the red circle.

a target lesion and remained stable throughout the study. This patient had the highest metabolic partial response on day 14 of cycle 1 in the FDG-PET. Progression with new lesions was assessed for the patient on day 79. In contrast to all other patients, this patient had highly elevated levels of heregulin, pHER3 and pAkt [heregulin mRNA expression: 4.03 (median 0.21, $n = 25$); pAkt (mean fluorescent intensity, MFI): 7107 (median 365, $n = 20$); pHER3 (MFI): 83 (median 9, $n = 11$), Fig. 3C–F] indicating a highly activated HER3 signal transduction cascade.

Partial metabolic response was observed in 9 of 38 (23.7%) and 1 of 23 (4.3%) patients at day 14 of cycle 1 and 4, respectively, in FDG-PET (Supplementary Fig. S5A and S5B).

Discussion

Here, we report the first-in-human study results of lumretuzumab, a novel humanized, glycoengineered anti-HER3 mAb.

Lumretuzumab showed a favorable safety profile across all dose groups. No DLTs occurred, and an MTD was not reached. To gain further insight into the safety and PK profile, patients in the extension cohort were treated with the highest dose explored in the dose-escalation phase of 2,000 mg.

In preclinical models, downregulation/internalization of HER3 in tumor tissue was demonstrated in a dose-dependent manner (17, 22). In addition, PK/PD models showed that antitumor efficacy was also dose dependent (22). Therefore, HER3 downregulation in tissue was assessed as a PD marker to

guide dose finding and dose optimization in this clinical study in combination with serum PK properties. After demonstrating initial PD activity in skin, which occurred at the first dose level tested (100 mg), serial tumor biopsies (pretreatment and on day 14 of cycle 1) were implemented from the 200-mg cohort onwards. Dose escalation demonstrated a plateauing of HER3 downregulation from 400 mg onwards. In addition, the PK profile showed linearity at doses of $\geq$400 mg and $\geq$95% target saturation was achieved from 400 mg over the entire dosing interval. Hence, the optimal biologic dose was determined to be $\geq$400 mg.

Despite glycoengineering to enhance ADCC function, the infusion of lumretuzumab was well tolerated in patients, given the low incidence rate of IRRs and associated severity. Analyses of peripheral blood immune cells showed a trend toward increased levels of CD16 expression of the $CD16^+$ population within the NK population. In parallel, the on-treatment biopsies did not demonstrate significant changes in tumor infiltrating immune cells. Overall, the ADCC-related PD effects are inconclusive. An *ex vivo* assay demonstrated an increased activation potential of NK lymphocytes by glycoengineered lumretuzumab as compared with a non-glycoengineered HER3 antibody. The influence of glycoengineering on the ADCC potential of lumretuzumab was clearly shown preclinically both *in vitro* and *in vivo* with a wild type HER3 MAb as a control (Mirschberger and colleagues, 17). However, the following factors might influence the apparent absence of an ADCC response in the clinical setting: (i) The effector-to-target ratio, which was certainly higher in the *in vitro* and *in vivo* preclinical system; (ii) the possibility that lumretuzumab-bound HER3 may have been internalized too rapidly (as indicated by the complete downregulation of HER3 14 days after administration).

The disease control rate in this study was 21%, which is similar to other phase I anti-HER3 antibody monotherapy studies (23–27). One patient with a triple-negative breast cancer showed a dramatic reduction of tumor load at 1,600 mg of lumretuzumab. Assessment of baseline tumor characteristics in this patient revealed highly elevated expression levels of heregulin mRNA and pHER3 and pAkt protein expression, indicating a highly activated HER3 pathway or HER3/heregulin autocrine loop as described previously in head and neck, ovarian and breast cancer tumors/cell lines (14, 16, 28). None of the other tumors analyzed in this trial showed similar baseline biomarker features, indicating that HER3 pathway activation may be correlated to response to HER3-targeted therapy and that heregulin gene expression may be a clinically measurable surrogate for HER3 pathway activation and a predictive biomarker. In contrast, the intensity of HER3 protein expression was not associated with clinical outcome.

The hypothesis that heregulin expression levels may serve as a potential response predictor for HER3-targeted therapy is strengthened by recent reports from other trials. Juric and colleagues (29) published data from a phase I trial in which the only two responding patients with head and neck cancer had the highest heregulin expression levels. In addition, randomized phase II trials in non–small cell lung and breast cancer patients treated with HER3-targeted therapy and with higher heregulin expression levels at baseline showed prolonged progression-free survival (30–32). Because the clinically relevant cutoff level of heregulin is unknown it may be challenging to select patients for clinical trials based on this biomarker especially if the prevalence is low or exceptionally high expression levels are needed. Nevertheless, it may be worth enriching the study population for tumor subtypes in which heregulin expression has been demonstrated to be elevated. Shames and colleagues (16) reported that heregulin expression was increased in a proportion of patients with head and neck cancer. Others have found heregulin to be highly expressed in BRAF-mutated thyroid cancer cells (33).

In conclusion, treatment with lumretuzumab was well tolerated up to doses of 2,000 mg. PK was linear from $\geq$400 mg, indicative of target-mediated drug disposition saturation, and PD activity was demonstrated. Serial biopsy sampling was implemented after observing initial PD effects in surrogate skin tissue. The PD effects observed in the serial tumor biopsies, in conjunction with the linear PK behavior of lumretuzumab, guided dose finding and optimization. Thus, this innovative phase I trial design demonstrates direct translation and implementation of preclinical knowledge into early clinical development. In addition, this phase I trial also demonstrates that paired tumor biopsies are feasible in the vast majority of patients with advanced cancer. Clinical activity was demonstrated in a breast cancer patient whose tumor showed HER3 pathway activation. Biomarkers such as heregulin, pHER3, and pAkt may serve as clinically measurable surrogates for HER3 pathway activation and ultimately as potential predictive biomarkers for HER3-targeted therapy. Clinical development of lumretuzumab is ongoing, focusing on biomarker-enriched patient populations and in combination with other HER family inhibitors.

Disclosure of Potential Conflicts of Interest

M. Lolkema reports receiving commercial research grants from Astellas Pharma, and is a consultant/advisory board member for Roche. A. Cervantes reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Roche. C. Adessi and M. Weisser hold ownership interest (including patents) in Roche. No potential conflicts of interest were disclosed by the other authors.

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