A First-in-Class mAb (BI-1607) Targeting FcγRIIB: Preclinical Data and First-in-Human Studies in Patients with HER2-Positive Advanced Solid Tumors

Javier Cortés[1,2,3], Araceli Priego[1], Elena Garralda[4,5,6,7], Katerin Rojas[4], Simon R. Lord[8], Thorsten O. Goetze[9], Sherko Kuemmel[10,11], Simon J. Crabb[12], Zinnia P. Parra-Guillen[13], Marie Borggren[13], Ingrid Karlsson[13], Danijela Lindahl[13], Linda Mårtensson[13], Robert Oldham[14], Anna Ropenga[13], Ingrid Teige[13], Johan Wallin[13], Björn Frendeus[13], and Andres McAllister[13]

ABSTRACT

Purpose: BI-1607 is a human mAb that specifically blocks FcγRIIB, the sole inhibitory Fc receptor and master regulator of humoral and innate immune homeostasis. These studies evaluated preclinical antitumor activity using a BI-1607 murine surrogate (mBI-1607) and the safety, tolerability, pharmacokinetics, and pharmacodynamics of the compound in combination with trastuzumab in patients with HER2-positive advanced solid tumors (NCT05555251).

Patients and Methods: Immunocompetent syngeneic mouse breast tumor (TUBO) and melanoma (B16-F10) models were used to evaluate *in vivo* antitumor activity in combination (anti-HER2 and anti-gp75). Ascending doses of BI-1607 administered intravenously every 3 weeks in combination with trastuzumab were evaluated in 18 patients with HER2-positive cancer. The primary objective was to assess the safety and tolerability of BI-1607 by determining dose-limiting toxicities and the maximum tolerated dose or maximum administered dose and identifying a recommended phase 2 dose.

Results: mBI-1607 enhanced tumor-targeting antibody efficacy and animal survival. BI-1607/trastuzumab was well tolerated, with dose-limiting toxicity (rash) in one patient (5.6%) at 900 mg; the maximum tolerated dose was not reached. Treatment-emergent adverse events grade ≥3 occurred in five patients (28%), including exanthema, increase in liver enzymes, urticaria, acute kidney injury, and aggravated condition. Overall best response was stable disease, observed in seven of the nine evaluable patients (78%). BI-1607 exhibits linear pharmacokinetics for doses above 500 mg, and full receptor saturation was observed throughout the 21 days at 700 mg. No antidrug antibodies were observed.

Conclusions: The enhancing effect on tumor direct-targeting antibodies observed preclinically, together with the favorable safety profile in patients, supports further investigation of BI-1607.

Introduction

Fcγ receptors (FcγR) are key regulators of the activity of IgG-type antibodies. Several activating (FcγRI, FcγRIIA, and FcγRIIIA) and a single inhibitory (FcγRIIB) receptors act in concert to regulate FcγR-expressing effector cell responses during therapy. Activating FcγR, like costimulatory T-cell checkpoints, promote immune effector cell activation. Conversely, FcγRIIB, the inhibitory receptor, blocks activation (1). Direct-targeting mAb such as trastuzumab, also termed cytotoxic mAb, bind to target cell-surface receptors and mark the cell for innate immune destruction. This mechanism is dependent on binding to FcγR via the constant Fc domain of the cytotoxic mAb, and the activating-to-inhibitory (A:I) FcγR engagement ratio determines the efficacy of the antibody-mediated target cell depletion (2). In mouse tumor models, genetic deletion of FcγRIIB enhanced *in vivo* therapeutic activity of cancer cell direct-targeting antibodies to CD20, HER2, and the melanoma antigen gp75 (3). Several mAb used in cancer therapy require functional activating FcγR for therapeutic activity, and conversely, the high expression of FcγRIIB reduces mAb efficacy (3). The approved mAb margetuximab (Margenza, MacroGenics) has a modified Fc region with enhanced binding to FcγRIIIA and moderately decreased binding to FcγRIIB, thereby increasing the antibody A:I ratio (4). This endows margetuximab with enhanced *in vitro* antibody-

[1]Faculty of Biomedical and Health Sciences, Department of Medicine, Universidad Europea de Madrid, Madrid, Spain. [2]International Breast Cancer Center (IBCC), Pangaea Oncology, Quironsalud Group, Barcelona, Spain. [3]IOB Madrid, Institute of Oncology, Hospital Beata Maria Ana, Madrid, Spain. [4]Research Unit for Molecular Therapy of Cancer (UITM), Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain. [5]Early Drug Development Group, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain. [6]Oncology Department, Hospital Universitario Vall d'Hebron (HUVH), Barcelona, Spain. [7]Phase I Unit – NEXT Oncology, Hospital Quirónsalud, Barcelona, Spain. [8]Department of Oncology, University of Oxford, Oxford, United Kingdom. [9]Institut of Clinical Cancer Research, UCT-University Cancer Center Frankfurt, Frankfurt, Germany. [10]Interdisciplinary Breast Unit, Kliniken Essen-Mitte, Essen, Germany. [11]Department of Gynecology with Breast Center, Charité – Universitätsmedizin Berlin, Berlin, Germany. [12]Southampton Experimental Cancer Medicine Centre, University of Southampton, Southampton, United Kingdom. [13]BioInvent International AB, Lund, Sweden. [14]Antibody and Vaccine Group, Centre for Cancer Immunology, University of Southampton, Southampton, United Kingdom.

Corresponding Author: Andres McAllister, BioInvent International AB, Ideon Science Park, Lund SE-223 70, Sweden. E-mail: andres.mcallister@bioinvent.com

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Translational Relevance

BI-1607 is a fully human anti-FcγRIIB antagonistic mAb with a modified Fc region that abrogates the binding of its Fc region to Fcγ receptors (FcγR). When administered in combination with a tumor-targeting antibody, BI-1607 will block the inhibitory impact of FcγRIIB, promoting the combination partner antibody's Fc-mediated binding to activating FcγR. This enhances the effector functions, for example, the antibody-dependent cellular phagocytosis of the combination antibody. Accordingly, BI-1607 mouse surrogate (mBI-1607) enhanced the therapeutic activity of tumor direct-targeting antibodies in murine syngeneic tumor models. The safe nature of this strategy to enhance clinically validated antibodies was demonstrated in this first-in-human study of BI-1607 combined with trastuzumab in patients with HER2-positive solid tumors. These results support the clinical investigation of additional BI-1607 combinations with established direct-targeting antibodies.

dependent cellular cytotoxicity (ADCC) and NK cell activation, *in vivo* antitumor activity (4), and may afford margetuximab survival advantage in patients with distinct FcγR polymorphisms (e.g., CD16-158FF; ref. 5). Similarly, the anti-EGFR antibody cetuximab is known to stimulate ADCC, and polymorphisms in *FCGR2A* and *FCGR3A* favoring activating engagement are associated with better clinical outcomes (6). The clinical importance of tumor-targeting antibodies with FcγR-dependent mechanisms of action is further illustrated by the approval of the claudin 18.2–specific antibody zolbetuximab for the treatment of HER2-negative metastatic gastric cancer (7).

To selectively target FcγRIIB immunosuppressive functions on effector cells present inside the tumor microenvironment, we generated an Fc:FcγR-muted anti-FcγRIIB antibody (BI-1607, 6G11 IgG1N297Q) based on a (8) highly FcγRIIB-specific antibody clone (**Fig. 1A** and **B**; ref. 4). An equally specific Fc-muted FcγRIIB-blocking mouse surrogate antibody [AT-130 $mIgG1_{N297A,}$ (mBI-1607)] was used for testing in immunocompetent tumor mouse models (9).

In this work, we demonstrate *in vivo* proof-of-concept that Fc-muted anti-FcγRIIB enhances the therapeutic efficacy and FcγR-dependent depleting activity of direct-targeting antibodies relevant to solid tumors. Furthermore, in this first-in-human (FIH) study, we demonstrate that BI-1607 can be safely administered and combined with trastuzumab (anti-HER2) at doses achieving sustained and complete FcγRIIB saturation. Given its mode of action, BI-1607 is not expected to have single-agent activity. The choice of trastuzumab as the combination agent in this trial was based on promising preclinical studies and a recognized need for additional options for patients who fail to respond or stop responding to trastuzumab. Ultimately, if shown to be safe and effective in combination with trastuzumab, BI-1607 can also be used in combination with other cytotoxic or immunomodulatory antibodies for cancer treatment.

Patients and Methods

In vitro characterization

The original clone directed against FcγRIIB was selected using the n-CoDeR single-chain variable fragment library (Lib 2000) and converted into an aglycosylated IgG1 antibody (containing the N297Q mutation) using in-house expression vectors. The resulting antibody was then expressed using the Lonza GS system. Chinese hamster ovary cells (CHOK1SV) were transfected with the pEE-JhG1N297Q-FCR241-006-G11-4 plasmid, and the clone L220 was chosen as the best cell line because of its growth characteristics, viability, and productivity during long-term cultivation.

In vitro binding of BI-1607 to FcγR proteins was tested in an ELISA. Briefly, recombinant human FcγRIIB/C, FcγRIIA, FcγRI, or FcγRIIIA, cynomolgus FcγRIIB, or mouse FcγRIIB was used for coating. Bound BI-1607 or BI-1206 was detected using anti-hIgG (H + L) horseradish peroxidase. A luminescence substrate was used for the readout.

In vitro ADCC assays were conducted. Briefly, ADCC assays were performed using an NK-92 cell line expressing hFcγRIIIA-158V. CLL cells, which naturally express FcγRIIB, from six different patients were used as target cells for BI-1607, BI-1206, and isotype control in the assay. Target cell lysis was analyzed by flow cytometry.

Preclinical *in vivo* studies

B-cell depletion

Assessment of target cell depletion *in vivo* for BI-1607 and BI-1206 was conducted in human FcγRIIB transgenic mice (hFcγRIIB ± x mFcγRII−/− C57BL/6 mice). Briefly, mice were injected intravenously with 10 mg/kg BI-1607 or BI-1206. Leukocyte analysis by flow cytometry was conducted before and 48 hours after receiving BI-1607 or BI-1206.

TUBO model

Female BalbC mice (RRID: IMSR_RJ:BALB-CJRJ) were injected subcutaneously with the HER2-expressing mouse breast cancer cell line TUBO (University of Bologna; 1×10^6 cells/mouse). Tumor growth was monitored (measured using a caliper), and when tumors reached approximately 7×7 mm, mice were randomized and treated intraperitoneally with either isotype control (mIgG2a 10 mg/kg), anti-HER2 (mIgG2a 1 or 10 mg/kg), mBI-1607 (mIgG1N297A 20 mg/kg; ref. 8), or a combination of mBI-1607 (20 mg/kg) and anti-HER2 (1 or 10 mg/kg). The mice were dosed three times (2–3 days apart), and mBI-1607 was always given 20 minutes ahead of the anti-HER2 mAb. Tumor growth was measured twice weekly until tumors reached a predetermined size (ethical endpoint), when the mice were euthanized.

