Title:

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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Translational Relevance

Human epidermal growth factor receptor (HER) 3 is implicated in tumor growth and maintenance in many solid tumor types and has been described as a potential escape mechanism of HER1- 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. Down-regulation of HER3 receptor in on-treatment tumor biopsies and linear serum PK indicated optimal biological 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.

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 advanced cancer patients.

Patients and Methods

Twenty-five patients with histologically confirmed HER3-expressing tumors received lumretuzumab (100, 200, 400, 800, 1600 and 2000 mg) q2w in 3+3 dose escalation phase. Additionally, 22 patients were enrolled into an extension cohort at 2000 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 ≥400 mg q2w indicating target-mediated drug disposition saturation.

Down-regulation 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 to 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 to 225 days).

Conclusion

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.

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 which activates several signal transduction pathways, particularly the phosphoinositid-3-kinase (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 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 to 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).

110 The current study evaluated the safety, efficacy and pharmacokinetics (PK) of
111 lumretuzumab. Potential biomarkers related to lumretuzumab were evaluated to
112 determine the optimal biological dose.

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 IV 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, and discernible HER3 membrane positivity in any neoplastic cell was considered diagnostically positive for HER3 protein expression.

Study drug administration

Patients received premedication 30 min prior to the start of the 1st infusion consisting of paracetamol (500 to 1000 mg p.o.) and diphenhydramine (25 to 50 mg p.o. or IV, or an alternative anti-histamine). Corticosteroids were allowed in case of ≥ grade 2 infusion-related reactions (IRRs). Lumretuzumab was administered as an IV infusion q2w starting at 50 mL/h, and escalating by 50 mL/h in 30-min 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

158 assessment was based on European Organisation for Research and Treatment of
159 Cancer (EORTC) criteria (18).

160 Tumor response assessment using Response Evaluation Criteria in Solid Tumors
161 1.1 (19) was conducted at screening and every 8 weeks thereafter.

162 Safety assessments included physical (ECOG performance status, vital signs) and
163 laboratory examinations and electrocardiogram (ECG). Adverse events (AEs) were
164 defined according to the Common Terminology Criteria for Adverse Events, version
165 4.0 (20). Lumretuzumab-specific anti-drug antibody (ADA) responses were assessed
166 prior to each infusion and at the end of the study using a state-of-the-art, bridging-
167 format immunoassay.

168 *Definition of dose-limiting toxicity (DLT)*

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

175 *Pharmacokinetic assessments*

176 PK evaluation was conducted for all patients on Day 1 of Cycle 1 (blood samples
177 taken before the infusion, at end of infusion [EOI], and at 2, 5, 24, 48, 100, 168 and
178 264 h after EOI), Day 1 of Cycle 4 (blood samples taken before the infusion, at EOI,
179 and at 2, 24, 48, 100 and 168 h after EOI), Day 1 of Cycle 8 (blood samples taken
180 before the infusion, at EOI, and at 24, 100 and 168 h 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., Cary, NC, USA).

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, Tucson, USA and performed by Source BioScience LtdSourceBioscience Ltd, Nottingham, UK). 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 down-regulation at Day 14 of Cycle 1 in skin biopsies. Changes in the co-expression of HER receptors (epidermal growth factor receptor [EGFR], HER2) and HER3 pathway activation (cMET, 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 semi-quantitatively using an immunoreactive score (IRS) according to: IRS = Staining Intensity (SI) x Percent Tumor Cells Stained (PS), where SI = 1 x “+” score + 2 x ”++” score + 3 x ”+++” score) / 100 and PS = (“+” score + ”++” score + ”+++” score) / 100.

Blood was obtained pre-infusion for all treatment cycles for (1) immunophenotyping of lymphocytes and (2) ADCC function assessed by an ex-vivo NK activation assay (21) where isolated peripheral lymphocytes were incubated with target cells

205 expressing HER3 and lumretuzumab or wild type anti-HER3 monoclonal antibody
206 (MAb). In addition, NK cell activation as measured by CD107a expression assay,
207 which measures the number of NK cells that are activated on exposure to antibody
208 and in the presence of T47D target cells expressing HER3, was quantified by flow
209 cytometry (Covance, Indianapolis, USA).

210 Heregulin mRNA expression was determined as a potential predictive biomarker for
211 lumretuzumab using a quantitative reverse transcription polymerase chain reaction
212 (qRT-PCR) assay developed by Roche Molecular Systems Inc. (Pleasanton, USA).
213 Heregulin mRNA expression was reported as delta cycle threshold (ΔCt) where ΔCt
214 = Ct(Reference) - Ct(Heregulin).

215 HER3, pHER3 and pAkt levels were determined in protein extracts from matched
216 fresh frozen tumor biopsies taken during screening and on Day 14 of Cycle 1, using
217 an exploratory Luminex assay methodology (NMI, Reutlingen, Germany).

218 *Statistical considerations*

219 All patients who received at least one dose of study medication were included in the
220 safety and efficacy populations. Descriptive statistics were used for demographics,
221 safety, and anti-tumor activity. Summary statistics of the PK and PD parameters
222 were presented using arithmetic mean, median, standard deviation (SD), coefficient
223 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 mg (n=3), 200 mg (n=3), 400 mg (n=3), 800 mg (n=7), 1600 mg (n=5), and 2000 mg (n=4). In the extension phase, 22 patients received 2000 mg. Forty-three patients (91.5%) discontinued the study due to progressive disease, two patients (4.3%) were withdrawn due to an AE (Grade 2 and Grade 3 IRR both in the 800-mg cohort) and two patients (4.3%) withdrew consent.

Safety

No DLTs were reported and the maximum tolerated dose (MTD) was not reached up to the highest dose tested (i.e. 2000 mg). A total of 379 AEs were reported in 46/47 patients (97.9%) (Table 2). Most AEs (90.2%) were Grade 1 or 2 in intensity. A total of 37 Grade 3/4 AEs were reported for 25 patients (53.2%). Only two events in two 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 one 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 toxicities were seen with lumretuzumab treatment with the exception of Grade ≥3 diarrhea which was only reported for patients from the extension cohort at 2000 mg.

In the present study, IRRs seem to be independent of the dose administered. Only one 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 prior to 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, Figure 1A and B). PK approached linearity

at the higher doses studied (400 to 2000 mg) (Table 3). Mean $t_{1/2}$ estimates increased with increasing doses from 100 mg 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 Figure 1). Similar PK estimates were observed following administration of 2000 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 one week (Figure 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 et al [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 to expression in paired pre-treatment screening samples (n=46, Supplementary Table 1). 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.

Down-regulation of HER3 membrane expression was observed for Cycle 1 Day 14 tumor biopsies compared to expression in paired pre-treatment screening samples at

all doses of lumretuzumab and in 35/38 (92%) of patients (Supplementary Table 1 and Figure 2).

There was no apparent relationship in the extent of HER3 down-regulation 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, whilst not mandatory, were available for 34/47 (72%) of patients in the study. HER3, EGFR and cMET expression were significantly higher in the fresh baseline sample compared to the archival sample (Supplementary Table 2).

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 to baseline, although this response was not sustained at Cycle 3 (Supplementary Table 3). In addition, there was a trend towards 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 to 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 to 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 4).

Similarly, no significant changes in immune cell infiltration were observed in tumor samples obtained pre-dose compared to Day 14 of Cycle 1 (Supplementary Table 5).

Antitumor activity

Of the 47 enrolled patients, ten (21.3%) had stable disease and 29 (61.7%) had progressive disease (Supplementary Table 6). Five patients (10.6%) had clinical progression and for three 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 to 225 days).

A triple-negative breast cancer patient from the 1600-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 (Figure 3A and B). A mixed lytic/blastic bone lesion was defined as 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], Figure 3C to F) indicating a highly activated HER3 signal transduction cascade.