In a separate experiment with the same treatment schedule, tumors were collected 1 day after the last treatment for analysis of tumor-immune infiltrates. Tumors were chopped into small pieces and enzymatically digested with a mixture of DNAse and Liberase at 37°C. Furthermore, the tumor solution was filtered through a cell strainer to obtain a single-cell solution. The cell solution was blocked with IVIG before staining. Immune cells were identified and quantified by FACS using the following markers: CD45, CD3, CD4, CD8, CD25, CD11b, Ly6C, Ly6G, MHCII, F4/80, CD49b, and NK 1.1 (all from BD Biosciences).

B16 model

Female C57/BL6 mice (RRID: IMSR_RJ:C57BL-6NRJ) were injected intravenously with the gp75-expressing mouse melanoma cell line B16F10 (RRID: CVCL_0159 5×10^4 cells/mouse). Four days after tumor injection, mice were randomized and treated intraperitoneally with either isotype control (mIgG2a 10 mg/kg), anti-gp75 (TA99 mIgG2a RRID: AB10949462 10 mg/kg), mBI-1607 (20 mg/kg),

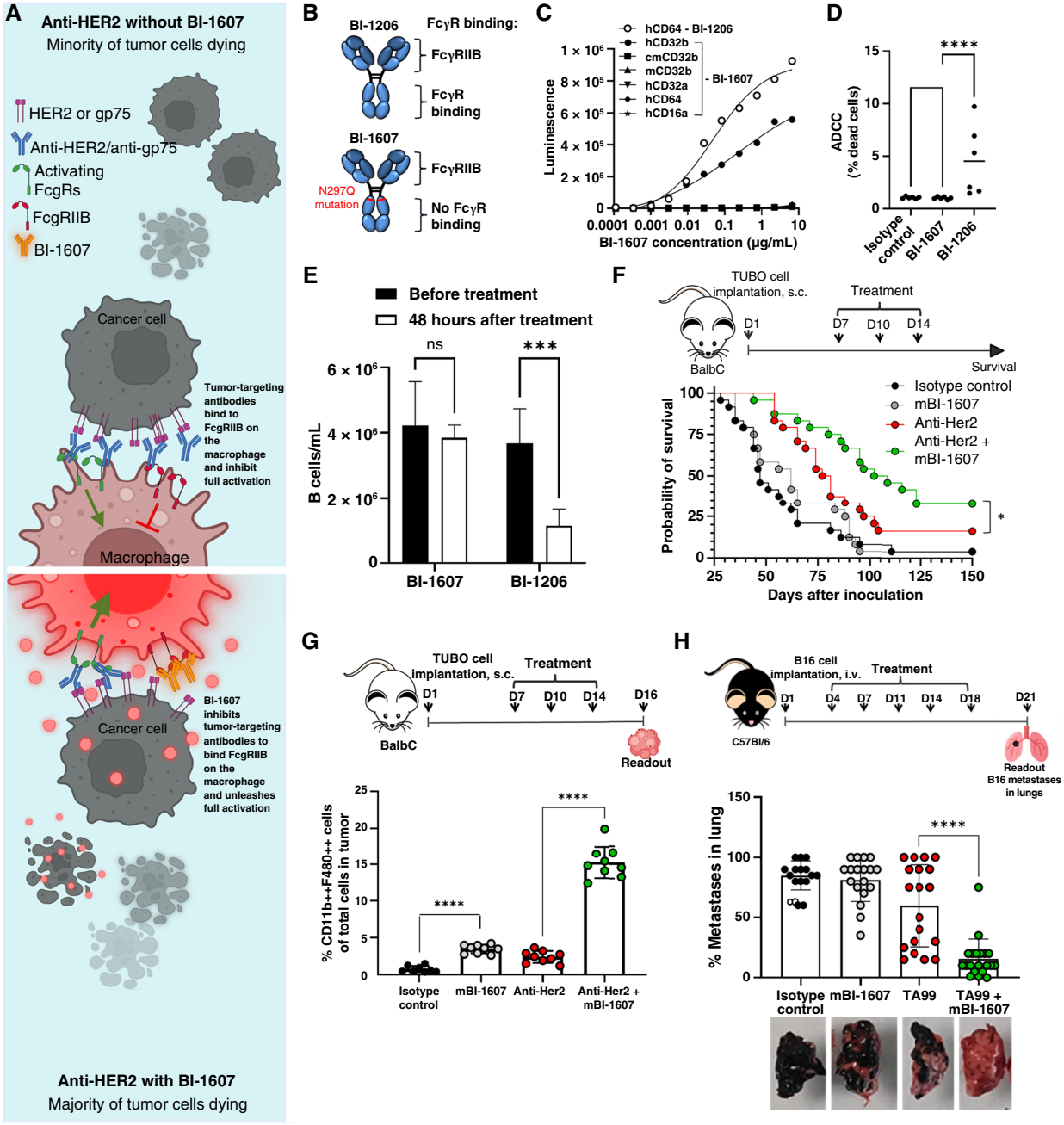

Figure 1.

A, Description of the mode of action of BI-1607 (anti-FcγRIIB$_{NA}$) in combination with tumor-targeting antibodies (anti-HER2/anti-gp75). By targeting the inhibitory FcγRIIB, BI-1607 will block the binding and inhibitory signaling through FcγRIIB of the tumor-targeting antibody. Instead, BI-1607 promotes the binding of the Fc domain of the tumor-targeting partner to activating FcγR, thereby enhancing effector functions of this antibody, such as antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis. **B,** BI-1607 is a fully human anti-FcγRIIB antagonistic mAb with a modified Fc region that abrogates the binding of its Fc region to FcγR. **C,** BI-1607 binding to human FcγRIIB, CD32a, CD64, and CD16a, cynomolgus FcγRIIB, and murine FcγRII. BI-1206 [the wild-type (WT) format of BI-1607 without mutation N297Q (thus has a functional Fc domain)] was used as a positive control binding to human CD64 via the Fc domain of the mAb. **D,** The cytotoxic ability of BI-1607 and BI-1206 in ADCC, using primary chronic lymphocytic leukemia (CLL) cells. Each dot represents one patient with CLL. ****, $P \leq 0.0001$ as assessed by paired Student *t* test. CLL, chronic lymphocytic leukemia. **E,** B-cell levels in hFcγRIIB transgenic mice before and 48 hours after treatment with BI-1607 or BI-1206. ***, $P \leq 0.001$. **F,** Anti-HER2 in combination with mBI-1607 demonstrated delayed tumor growth and increased survival compared with anti-HER2 treatment alone in the TUBO breast cancer model (two separate experiments, pooled log rank *, $P \leq 0.05$). **G,** Tumors analyzed for immune cell content by FACS showed that the combined treatment of anti-HER2 and mBI-1607 resulted in an increased CD11b++/F4/80++ population compared with single anti-HER2 treatment. **H,** In the B16F10 melanoma model, mBI-1607 improved the antitumor effect of the B16F10-specific TA99 antibody. This was demonstrated by significantly lower tumor burden in the lungs of mice treated with TA99 in combination with mBI-1607. (**A,** Created in BioRender. It, B. [2025] https://BioRender.com/xu3axf7.)

or a combination of TA99 (10 mg/kg) and mBI-1607 (20 mg/kg). The treatment was given five times, 2 to 3 days apart, and mBI-1607 was always given 20 minutes ahead of the anti-HER2 mAb. Three weeks after tumor injection, the mice were euthanized, and B16F10 metastasis formation in the lungs was quantified.

Clinical trial information

The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice Guidelines, and applicable local regulations. The protocol was approved by each Institutional Review Board or independent Ethics Committee before patient recruitment. Written informed consent was obtained for all patients before performing study-related procedures. This study was registered at clinicaltrials.gov (NCT05555251) and EU (2021-005646-15).

Study participants

Eligible participants were ages ≥18 years and had HER2-positive (10) locally advanced, unresectable, or metastatic solid tumors with disease progression after standard anticancer treatment, including prior lines of trastuzumab and at least one line of trastuzumab antibody–drug conjugate [e.g., trastuzumab emtansine (Creative Biolabs, cat. No. TAB-H70, RRID: AB_3112043) or trastuzumab deruxtecan]. According to the protocol, HER2-positive tumor status [according to 2018 ASCO/CAP JER2 test guidelines (10)] could have been from the most recent archival tissue sample or new tissue material from a recently obtained surgical or diagnostic biopsy. In addition, to be eligible, subjects had to have at least one measurable disease lesion as defined by RECIST version 1.1 criteria, left ventricular ejection fraction ≥50%, a life expectancy of ≥12 weeks, an Eastern Cooperative Oncology Group performance status of 0 or 1, and adequate organ function as confirmed by laboratory values. Key exclusion criteria included the following: the need of high doses of prednisolone (>10 mg daily, or other equipotent corticosteroids); active central nervous system metastases and/or carcinomatous meningitis; known or suspected hypersensitivity or contraindications to trastuzumab; or an active, known, or suspected autoimmune disease. The complete list of inclusion and exclusion criteria is available as supplementary material (SM1).

No anticancer therapy was permitted within 2 weeks before initiation of BI-1607. Prior treatment with immunotherapy or biological therapy was not permitted within 4 weeks or five half-lives of the respective drug before the first dose of BI-1607.

Study design and treatment

This was a FIH, phase I, open-label, multicenter, dose-escalation study to evaluate BI-1607 treatment in combination with trastuzumab. The primary objective of this study was to evaluate the safety and tolerability profile of BI-1607 when administered intravenously every 3 weeks in combination with trastuzumab in patients with HER2-positive locally advanced unresectable or metastatic solid tumors to characterize the dose-limiting toxicities (DLT), determine the maximum tolerated dose or maximum administered dose, and determine the recommended phase 2 dose for subsequent studies. Secondary objectives included pharmacokinetic (PK) parameters, immunogenic potential, receptor occupancy (RO) of BI-1607 on B cells, and preliminary assessment of the antitumor activity of BI-1607 in combination with trastuzumab. Exploratory objectives included assessment of the expression level of immunologic and predictive biomarkers and exploring the potential correlation of genetic FcγR isoforms with clinical responses.

Dose escalation of BI-1607 was according to the mTPI-2, a Bayesian design based on the modified toxicity probability interval to allow for a more efficient estimation of the maximum tolerated dose and reduce the risk of overdosing (11). The starting dose of 75 mg was based on an aggregate of preclinical PK and pharmacodynamic (PK/PD) data from *in vitro* studies and *in vivo* data combined with a full preclinical toxicology package. Following minimal anticipated biological effect level principles (12), a starting dose targeting a 50% RO 24 hours after administration was selected. The selected dose of 75 mg (1 mg/kg in 75 kg subject) provides a 250-fold safety margin compared with the well-tolerated dose of 250 mg/kg in mice established in a Good Laboratory Practice (GLP) toxicologic study. Given the mode of action for BI-1607 and the preclinical safety package, there was no prior concern for cytokine release syndrome or other severe infusion-related reactions (IRR), and the planned dose-escalation steps were guided by a desire to reach therapeutic concentrations without unnecessarily underdosing patients. Nonetheless, to minimize the incidence and severity of potential IRR, BI-1607 was administered as a slow intravenous infusion starting at a rate of 30 mg/hour and sequentially increasing the infusion rate.

Trastuzumab was also administered every 3 weeks at an 8 mg/kg dose for cycle 1 and 6 mg/kg for cycle 2 onward, 1 day after BI-1607 administration.

Safety and tumor assessment

All patients who received at least one dose of BI-1607 were included in the safety analysis. Safety assessments included physical exams, vital signs, hematology, blood chemistry, urinalysis, and ECG. Adverse events (AE), graded for severity according to the NCI Common Terminology Criteria for AE version 5.0, were documented until 30 days after the last dose of study treatment. Occurrence of DLT was assessed by the investigator as related to BI-1607 and occurring during the DLT assessment window (days 1–21 of cycle 1) and following the approved clinical study protocol criteria.

Tumors were assessed by CT/PET or MRI scan at screening, weeks 6, 12, 18, and 27, and every 12 weeks thereafter or as clinically indicated, for up to 2 years from the first dose of BI-1607. Treatment was continued until disease progression or unacceptable toxicity. Disease status was assessed by the investigator using RECIST version 1.1 (13). For response assessment, all patients who received at least one dose of BI-1607 and trastuzumab and had at least one evaluable postbaseline disease assessment (i.e., at least one valid postdose assessment collected at least 42 days after the initial dose) were included.

PK, PD, and immunogenicity assessments

Blood samples for serum BI-1607 concentration were collected from all participants treated with BI-1607/trastuzumab at predetermined time points at baseline, during and after infusion, and with a dense sampling after cycles 1 and 4. A validated ELISA with a lower limit of quantification of 150 ng/mL was used to determine the serum concentrations of BI-1607 in participants. Immunogenicity assessments were made for the presence/absence of antidrug antibodies to BI-1607 using pre- and posttreatment samples of infusion cycles 1 to 4 and cycles 6, 8, 12, and 16. Cytokine and chemokine levels were assayed in plasma collected in cycle 1 (predose, end of infusion, and 4 and 24 hours after infusion) using Meso Scale Discovery catalog kits K15049 and K15067. FcγRIIB RO was evaluated on $CD19^+$ B cells in whole blood collected in the same

manner as serum for PK assessment in cycles 1 to 4 and assessed by flow cytometry. Around 80% RO in this assay was reported as full saturation due to the limits of this analysis.

Paired fresh tumor biopsy specimens were collected in a subset of patients and assayed using whole-transcriptome analysis. A blood sample for DNA evaluation of FcγR isoforms was collected from participants predosed in cycle 1. The polymorphisms *FCGR2A*-H131R, *FCGR2B*-I232T, *FCGR2C*, and *FCGR3A*-V158F were assessed using allele-specific PCR methods.

PK, immunogenicity, and biomarker analyses were conducted using analytically validated methodologies and sponsor-defined protocols for sample handling and processing in central laboratories. All patients from the safety population were included in the analyses if at least one postdose measurement was available in addition to baseline.

Statistical analyses

PK and RO assessments were analyzed using nonlinear mixed effects modeling in NONMEM 7.5. (RRID: SCR_016986; ref. 14) to characterize both, the typical time course tendency together with its associated variability. For the analysis, PK measurements were logarithmically transformed, and different error models were considered for each type of measurement. Measurements below the limit of quantification were included and treated as censored information using the M3 method (15). Interindividual variability was modeled exponentially, except for bounded parameters in which additive models in the logit domain were used. Nondiagonal elements of the variance–covariance matrix were tested for significance. Model selection and model evaluation were performed according to state-of-the-art methods, including objective function value, parameter precision, goodness of fit, and simulation-based techniques (16). The results were visualized in R version 4.3.3 (RRID: SCR_001905) through RStudio graphical interface version 2023.12.1 (RRID: SCR_000432).

As trastuzumab-containing therapies may carry a risk for QT prolongation, the potential for cardiac effects of BI-1607 was explored through exposure–safety analysis for heart rate and Fridericia-corrected QT time. A linear mixed effects model approach was applied to explore a potential positive correlation between time-matched PK samples and ECG (17).

Results

Preclinical data

The binding specificity, FcγRIB-blocking, and function-modulating effects of the clinical BI-1607 candidate (aka 6G11-N297Q) have been previously described in detail (8). In this study, BI-1607's highly specific binding to selected human, murine, and cynomolgus FcγR proteins was verified by ELISA (**Fig. 1C**). BI-1607 bound with high affinity to human FcγRIIB and showed no binding to other FcγR proteins, indicating that BI-1607 is specific for human FcγRIIB and does not cross-react with closely related proteins. The lack of binding of BI-1607 to the FcγRIA receptor, which binds with high affinity to IgG, confirmed the FcγR binding–impairing effect of the single mutation (N297Q) in BI-1607's Fc domain. In contrast, BI-1206, a mAb identical to BI-1607 in the variable Fab domain but with a WT Fc domain, binds with expected affinity to FcγRIA. Consistent with BI-1607's Fc domain having been engineered for impaired FcγR binding, functional assessment of BI-1607 indicated no *in vitro* ADCC or *in vivo* B-cell–depleting activity (**Fig. 1D** and **E**).

When preclinically evaluating BI-1607 efficacy, the murine surrogate antibody (mBI-1607) improved the therapeutic effect of two tumor-targeting antibodies in relevant tumor antigen-expressing mouse models. The therapeutic effect of anti-HER2 in combination with mBI-1607 was compared with that of an isotype control antibody and with anti-HER2 as a single treatment in the TUBO breast cancer model. Combination with mBI-1607 delayed tumor growth and increased survival compared with anti-HER2 single-agent treatment (**Fig. 1F**; Supplementary Fig. S1). Tumor single-cell suspensions analyzed for immune cell content by FACS showed that the combined treatment of anti-HER2 and mBI-1607 resulted in increased $CD11b^{++}$ $F4/80^{++}$ cells compared with single-agent anti-HER2 treatment (**Fig. 1G**). In addition, an increase in infiltration of monocytes, NK cells, and B cells was seen in this group (Supplementary Fig. S2). In the B16F10 melanoma model, mBI-1607 improved the antitumor effect of the B16F10-specific TA99 antibody. This was shown by significantly lower tumor burden in the lungs of mice treated with TA99 in combination with mBI-1607 compared with isotype control or to TA99 single-agent treatments (**Fig. 1H**).

Patient characteristics and disposition

A total of 18 participants were included in the dose-escalation cohorts (75, 225, 500, 900, and 700 mg every 3 weeks) with a median age of 63 years (range, 46–77), and four (22%) were male (**Table 1**). The most common tumor types included breast tumors (44%), followed by gastro-gastroesophageal (33%). The extent and nature of prior cancer therapy varied, but all patients had previously received trastuzumab therapy. An overview of patient representation can be found in Supplementary Table S1.

Dose escalation and safety

Dose escalation proceeded stepwise until the top dose was reached. One patient at the 900-mg BI-1607 dose experienced a DLT leading to treatment discontinuation (rash, grade 3). A 700 mg every 3 weeks dose level was included in the protocol as an additional escalation dose. As this dose level was well tolerated, 700 mg per 3 weeks was determined as the higher end of the recommended dose range for further exploration in subsequent phase 2 studies.

Of the 18 subjects in the safety population, 17 (94.4%) experienced at least one treatment-related AE (TEAE) with no dose-dependent trends observed (**Table 2**). The full list of TEAE by system organ class stratified by dose level is provided in Supplementary Table S2. Nonetheless, most subjects experienced TEAE with a maximum grade of grade 1 (five subjects; 27.8%) or grade 2 (seven subjects; 38.9%). Grade ≥3 TEAE occurred in five patients (28%), with only three treatment-emergent serious AE, all unrelated to the study drugs, being reported in a total of two patients, namely cholangitis, acute kidney injury, and aggravated condition.

Premedication for IRR was allowed; however, it was recommended only if medically indicated. IRR were reported in eight subjects (44%), including erythema, infusion site pruritus, infusion site urticaria, rash, rash macular, rash maculopapular, and urticaria, and all were related to the study drugs. IRR were managed with intravenous corticosteroids and reduction of the infusion rate, which allowed drug administration to be resumed in all patients except for a patient with grade 3 urticaria, which was described as a DLT.

However, only two grade 3 IRR were recorded in two patients in the 900-mg cohort. Concentration–QT analysis revealed no

Table 1. Patient demographics and tumor response.

Cohort	Age/sex	Disease/year of diagnosis	No. prior treatments	Prior trastuzumab treatment	Prior TDX treatment	Last treatment prior to study	Duration on study in cycles	Best response on the study
Cohort 1	57/F	Breast/2002	21	16	1	TRAS + TUCA	2	PD
(75 mg)	53/F	Breast/2018	6	3	2	TDX	2	NE (PD)[a]
	63/F	Uterus/2018	6	1	—	BEV	2	NE (PD)[a]
Cohort 2	72/F	Breast/2011	10	5	1	TDX	2	PD
(225 mg)	70/F	Uterus/2020	3	2	—	TRAS + DUO	2	NE (PD)[a]
	65/F	Breast/2011	4	2	1	TDX	6	SD
Cohort 3	53/F	Breast/2007	7	3	1	TDX + ZO + LET	7	SD
(500 mg)	55/M	Paget's disease extramammary/2021	2	1	—	TRAS + PAC	2	NE (PD)[a]
	62/M	Esophagus/2023	2	1	—	DOC + FLU + FOL + OXA	1	NA
Cohort 4	46/F[b]	Breast/2016	3	2	1	TDX	1	NA
(900 mg)	77/F	GEJ/2020	4	1	1	TDX	3	SD
	48/F	Breast/2013	11	7	1	TRAS + DOC	9	SD
Cohort 5	69/M	Stomach/2018	3	2	—	RAM + PAC	2	NE (PD)[a]
(700 mg)	63/F	Breast/2015	5	2	—	TDM-1 + FUL	4	SD
	64/F	Stomach/2021	2	1	—	RAM + PAC	1	NA
	62/F	Colorectal/2017	6	1	—	IRI	4	SD
	72/M	Gastroesophageal adenocarcinoma/2017	5	4	—	TRAS	4	SD
	71/F	Stomach 2019	3	1	1	Clinical trial ADC	2	NE (PD)[a]

Abbreviations: ADC, antibody–drug conjugate; BEV, bevacizumab; DOX, docetaxel; DUO, duocarmazine; FLU, fluorouracil; FO, folinic acid; FUL, fulvestrant; GEJ, gastroesophageal junction; IRI, irinotecan; LET, letrozol; NA, not available; NE, not evaluable; OXA, oxaliplatin; PAC, paclitaxel; PD, progressive disease; RAM, ramucirumab; SD, stable disease; TDM-1, trastuzumab emtansine; TDX, trastuzumab deruxtecan; TRAS, trastuzumab; TUCA, tucatinib; ZO, Zometa.
[a]Patient did not meet the criteria for response evaluation because of progressive disease prior to the 42 days landmark.
[b]Patient received 380-mg dose.

statistically significant positive correlation between time-matched PK measurements and heart rate or Fridericia-corrected QT time captured from triplicate ECG (**Fig. 2**).