342 Partial metabolic response was observed in 9 of 38 (23.7%) and 1 of 23 (4.3%)
343 patients at Day 14 of Cycle 1 and 4, respectively, in FDG-PET (Supplementary
344 Figure 5A and B).

Discussion

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

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 2000 mg.

In preclinical models, down-regulation/internalization of HER3 in tumor tissue was demonstrated in a dose-dependent manner (17, 22). In addition, PK/PD models showed that anti-tumor efficacy was also dose-dependent (22). Therefore, HER3 down-regulation 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 down-regulation from 400 mg onwards. In addition, the PK profile showed linearity at doses of ≥400 mg and ≥95% target saturation was achieved from 400 mg over the entire dosing interval. Hence, the optimal biological dose was determined to be ≥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 towards 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 to 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 et al, 2013). However, the following factors might influence the apparent absence of an ADCC response in the clinical setting: (a) The effector-to-target ratio, which was certainly higher in the in vitro and in vivo preclinical system; (b) 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 1600 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 et al. published data from a phase 1 trial in which the only two responding patients with head and neck cancer had the highest heregulin expression levels (29). In addition, randomized phase 2 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). Since the clinically relevant cut-off 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 et al. reported that heregulin expression was increased in a proportion of head and neck cancer patients (16). 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 2000 mg. PK was linear from ≥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 advanced cancer patients. 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.

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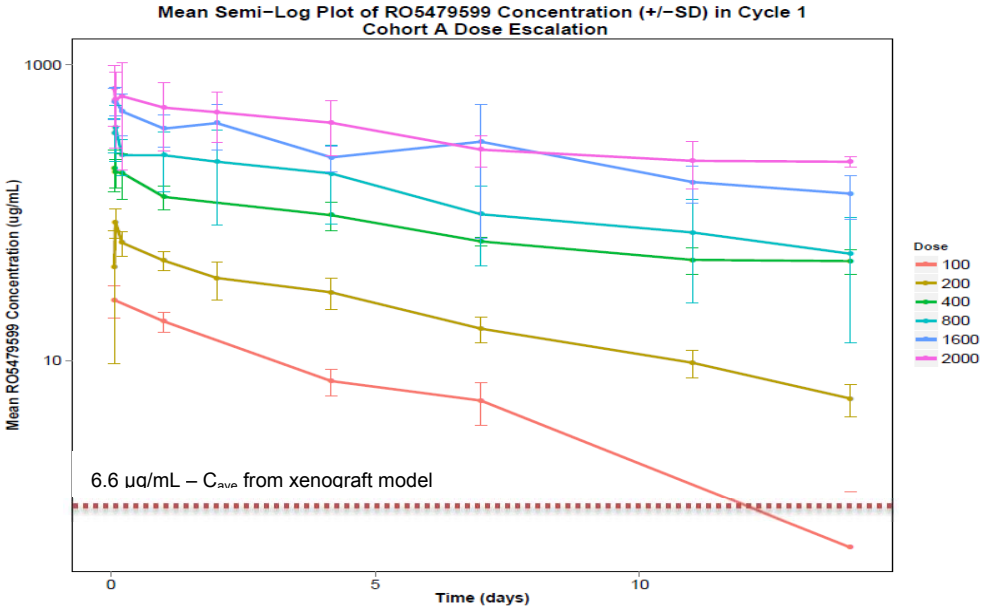
Figure 1 (a) Mean (±SD) serum lumretuzumab profiles following single ascending doses from 100 mg up to 2000 mg for Cycle 1. (b) AUC_{last}/Dose normalized versus dose (100 mg – 2000 mg) box plot. Black lines indicate the median and boxes indicate the inter-quartile range.

Figure 2 HER3 expression in a liver lesion biopsy from a patient with colon cancer sampled prior and 14 days post treatment with 400 mg lumretuzumab. (x20 objective, x10 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 degree of cytoplasmic staining in the post treatment 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).

Figure 3 CT scan of a triple-negative breast cancer patient in the 1600-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 (C) HER3 (N=27), (D) pHER3 (N=11) and (E) pAKT (N=20) in fresh frozen tumor biopsies determined by Luminex immunoassay and (F) heregulin mRNA (N=25) in formalin-fixed paraffin embedded tumor biopsies determined by qRT-PCR. Biopsy samples were obtained prior to dosing with lumretuzumab. The triple-negative breast cancer patient from the 1600-mg cohort is identified by the red circle.

Figure 1

(a)



Note: Lumretuzumab C_{min} levels from 100 up to 2000 mg. Bottom horizontal red line is the trough corresponding to maximal efficacy (C_{ave}) in mice.

(b)

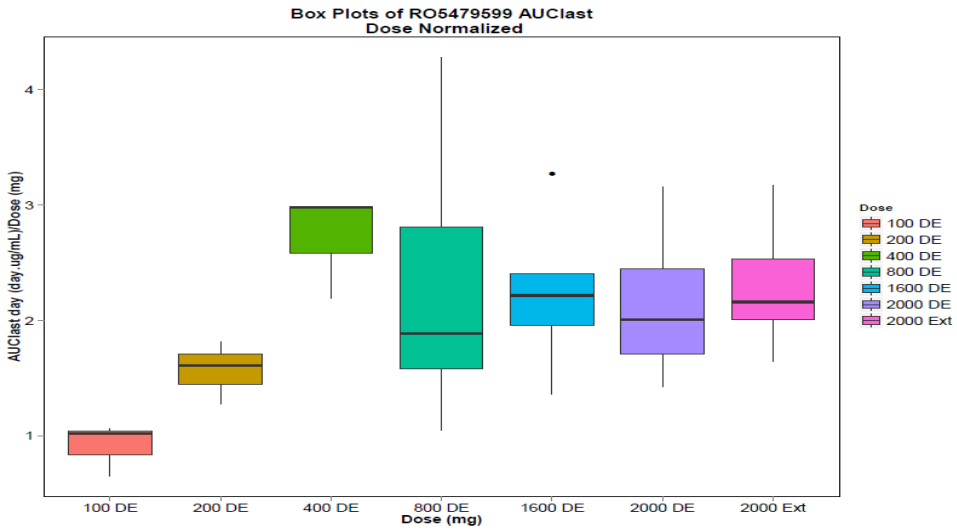
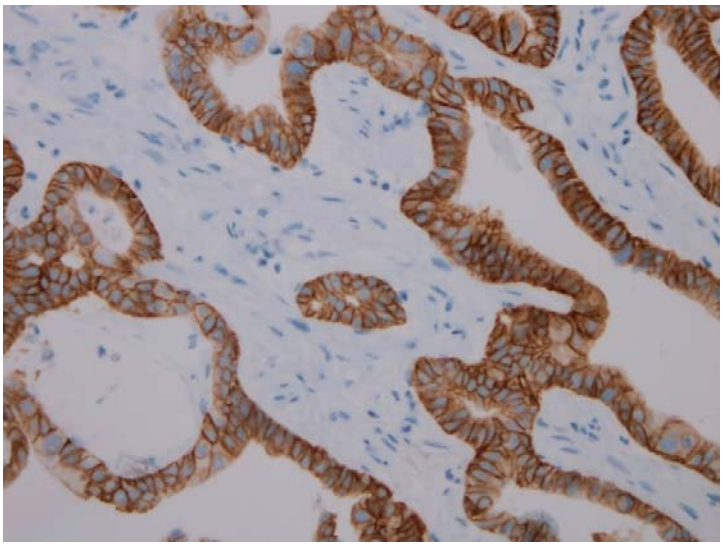
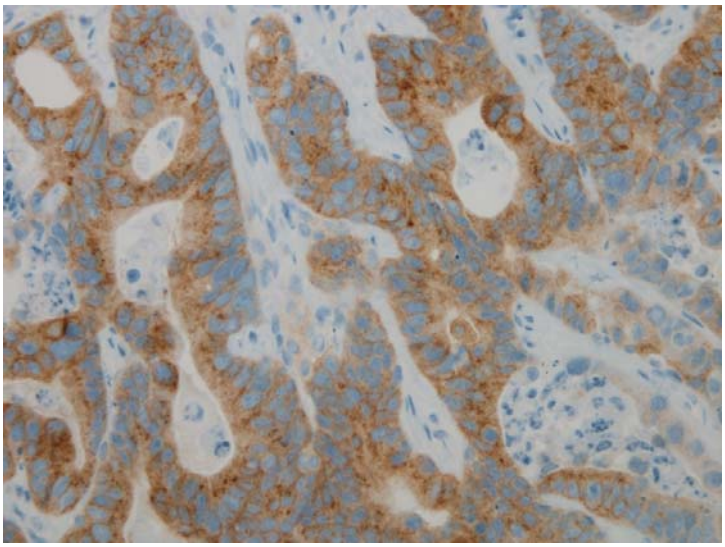