No significant depletion of CD19+ B cells was observed in the clinical study, as expected given that BI-1607 was engineered for impaired FcγR binding (Supplementary Fig. S3).

Antitumor activity

Of the 18 treated patients, nine met the criteria for response evaluation (see the methods section). Tumor progression in target lesions was observed in two patients, both from the low-dose cohort groups (**Fig. 3**). Although none achieved an objective response, seven patients (78%) exhibited stable disease, with the longest duration of 6 months.

Table 2. TEAE in all treated patients ($n = 18$).

	BI-1607					
	Dose cohort					
Category	75 mg	225 mg	500 mg	900 mg	700 mg	Total
Number of subjects	3	3	3	3	6	18
Subjects with at least one TEAE of any grade	3 (100%)	3 (100%)	3 (100%)	3 (100%)	5 (83%)	17 (94%)
Subjects with ≥grade 3 TEAE	0	0	1 (33%)	2 (66%)	2 (33%)	5 (28%)
Subjects with TEAE related to BI-1607	2 (66%)	2 (66%)	3 (100%)	3 (100%)	3 (50%)	13 (72%)
Subjects with TEAE related to trastuzumab	2 (66%)	3 (100%)	3(100%)	0	2 (33%)	10 (56%)
Subjects with ≥grade 3 TEAE related to study BI-1607	0	0	0	2 (66%)	1 (17%)	3 (17%)
Subjects with ≥grade 3 TEAE related to trastuzumab	0	0	0	0	1 (17%)	1 (6%)
Subjects with serious TEAE related to study treatment	0	0	0	0	0	0
Subjects with treatment-related AE that led to discontinuation	0	0	0	1 (33%)	0	1 (6%)
TEAE of interest related to BI-1607 in combination with trastuzumab						
Subjects with any-grade IRR	1 (33%)	2 (66%)	1 (33%)	3 (100%)	1 (17%)	8 (44%)
Subjects with ≥grade 3 IRR	0	0	0	2 (66%)	0	2 (11%)
Subjects with ≥grade 3 increased ALT/AST	0	0	0	0	1 (33%)	1 (6%)

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase.

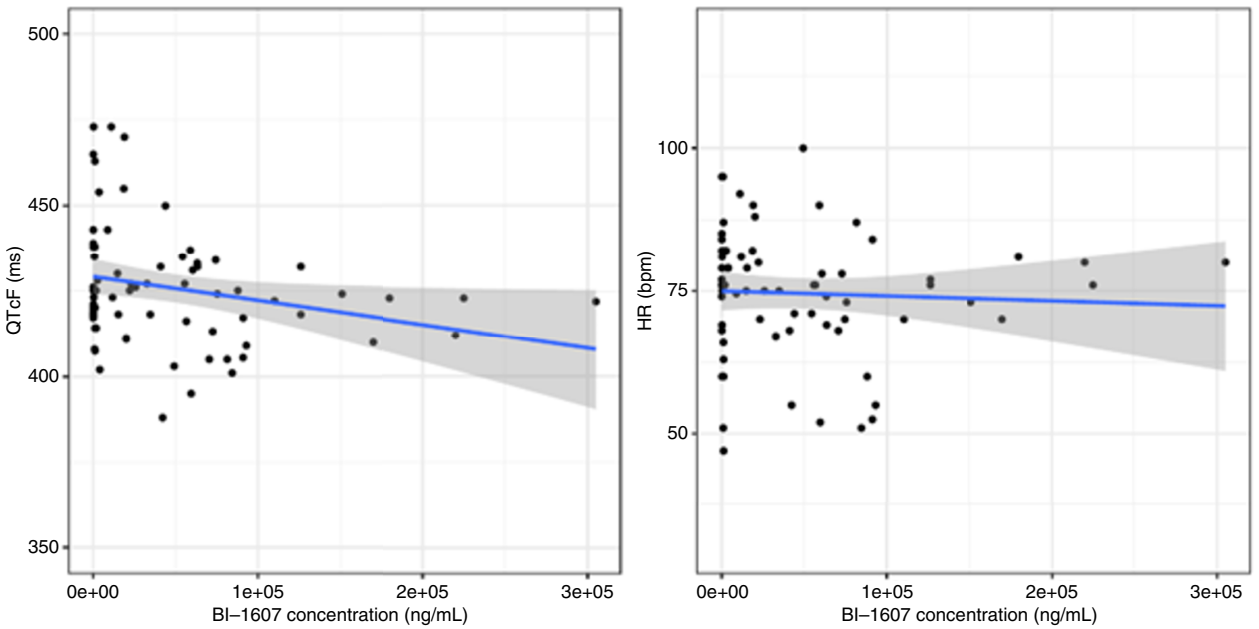

Figure 2.

Fridericia-corrected QT time (QTcF) and heart rate vs. BI-1607 serum concentration. The black dots show individual data points, the blue line depicts the linear mixed-effects model fit, and the gray shaded area represents the model's 95% confidence interval.

Of the remaining nine patients, three dropped out of the study before response assessments, and six exhibited progressive disease, although before the 42-day landmark, and thus could not be included in the response population but are reported here for completeness.

PK and RO

PK and FcγRIIB RO levels from 18 patients were available for model building. A clear nonlinear increase in serum BI-1607 levels was observed with dose, as well as a dose-dependent increase in RO, reaching a maximum that was sustained during the whole dosing interval at doses of 700 mg and higher (**Fig. 4**).

A two-compartment model with Michaelis–Menten elimination provided an adequate description of the nonlinear PK behavior, with reasonable parameter estimates and precision (Supplementary Fig. S4). Accounting for additional complexities, such as target-mediated disposition, did not improve model performance ($P > 0.05$). A value of 6,100 ng/mL was estimated for K_M (concentration at which 50% of the maximum elimination rate is attained). This concentration is reached already at the lowest dose level of 75 mg, suggesting linear PK at clinically relevant doses.

Regarding FcγRIIB RO, data were well described with a direct saturable model driven by serum BI-1607 concentrations, reporting maximum RO of 80.8% and a low value of C_{50} (concentration at which 50% of maximum RO is attained) of 637 ng/mL. Variability was only supported by the linear clearance term, the volume of the central compartment, and the maximum RO. Given the low number of patients and their heterogeneity, no meaningful covariates were identified.

Immunogenicity and biomarkers

Across all evaluable patients, no signs of immunogenicity were observed, and no treatment-emergent or treatment-induced anti-drug antibodies against BI-1607 were detected.

Cytokines were analyzed in the peripheral blood of a subset of patients up to 24 hours after infusion, and no analytes were affected by BI-1607/trastuzumab infusion. Cytokines analyzed included IFNγ, IL10, IL6, IL8, MIP-1β, TNFα, IL1β, IL23, IL12p70, IL2, IL4, TARC, and VEGF. The absence of cytokines has previously been confirmed in several *in vitro* assays of BI-1607 and peripheral blood mononuclear cells (Supplementary Figs. S5–S8).

The effect of *FCGR2A*, *FCGR2B*, *FCGR2C*, and *FCGR3A* polymorphisms on clinical response to BI-1607/trastuzumab treatment was analyzed. No association was found between polymorphisms and response to treatment, but given the low number of patients, the results are at this time inconclusive.

Discussion

The efficacy of tumor-targeting and immunomodulatory mAb is determined by the interaction of their Fc domain with Fc receptors that can be either activating (i.e., FcγRI, FcγRIIA, or FcγRIIIA) or inhibitory (FcγRIIB; refs. 18, 19). Accordingly, Fc-engineering to enhance binding to activating FcγR is a validated approach to increase tumor-targeting antibody activity, e.g., anti-CD20 (20). Here, we report the first human data on an alternative strategy, namely blocking the single inhibitory FcγRIIB to enhance therapeutic antibody efficacy (8, 18). More specifically, we demonstrate that BI-1607, a first-in-class Fc-inactivated mAb to human FcγRIIB, is well tolerated across doses ranging from 75 up to 900 mg every 3 weeks, and adequate exposure is associated with complete and sustained FcγRIIB cell-surface receptor saturation.

This approach has several potential advantages to Fc-engineering for enhanced binding to activating FcγR. First, regarding efficacy, the inhibitory FcγRIIB is strongly upregulated on tumor and effector cells in resistant niches, for example, in the bone marrow of

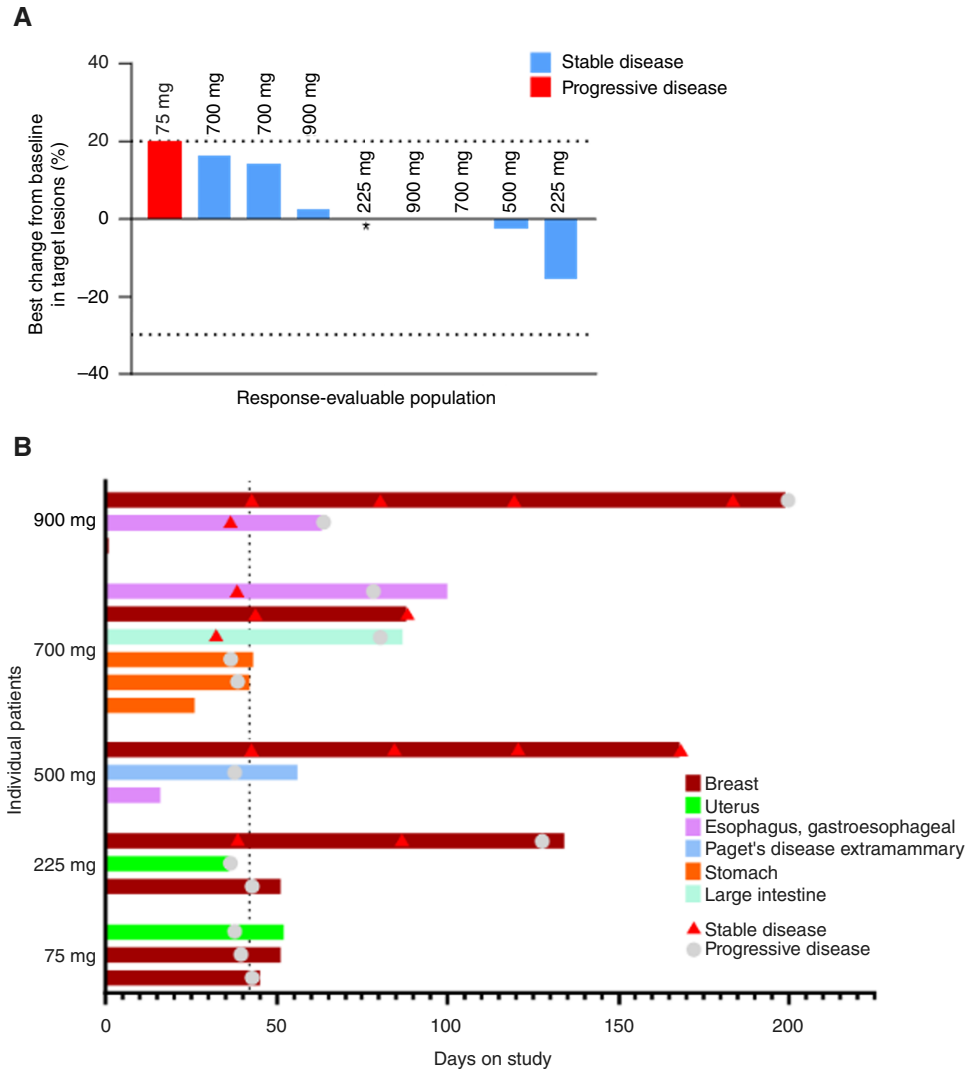

Figure 3.