Figure 2

(a)

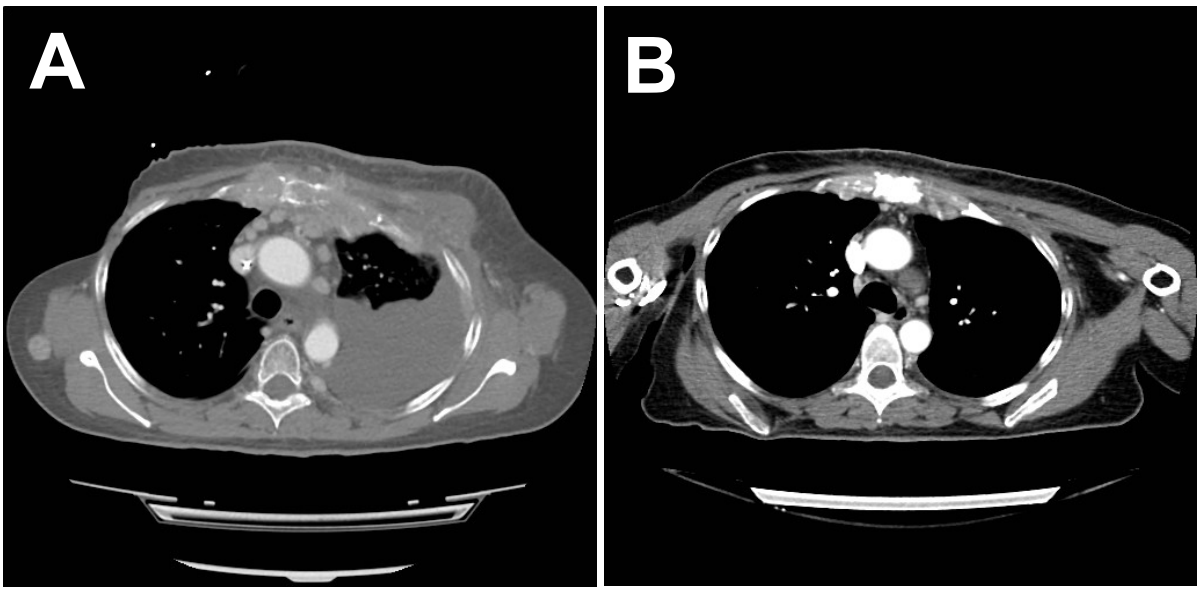


(b)