Tumor response. **A,** Waterfall plot of the best overall responses in the evaluable patient population with respect to tumor size. Patients with at least one valid postdose assessment (CT/PET or MRI scan) at day 42 or later were included in the evaluable patient population ($n = 9$). * indicates the patient with the best overall response progressive disease despite no increase in target lesion. **B,** Swimmer plot of time to tumor responses in all treated patients ($n = 18$). The dotted line in the swimmer plot is day 42 after initial treatment.

patients with leukemia (21) and the solid cancer tumor microenvironment (22), promoting resistance to antibody therapy (22–24). The activating FcγR, in contrast, are heterogeneously expressed by different cell types and may be downregulated in tumors (22). Targeting FcγRIIB has the potential to enhance effector cell activation and cytotoxic efficacy regardless of which activating FcγR (i.e., FcγRI, FcγRIIA, or FcγRIIIA) are expressed in a particular patient or a patient's tumor (2).

Second, regarding tumor selectivity and tolerability, FcγRIIB is expressed only on ~ 5% of circulating monocytes, not NK cells or granulocytes (8). This is in sharp contrast with the expression of activating FcγR including FcγRIIIA, targeted by most antibody engineering approaches (e.g., by afucosylation or amino acid substitution), which are abundantly expressed on proinflammatory immune effector cells in the blood (22). As the engagement of activating FcγR on blood effector cells is associated with decreased tolerability and occurrence of IRR (25), specific targeting of FcγRIIB should provide both a safer and more efficacious approach to enhance the activity of the combining agent.

In this regard, this approach could potentially be extended to other tumor-targeting mAb with activity dependent on antibody-dependent cellular phagocytosis. FcγRIIB blockade could provide a means to enhance multiple validated tumor-targeting and immune-modulatory antibodies with FcγR-dependent mechanisms of action. In preclinical experiments, using BI-1607's murine surrogate antibody and immune-competent mouse tumor models, we demonstrate *in vivo* proof-of-concept that FcγRIIB blockade enhances tumor-targeting antibodies relevant to breast cancer and metastatic melanoma, indicating a broad therapeutic potential. Our preclinical data demonstrated that FcγRIIB blockade, although not exhibiting antitumor efficacy of its own, does enhance anti-HER2 efficacy. Interestingly, our preclinical data showed that combined FcγRIIB blockade with subtherapeutic "low" anti-HER2 doses, which targeted only a fraction of tumor-expressed HER2 receptors, also resulted in increased animal survival. This suggests that anti-FcγRIIB may be a means to enhance the efficacy of tumor-targeting antibodies and extend it to lower antigen–expressing cancers, such as low HER2–expressing tumors. Our ongoing efforts aim to evaluate this potential.

Whereas the introduction of trastuzumab has dramatically changed outcomes in patients with HER2-positive cancer, primary

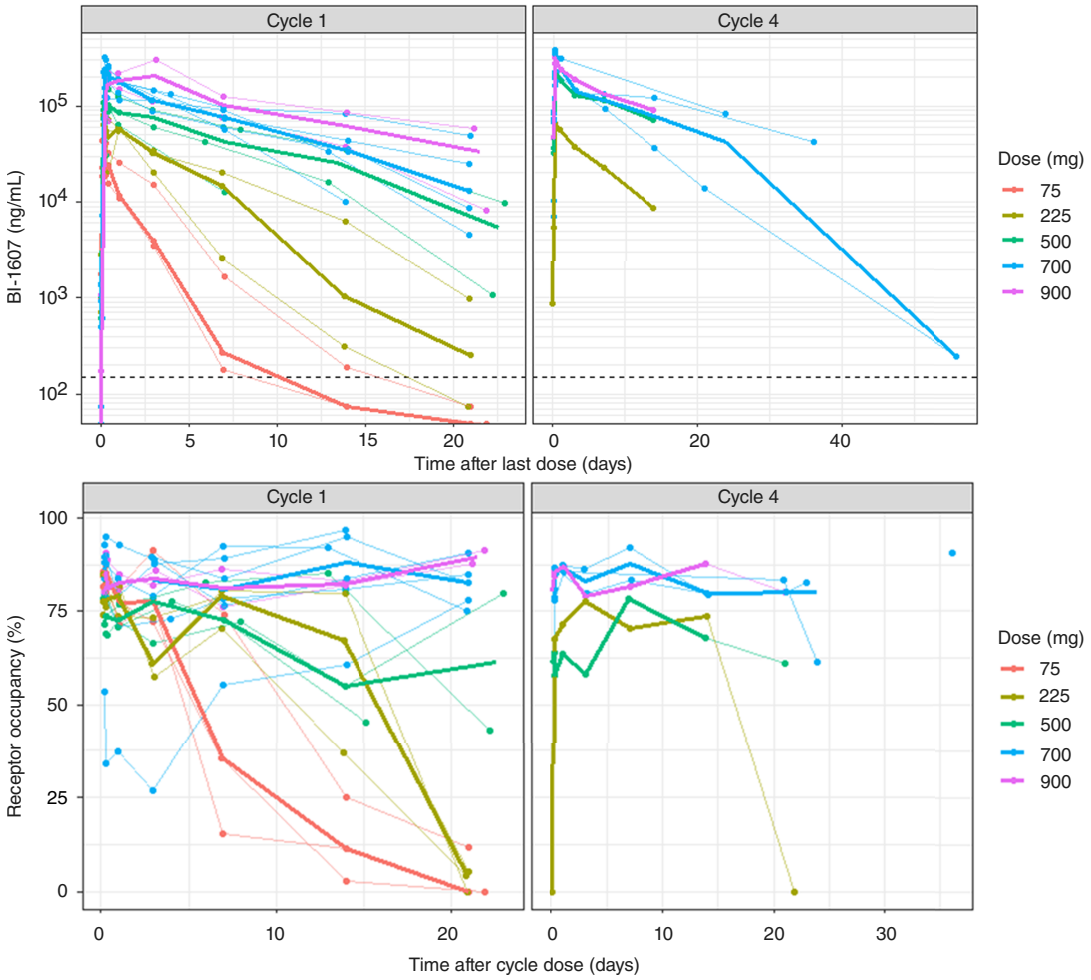

Figure 4.

Time course of serum BI-1607 concentrations and CD32 RO. Colored lines represent the median of the raw data at each dose level and cycle. The dashed solid line in the top represents the lower limit of quantification (LLOQ). Note, first data below the LLOQ are set to LLOQ/2, and the rest are set to 0.

or acquired resistance to trastuzumab has been recognized as a significant obstacle in the clinical management of this disease. Combining anti-HER2 antibodies with other immunotherapies will likely improve the quantity and quality of responses. Our findings from this FIH study demonstrate that BI-1607 exhibits a desirable PK behavior and is well-tolerated by human patients with cancer at doses associated with complete and sustained FcγRIIB saturation (500 mg and above), supporting that FcγRIIB blockade is an attractive strategy to enhance treatment efficacy. Most of the AE observed were mild or moderate (Common Terminology Criteria for AE grade 1 or 2). IRR occurred only during cycle 1; one subject who experienced grade 3 urticaria (DLT) was withdrawn from further study treatment. All other subjects who experienced an IRR continued with the study, and IRR were not observed in subsequent cycles. In most cases, dexamethasone was added as premedication following the occurrence of an IRR. Three subjects (17%) had serious AE reported; all were unrelated to the study treatment. Four deaths were reported due to disease progression, condition aggravation, and acute kidney injury, all unrelated to the study treatment. Trastuzumab treatment is associated with cardiotoxicity, primarily related to inhibiting HER2-mediated cardioprotective effects in cardiomyocytes, but may also involve recruitment of inflammatory cells (26). The fact that no enhancement of trastuzumab toxicity was observed in BI-1607–treated patients demonstrates the safe nature of sustained FcγRIIB blockade and is consistent with the notion that BI-1607 selectively enhances FcγR-dependent antibody functions in body compartments in which FcγRIIB is upregulated (e.g., in tumor tissue). These observations further identify FcγRIIB blockade as an attractive approach to enhance the efficacy of clinically validated mAb in a tumor-selective manner (27).

Our choice to study BI-1607 in combination with trastuzumab relates to its potential use in many other solid tumors. Previously, margetuximab, which has been Fc-engineered for a higher A:I ratio but which retains FcγRIIB binding (4), was approved in combination with chemotherapy for the treatment of patients with metastatic breast cancer who had received at least two anti-HER2 regimens. Our preclinical findings with mBI-1607 demonstrated enhanced therapeutic activity of the well-characterized anti-HER2 (28) antibody 16.7.4 in the high A:I mIgG2a isotype format, supporting the notion that BI-1607 can enhance not only WT IgG Fc

antibodies, such as trastuzumab and zanidatamab, but also Fc-engineered antibodies like margetuximab. In early lines of therapy, such BI-1607–enhanced therapies could provide attractive treatment alternatives to antibody–drug conjugates, with a favorable safety profile. Finally, antibodies often used for the treatment of hematologic malignancies, such as rituximab or obinutuzumab, could also benefit from FcγRIIB blockade. This hypothesis is currently being tested in the ongoing clinical study (NCT03571568) in which BI-1206, a fully competent IgG1 mAb directed against FcγRIIB, is being combined with rituximab for the treatment of non–Hodgkin lymphoma.

Concentration–QT analysis showed no contribution to QT time caused by BI-1607. Population PK/PD analysis was performed to quantitatively describe the observed nonlinear PK, common in mAb due to the target-mediated disposition (29). Interestingly, the estimated K_M value was one order of magnitude higher than the concentration at which 50% of the maximum RO is attained. This result suggests that the circulating B cells might not be the main driver of drug nonlinear clearance from the system. Instead, other FcγRIIB-expressing cells, such as liver Kupffer and sinusoidal endothelial cells, might be involved and act as a sink (30, 31). In any case, linearity is observed in well-tolerated and therapeutically relevant doses above 500 mg. Regarding RO, a direct and saturable relationship driven by plasma BI-1607 levels was identified, with an estimated C_{50} in line with preliminary *ex vivo* studies (Supplementary Fig. S9). Given this direct relationship and the low C_{50} value (637 ng/mL), maximum RO is practically attained instantaneously after intravenous infusion for all doses explored. Thus, sequential administration of the drugs, with BI-1607 given first, is sufficient; earlier administration of BI-1607 is unnecessary. More importantly, a sustained and almost complete (>90%) receptor saturation can be attained over 14 days at doses of 500 mg and above or even over 21 days at doses of 700 mg and above.

Although no objective responses were observed in enrolled patients and given the recent change in the treatment paradigm of HER2-positive breast cancer, the dose-expansion phase was not initiated. However, several patients in this heavily pretreated population exhibited disease control for an extended period. Two patients with breast cancer receiving 500- or 900-mg doses and who had previously received seven and 11 prior lines of treatment—that included trastuzumab (three and seven treatment lines, respectively), TDM-1 (one and two treatment lines, respectively), and trastuzumab deruxtecan (one treatment line each)—had stable disease for over seven and nine cycles, respectively (or 5 and 6 months, respectively). We should, however, acknowledge certain study limitations, including the small sample size, the use of archival tissue samples to confirm HER2-positive status in the absence of performed biopsies at screening in nearly all patients, and the limited number of participants with any one tumor type.

In conclusion, data presented in this FIH study demonstrate that FcγRIIB blockade can be safely achieved and combined with an FcγR-engaging therapeutic antibody, both administered at therapeutically relevant doses. Moreover, our supporting preclinical data demonstrate that FcγRIIB blockade can enhance the efficacy of antibodies relevant to different cancers and extend their use to additional cancer types and patients with cancer. As such, FcγRIIB blockade is a potentially safe and well-tolerated strategy that could bring the clinical benefit of validated antibody drugs to more cancer types and patients, though further studies are required.

Data Availability

Data can be made available upon reasonable request to the corresponding author. Due to patient privacy concerns, they are not publicly available.

Authors' Disclosures

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Authors' Contributions

J. Cortés: Conceptualization, resources, investigation, writing–review and editing. **A. Priego:** Resources, investigation, writing–review and editing. **E. Garralda:** Resources, investigation, writing–review and editing. **K. Rojas:** Resources, investigation, writing–review and editing. **S.R. Lord:** Resources, investigation, writing–review and editing. **T.O. Goetze:** Resources, investigation, writing–review and editing. **S. Kuemmel:** Resources, investigation, writing–review and editing. **S.J. Crabb:** Resources, investigation, writing–review and editing. **Z.P. Parra-Guillén:** Data curation, formal analysis, investigation, writing–original draft, writing–review and editing. **M. Borggren:** Data curation, formal analysis, investigation, methodology, writing–original draft, writing–review and editing. **I. Karlsson:** Conceptualization, formal analysis, supervision, investigation, methodology, writing–original draft, writing–review and editing. **D. Lindahl:** Data

curation, formal analysis, investigation, writing–original draft, writing–review and editing. **L. Mårtensson:** Formal analysis, investigation, methodology, writing–original draft, writing–review and editing. **R. Oldham:** Resources, investigation, writing–review and editing. **A. Ropenga:** Conceptualization, data curation, formal analysis, investigation, writing–original draft, project administration, writing–review and editing. **I. Teige:** Conceptualization, formal analysis, supervision, investigation, methodology, writing–original draft, writing–review and editing. **J. Wallin:** Conceptualization, formal analysis, supervision, investigation, methodology, writing–original draft, writing–review and editing. **B. Frendeus:** Conceptualization, resources, formal analysis, supervision, investigation, methodology, writing–original draft, writing–review and editing. **A. McAllister:** Conceptualization, resources, formal analysis, supervision, investigation, methodology, writing–original draft, writing–review and editing.

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Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Table S1. Representativeness of study participants.

Cancer type	HER2-Positive Solid Tumors
Considerations related to:	
Sex	In 2022, it was estimated that there were almost 20 million cancer cases worldwide. Of these, 10.3 million cases were in men (accounting for ~51.6% of cases) and 9.7 million cases in women (accounting for ~48.4% of cases)[1].
Age	Advancing age is the most important risk factor for cancer overall and for many individual cancer types. The incidence rates for cancer overall climb steadily as age increases, from fewer than 26 cases per 100,000 people in age groups under age 20, to more than 1,000 per 100,000 people in age groups 60 years and older [2]. In the US, the median age at diagnosis is 67 years [2]. Similar trends are also observed in Europe [1].
Race/Ethnicity	In the U.S., White patients have the highest rate of new cancer diagnoses across all cancer types, Black patients have higher cancer death rates, and Asian/Pacific Islander patients have the lowest rates[3]. In Europe, incidence and mortality rates are available by country, socio-economic status, sex or age, but not by race.
Geography	Cancer is the second leading cause of death both in EU and US [4,5].
Other considerations	Solid tumors represent the majority of newly diagnosed cancers with lung, breast, colorectum, prostate and stomach covering almost 50 % of new cases among both sexes and ages in 2022[1]. In a pan-tumor study carried out in samples from over 65,000 patients [6], a HER2+ (IHC 3+) positivity rate of ~ 3 % was reported, with the highest prevalence in bladder (13.9 %), uterus (13.6 %), esophagogastric junction (12.1 %), breast (7.8 %) and gastric cancers (6.6 %). Participants enrolled in the study were diagnosed with HER2+ breast (n=8 patients), uterus (n=2), stomach (n=3), colorectal (n=1), Paget's disease extramammary (n=1) or gastroesophageal (n=3) cancer. Taking into account the small sample size, tumor representation in our study is aligned with global incidence of cancer and the prevalence of HER2+. In our study, 4 (22 %) of enrolled patients were male. This is due to the higher enrolment of breast and uterus cancer (10 out of 18, 55.6 %) compared to the other HER2+ cancer types. Approximately 99 % of breast cancers occur in women. From the remaining 8 patients, 4 were male (50 %)

	and 4 were female (50 %) aligned with the cancer incidence by sex, despite they mainly belong to digestive tumors, for which higher incidence in male (almost double in stomach) have been described compared to female [1]. The median age of participants in the study was 63 years (range 46-77), consistent with the higher incidence of cancer observed with age. However, it has been recognized that there is reduced inclusion or participation of older cancer patients in phase 1 clinical trials, which could affect the generalizability of the results of this study. This study was conducted across 3 countries, Spain (n=13), United Kingdom (n=3) and Germany (n=2). Participants in our study were mainly White and not Hispanic or Latino (15 subjects, 83.3 %), the other 3 were unknown or not reported. These figures are aligned with the background population distributions and race representations trends in European Breast cancer trials, featuring a White-predominant population (>90 %). Nonetheless, our study has a very small number of participants which accounts for it not being fully representative of the global cancer population demographics.

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1. Study Population

1.1. Inclusion Criteria

The following inclusion criteria must be met for a subject to be eligible for inclusion in the study:

Phase 1 and Phase 2a

1. Is willing and able to provide written informed consent for the trial.
2. Is ≥18 years of age on day of signing informed consent.
3. Can attend the clinical site for administration of the experimental treatment.
4. Has a HER2+ locally advanced unresectable or metastatic solid tumor and has received standard of care or is intolerant to standard of care antineoplastic therapy. Subjects who are intolerant to trastuzumab cannot be enrolled in the study.
5. Has at least 1 measurable disease lesion as defined by RECIST v1.1 criteria.
6. Has a locally confirmed HER2+ tumor (according to 2018 ASCO/CAP HER2 test guideline) by an accurate and validated assay (according to Herceptin®/Trazimera® SmPC/PI). This can be from the most recent archival tissue sample or new tissue material from a recently obtained surgical or diagnostic biopsy. Tissue obtained for the biopsy must not have been previously irradiated. Subjects who do not have an archival or new tissue sample at Screening may still be enrolled in the study provided HER2 positivity can be established.
7. Must have progressive disease after the last line of treatment. In addition, subjects must have received the following previous lines of treatment:
    a. Prior lines of treatment including trastuzumab and chemotherapy.
    b. At least one prior line of treatment with an antibody-drug conjugate (ADC) (eg, trastuzumab-emtansine [TDM-1, or trastuzumab-deruxtecan]) if it is part of the standard of care.
8. Has left ventricular ejection fraction ≥50%.
9. Has a life expectancy of ≥12 weeks.
10. Has an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
11. Has adequate organ function as confirmed by laboratory values listed in the table below.

Table 5: Hematological and Biochemical Indices for Eligibility

Laboratory Test	Value Required
Hemoglobin (Hb)	≥9.0 g/dL or ≥5.6 mmol/L (Criteria must be met without erythropoietin dependency and without packed red blood cell transfusion within last 2 weeks)
ANC	≥1500/μL
Platelet count	≥100,000/μL
Total bilirubin	≤1.5 ×ULN OR direct bilirubin ≤ ULN for subjects with total bilirubin levels >1.5 × ULN
ALT and AST	≤2.5 × ULN (≤5 × ULN for subjects with liver metastases)
Measured or calculated creatinine clearance (per institutional standard [GFR can also be used in place of creatinine or creatinine clearance])	≥30 mL/min for subject with creatinine levels >1.5 × institutional ULN
INR OR PT, aPTT	≤1.5 × ULN unless subject is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants

ALT=alanine aminotransferase; ANC=antinuclear antibody; aPTT=activated partial thromboplastin time; AST=aspartate aminotransferase; GFR=glomerular filtration rate; INR=international normalized ratio; PT=prothrombin time; ULN=upper limit of normal.

Phase 2a Only

In addition, the following requirements are necessary for a subject to be enrolled in Phase 2a:

12. **Cohort 1** (HER2+ locally advanced or metastatic breast cancer):
    a. Has histologically confirmed breast adenocarcinoma that is unresectable locoregional, or metastatic.
    c. Must have received a minimum of 1 and a maximum of 3 prior anti–HER2-based regimens with documented progression on the most recent regimen.
13. **Cohort 2** (HER2+ metastatic gastric or gastroesophageal junction adenocarcinoma):
    a. Has histologically confirmed metastatic gastric or gastroesophageal junction adenocarcinoma.
    d. Must have received a minimum of 1 and a maximum of 2 prior anti–HER2-based regimens with documented progression on the most recent regimen.

1.2. Exclusion Criteria

A subject who meets any of the following exclusion criteria will not be eligible for inclusion in the study:

1. Needs doses of prednisolone >10 mg daily (or equipotent doses of other corticosteroids) while on the trial other than as premedication. During the Screening period, doses of up

to 20 mg/day may be given, but the dose must be reduced to 10 mg/day within 7 days before the first dose of study drug. Steroids are allowed as premedication and in subjects with allergies to contrast scans.