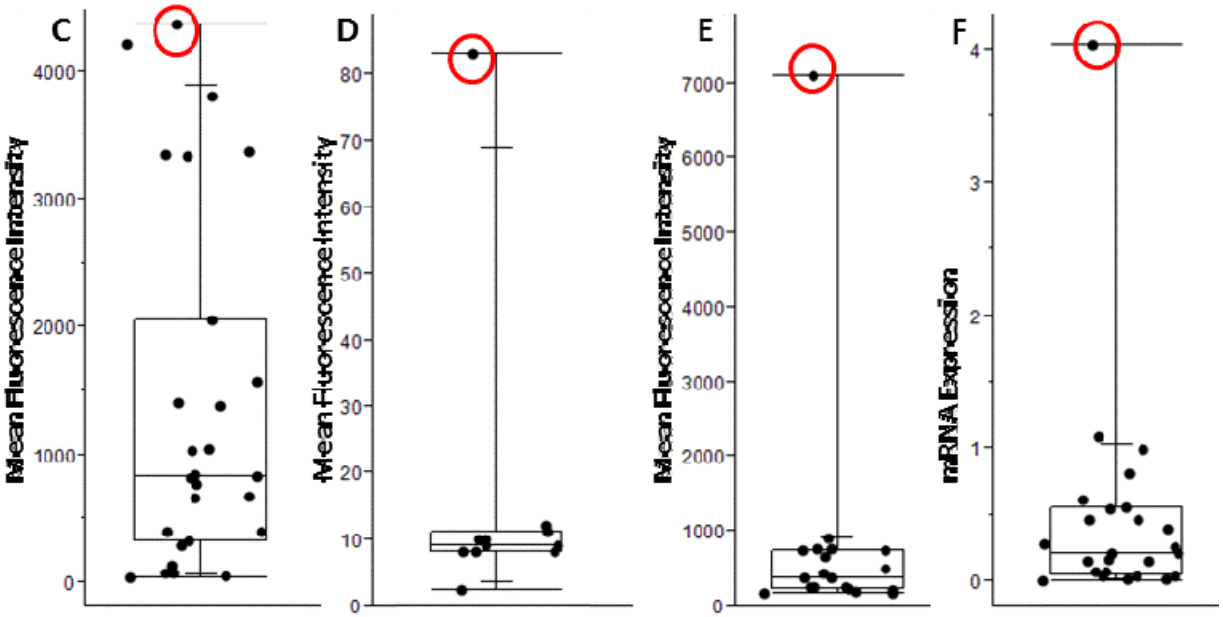


21 Figure 3

22



23



24

25 Table 1 Baseline patient demographics and characteristics

Characteristic	Total (N=47)
Sex, n (%)	
Male	25 (53.2)
Female	22 (46.8)
Age (years), 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.

26 Table 2 Summary of adverse events of any grade and of grade ≥3 adverse

27 events

Adverse event	No. of patients having an adverse event (%)			
	All adverse events		Study drug-related adverse events	
	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
Infusion-related reaction	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)

Please note: Only adverse events reported by >5% of the patients are shown.

28

29 Table 3 Lumretuzumab serum PK parameters at Cycle 1 following administration

30 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	3640	1040	2.40
	CV%	24.9	24.6	49.5	18.3	42.5
200	N	3	3	3	3	3
	Mean	86.6	314	3740	586	4.50
	CV%	22.7	17.2	9.36	20.3	12.0
400	N	3	3	3	3	3
	Mean	215	1090	3590	222	11.5
	CV%	28.6	16.8	20.6	24.1	28.5
800	N	7	7	7	7	7
	Mean	439	1830	3780	383	8.90
	CV%	23.4	48.1	45.3	38.7	37.4
1600	N	5	5	4	4	4
	Mean	658	3590	4980	329	10.4
	CV%	18.5	31.1	44.8	16.6	34.1
2000	N	4	4	4	4	4
	Mean	699	4300	4340	292	12.0
	CV%	42.4	34.6	18.6	51.1	38.7
2000 Extension	N	22	22	22	22	22
	Mean	970	4530	4390	264	11.7
	CV%	52.2	17.9	24	29.7	38.3

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

31

AACR American Association for Cancer Research

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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