14. Has known active CNS metastases and/or carcinomatous meningitis. Subjects with previously treated CNS metastases may participate provided they are radiologically stable (without evidence of progression for at least 4 weeks by repeat imaging; note that the repeat imaging should be performed during study screening); has no newly onset or worsening symptomatology of brain metastases; and has not required steroids for at least 14 days before study treatment.
15. Has known or suspected hypersensitivity or contraindication to trastuzumab (refer to Herceptin® /Trazimera® SmPC/PI), BI-1607, or any of their excipients. Previous isolated IRRs are not to be considered a reason for exclusion unless Grade 4 in intensity.
16. Has cardiac or renal amyloid light-chain amyloidosis.
17. Has received the following:
    a. Chemotherapy or small molecule products within 2 weeks of first dose of BI1607.
    e. Radiotherapy within 2 weeks of first dose of BI-1607. A 1-week washout is required for palliative radiation (≤2 weeks of radiotherapy) for non-CNS disease. Subjects who have previously had radiation pneumonitis are not allowed.
    f. Immunotherapy or biological therapy within 4 weeks or 5 half-lives of the respective drug, whichever is longer, before the first dose of BI1607.
18. Has not recovered from AEs to at least Grade 1 by NCI CTCAE v5.0 due to prior anticancer therapies. Exceptions to this are alopecia or certain Grade 1 toxicities that in the opinion of the Investigator should not exclude the subject. Subjects with ≤ Grade 2 neuropathy are eligible for inclusion.
19. Has had clinically significant lung disease requiring systemic corticosteroid treatment within the last 6 months of enrollment (eg, interstitial pneumonia, pneumonitis due to other causes other than radiation, and pulmonary fibrosis) or who are suspected to have these diseases by imaging at screening period or severe dyspnea at rest due to complications of advanced malignancy or requiring supplementary oxygen therapy.
20. Has an active, known, or suspected autoimmune disease. However, subjects with type 1 diabetes mellitus, hypothyroidism only requiring hormone replacement, skin disorders (such as vitiligo, mild psoriasis, or alopecia not requiring systemic treatment), or conditions not expected to recur in the absence of an external trigger will be permitted to participate.
21. Is a female subject and has the ability to become pregnant (or already pregnant or lactating/breastfeeding). Subjects should not breastfeed during treatment and for 7 months after the last dose of study treatment. However, those female subjects who have a negative serum or urine pregnancy test before enrollment and agree to use a highly effective method of birth control for 4 weeks before entering the trial, during the trial,

and for 12 months after last dose of BI-1607 are considered eligible. Highly effective methods of birth control include:

a. Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation:
    - Oral
    - Intravaginal
    - Transdermal
g. Progestogen-only hormonal contraception associated with inhibition of ovulation:
    - Oral
    - Injectable
    - Implantable
h. Intrauterine device
i. Intrauterine hormone-releasing system
j. Bilateral tubal occlusion
k. Vasectomized partner (given that the partner is the sole sexual partner of the subject and that the vasectomized partner has received medical assessment of the surgical success).
l. Sexual abstinence (defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the preferred and usual lifestyle of the subject).

Note: A woman of childbearing potential is defined as any female who has experienced menarche, who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy), and who is not postmenopausal. Menopause is defined as 12 months of amenorrhea in a woman over age 45 in the absence of other biological or physiological causes. In addition, females under the age of 55 years must have a documented serum follicle stimulating hormone level >40 mIU/mL to confirm menopause.

22. Is a male subject with partner(s) of childbearing potential (unless he agrees to use a barrier method of contraception [condom plus spermicidal gel] with the female partner(s) who is using one highly effective method of contraception during the trial and for 12 months after completing treatment). Men with pregnant or lactating partners should be advised to use barrier method contraception (condom plus spermicidal gel) to prevent exposure to the fetus or neonate. All males should refrain from sperm donation for 12 months after last dose of study drug.
23. Has had major surgery from which the subject has not yet recovered.
24. Is at high medical risk because of nonmalignant systemic disease including severe active infections on treatment with antibiotics, antifungals, or antivirals.
25. Has presence of chronic graft versus host disease.

26. Has had an allogenic tissue/solid organ transplant.
27. Has evidence of chronic active hepatitis B virus (HBV) infection (not including subjects with prior hepatitis B vaccination or positive serum hepatitis B surface antibody) or chronic active hepatitis C virus (HCV) infection or known history of HIV. If hepatitis B core antibody or HCV antibody is positive, the subject must be evaluated for the presence of HBV DNA, or HCV RNA by PCR. Subjects with a history of chronic HCV whose viral load has become negative with adequate medical treatment can be enrolled.
28. Has received a live vaccine within 30 days before the first dose of study treatment. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed. Similarly, COVID-19 vaccines based on viral RNA or protein fragments, or killed viruses, are allowed. COVID-19 vaccines based on live replicating viral or bacterial vectors (eg, adenoviruses, adeno-associated viruses, vesicular stomatitis virus, vaccinia viruses, or measles virus) are not allowed.
29. Has uncontrolled or significant cardiovascular disease including, but not limited to, any of the following:
    a. Myocardial infarction or stroke/transient ischemic attack within the past 6 months.
    m. Uncontrolled angina within the past 3 months.
    n. Any history of clinically significant arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or torsades de pointes).
    o. QT interval prolongation >480 msec (corrected for heart rate using Fridericia's formula [QTcF]).
    p. History of other significant heart disease (eg, cardiomyopathy, congestive heart failure with New York Heart Association functional classification III or IV, pericarditis, significant pericardial effusion, or myocarditis).
30. Has a known psychiatric or substance abuse disorder that would interfere with the subject's ability to cooperate with the requirements of the study.
31. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the study, interfere with the subject's participation for the full duration of the study, or is not in the best interest of the subject to participate, in the opinion of the treating Investigator.
32. Is participating or planning to participate in another interventional clinical trial, or has participated in a trial of an investigational agent or has used an investigational device within 4 weeks or 5 half-lives of the respective drug, whichever is longer, before first dose of study drug. Subjects who have entered the follow-up phase of an investigational study may participate as long as it has been 4 weeks or 5 half-lives of the respective drug,

whichever is longer, after the last dose of the previous investigational agent. Participation in an observational trial is acceptable.

33. Has a known additional malignancy of another type, except for adequately treated cone-biopsied carcinoma in situ (eg, breast carcinoma, cervical cancer in situ), adequately controlled superficial bladder cancer, and basal or squamous cell carcinoma of the skin. Male subjects with asymptomatic prostate cancer without known metastatic disease and with no requirement for therapy or requirement for therapy or requiring only hormonal therapy and with normal prostate-specific antigen for >1 year before start of trial therapy are eligible. Subjects who have undergone potentially curative therapy for a prior malignancy, have no evidence of that disease for ≥1 year and are deemed at negligible risk for recurrence are also eligible.
34. Has a diagnosis of primary or acquired immunodeficiency disorder or is taking any other form of immunosuppressive therapy within 7 days or 5 half-lives of the respective drug, whichever is longer, before the first dose of study drug.
35. Has active or chronic corneal disorder, including but not limited to Sjogren's, Fuch's corneal dystrophy, history of corneal transplantation, active herpetic keratitis, as well as other active ocular conditions requiring ongoing therapy. Has any clinically significant corneal disease that prevents adequate monitoring of drug-induced keratopathy.

Table S2: Treatment-Emergent Adverse Events (TEAE) by System Organ Class and Preferred Term for Phase 1

	Dose Cohort					
System Organ Class	**75 mg**	**225 mg**	**500 mg**	**700 mg**	**900 mg**	**Total**
Preferred Term, n (%)	**(N = 3)**	**(N = 3)**	**(N = 3)**	**(N = 6)**	**(N = 3)**	**(N = 18)**
Subjects with at Least 1 TEAE	3 (100)	3 (100)	3 (100)	5 (83.3)	3 (100)	17 (94.4)
Blood And Lymphatic System Disorders	2 (66.7)	2 (66.7)	1 (33.3)	3 (50.0)	0	8 (44.4)
Anaemia	2 (66.7)	0	0	3 (50.0)	0	5 (27.8)
Neutropenia	0	1 (33.3)	1 (33.3)	1 (16.7)	0	3 (16.7)
Thrombocytopenia	0	1 (33.3)	0	0	0	1 (5.6)
General Disorders and Administration Site Conditions	2 (66.7)	2 (66.7)	0	3 (50.0)	1 (33.3)	8 (44.4)
Asthenia	2 (66.7)	2 (66.7)	0	1 (16.7)	0	5 (27.8)
Condition aggravated	0	0	0	1 (16.7)	0	1 (5.6)
Fatigue	0	0	0	1 (16.7)	0	1 (5.6)
Infusion site pruritus	0	0	0	0	1 (33.3)	1 (5.6)
Infusion site urticaria	0	0	0	0	1 (33.3)	1 (5.6)
Pyrexia	1 (33.3)	0	0	0	0	1 (5.6)
Gastrointestinal Disorders	3 (100)	2 (66.7)	1 (33.3)	1 (16.7)	0	7 (38.9)
Ascites	0	1 (33.3)	1 (33.3)	1 (16.7)	0	3 (16.7)
Abdominal pain	1 (33.3)	1 (33.3)	0	0	0	2 (11.1)
Abdominal pain upper	1 (33.3)	0	0	0	0	1 (5.6)
Constipation	1 (33.3)	0	0	0	0	1 (5.6)
Diarrhoea	0	1 (33.3)	0	0	0	1 (5.6)
Flatulence	0	0	0	1 (16.7)	0	1 (5.6)
Gingival bleeding	0	1 (33.3)	0	0	0	1 (5.6)
Investigations	1 (33.3)	0	2 (66.7)	3 (50.0)	1 (33.3)	7 (38.9)
Aspartate aminotransferase increased	0	0	2 (66.7)	2 (33.3)	0	4 (22.2)
Alanine aminotransferase increased	0	0	1 (33.3)	1 (16.7)	0	2 (11.1)
Blood alkaline phosphatase increased	0	0	0	1 (16.7)	0	1 (5.6)
Blood bilirubin increased	0	0	0	1 (16.7)	0	1 (5.6)
Blood creatinine increased	1 (33.3)	0	0	0	0	1 (5.6)
Platelet count decreased	0	0	1 (33.3)	0	0	1 (5.6)
Transaminases increased	0	0	0	0	1 (33.3)	1 (5.6)
White blood cell count decreased	0	0	1 (33.3)	0	0	1 (5.6)

System Organ Class Preferred Term, n (%)	Dose Cohort 75 mg (N = 3)	 225 mg (N = 3)	 500 mg (N = 3)	 700 mg (N = 6)	 900 mg (N = 3)	 Total (N = 18)
Skin and Subcutaneous Tissue Disorders	2 (66.7)	2 (66.7)	0	1 (16.7)	1 (33.3)	7 (38.9)
Rash maculo-papular	0	0	0	1 (16.7)	1 (33.3)	2 (11.1)
Urticaria	1 (33.3)	1 (33.3)	0	0	0	2 (11.1)
Dry skin	1 (33.3)	0	0	0	0	1 (5.6)
Erythema	0	1 (33.3)	0	0	0	1 (5.6)
Pruritus	0	1 (33.3)	0	0	0	1 (5.6)
Rash	0	0	0	0	0	1 (5.6)
Rash macular	0	1 (33.3)	0	0	0	1 (5.6)
Metabolism and Nutrition Disorders	1 (33.3)	0	0	2 (33.3)	0	3 (16.7)
Hypoglycaemia	1 (33.3)	0	0	1 (16.7)	0	2 (11.1)
Hypocalcaemia	0	0	0	1 (16.7)	0	1 (5.6)
Hypokalaemia	0	0	0	1 (16.7)	0	1 (5.6)
Musculoskeletal and Connective Tissue Disorders	2 (66.7)	1 (33.3)	0	0	0	3 (16.7)
Musculoskeletal chest pain	2 (66.7)	0	0	0	0	2 (11.1)
Back pain	0	1 (33.3)	0	0	0	1 (5.6)
Nervous System Disorders	0	1 (33.3)	1 (33.3)	0	1 (33.3)	3 (16.7)
Amnesia	0	0	0	0	1 (33.3)	1 (5.6)
Headache	0	0	0	0	1 (33.3)	1 (5.6)
Neuropathy peripheral	0	0	1 (33.3)	0	0	1 (5.6)
Tremor	0	1 (33.3)	0	0	0	1 (5.6)
Respiratory, Thoracic and Mediastinal disorders	0	1 (33.3)	0	2 (33.3)	0	3 (16.7)
Cough	0	1 (33.3)	0	1 (16.7)	0	2 (11.1)
Epistaxis	0	0	0	1 (16.7)	0	1 (5.6)
Infections and Infestations	0	1 (33.3)	0	1 (16.7)	0	2 (11.1)
Urinary tract infection	0	1 (33.3)	0	1 (16.7)	0	2 (11.1)
Parotitis	0	1 (33.3)	0	0	0	1 (5.6)
Neoplasms Benign, Malignant and Unspecified (Including Cysts and Polyps)	1 (33.3)	0	0	1 (16.7)	0	2 (11.1)
Tumour pain	1 (33.3)	0	0	1 (16.7)	0	2 (11.1)

System Organ Class Preferred Term, n (%)	Dose Cohort 75 mg (N = 3)	 225 mg (N = 3)	 500 mg (N = 3)	 700 mg (N = 6)	 900 mg (N = 3)	 Total (N = 18)
Hepatobiliary Disorders	0	0	1 (33.3)	0	0	1 (5.6)
Cholangitis	0	0	1 (33.3)	0	0	1 (5.6)
Injury, Poisoning and Procedural Complications	0	0	1 (33.3)	0	0	1 (5.6)
Infusion-related reaction	0	0	1 (33.3)	0	0	1 (5.6)
Renal and Urinary Disorders	0	0	1 (33.3)	0	0	1 (5.6)
Acute kidney injury	0	0	1 (33.3)	0	0	1 (5.6)
Reproductive System and Breast Disorders	0	1 (33.3)	0	0	0	1 (5.6)
Breast pain	0	1 (33.3)	0	0	0	1 (5.6)
Vascular Disorders	0	0	0	1 (16.7)	0	1 (5.6)
Embolism venous	0	0	0	1 (16.7)	0	1 (5.6)

Supplementary Figures

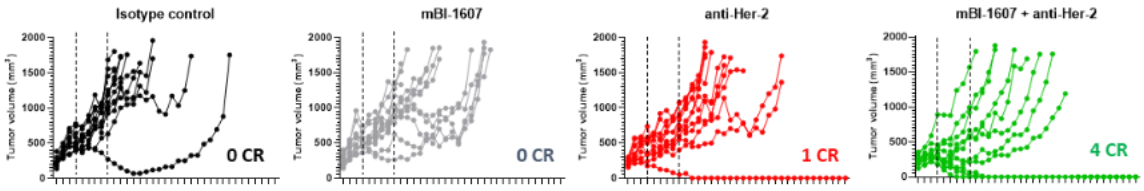


Figure S1. Individual tumor growth curves in the subcutaneous TUBO breast cancer model (n=12). Anti-HER2 in combination with mBI-1607 demonstrates delayed tumor growth and an increased number of complete responders (CR) compared to anti-HER2 treatment alone.

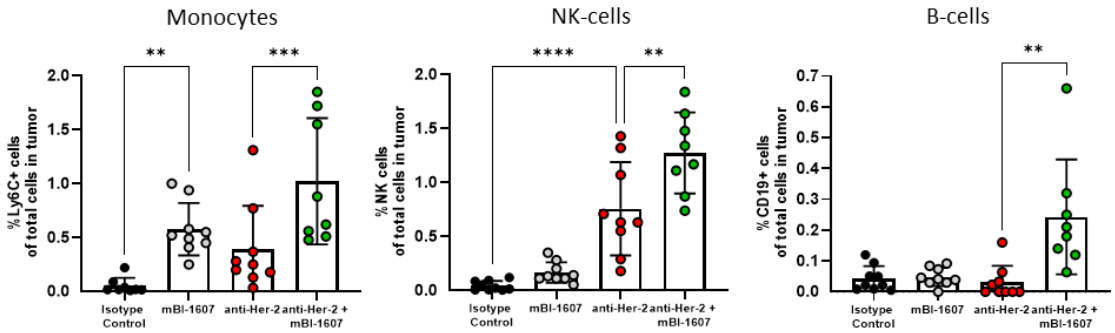


Figure S2. Combined treatment of anti-HER2 and mBI-1607 results in an increased influx/expansion of monocytes, NK-cells and B-cells compared to single treatment with anti-HER2. Tumors were dissected out from TUBO bearing mice 8 days post treatment start. Single cell suspensions were analyzed by FACS. Each dot is one animal. ***= p<0,001 *=p<0,05 as calculated using one-way ANOVA.

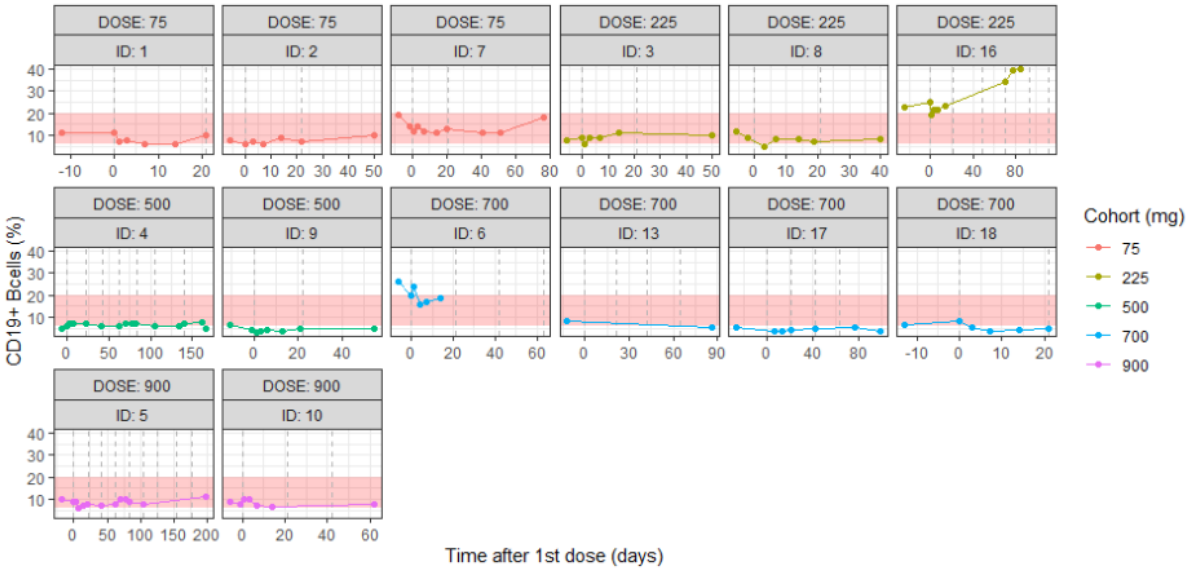
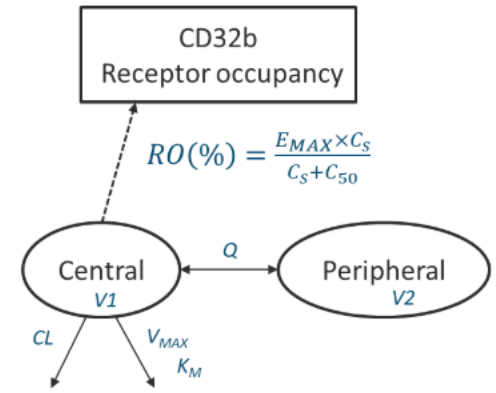


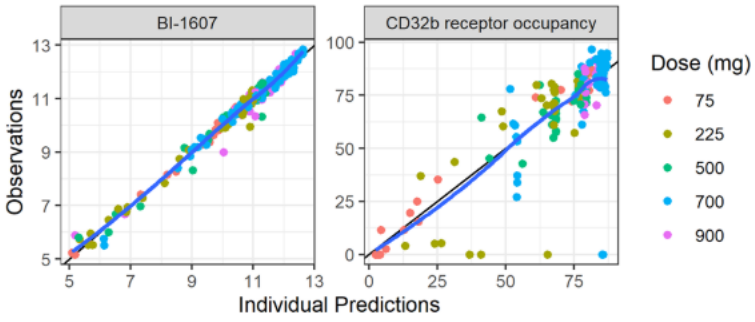
Figure S3: Individual B-cells (%) over time stratified by dose cohort level and subject. Only subjects with more than one B-cell measurement are displayed. The red area marks normal B-cell ranges.

A.



Parameter	Typical value (RSE, %)	IIV [%] (RSE, %)
CL (L/d)	0.264 (54.9)	116 (57.4)
V1 (L)	4.03 (26.6)	40.7 (27.9)
Q (L/d)	0.120 (14.6)	
V2 (L)	2.11 (10.6)	
V_{MAX} (mg/d)	13.3 (24.1)	
K_M (ng/mL)	6100 (28.5)	
E_{MAX} (%) [1]	80.8 (8.2)	5.1 (34.7)
C_{50} (ng/mL)	637 (51)	
$RUV_{BI\text{-}1607}$ (log ng/mL)	0.241 (5.1)	
RUV_{RO} (%)	10.7 (7.7)	

B.



C.

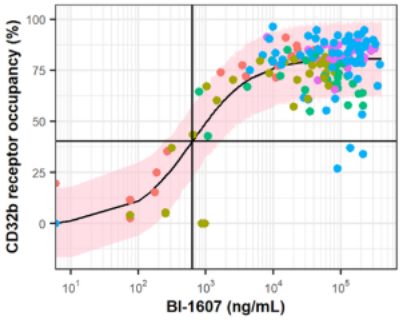


Figure S4. Pharmacokinetic (PK) and receptor occupancy (RO) modeling results. (A) Schematic representation of the final model and table of parameter estimates. (B) Observations versus individual model predictions colored by dose, solid lines represent the identity line. (C) Relationship between observed (points) and model predicted (areas) BI-1607 serum levels and receptor occupancy. Pink area covers the 90 % model prediction interval.
CL: clearance, C_S; BI-1607 serum levels, C_{50}; BI-1607 serum levels triggering 50% of the maximum receptor occupancy; E_{MAX}: maximum model predicted receptor occupancy; IIV: interindividual variability; K_M: BI-1607 serum levels triggering 50% of the maximum non-linear drug clearance; Q: intercompartmental clearance; RO: receptor occupancy; RSE: relative standard error computed as NONMEM reported standard error over parameter estimate; RUV: residual unexplained variability; V1: volume of the central compartment; V2: volume of the peripheral compartment; V_{MAX}: maximum rate of non-linear drug clearance.

[1] Parameter variability modeled in the logit scale.

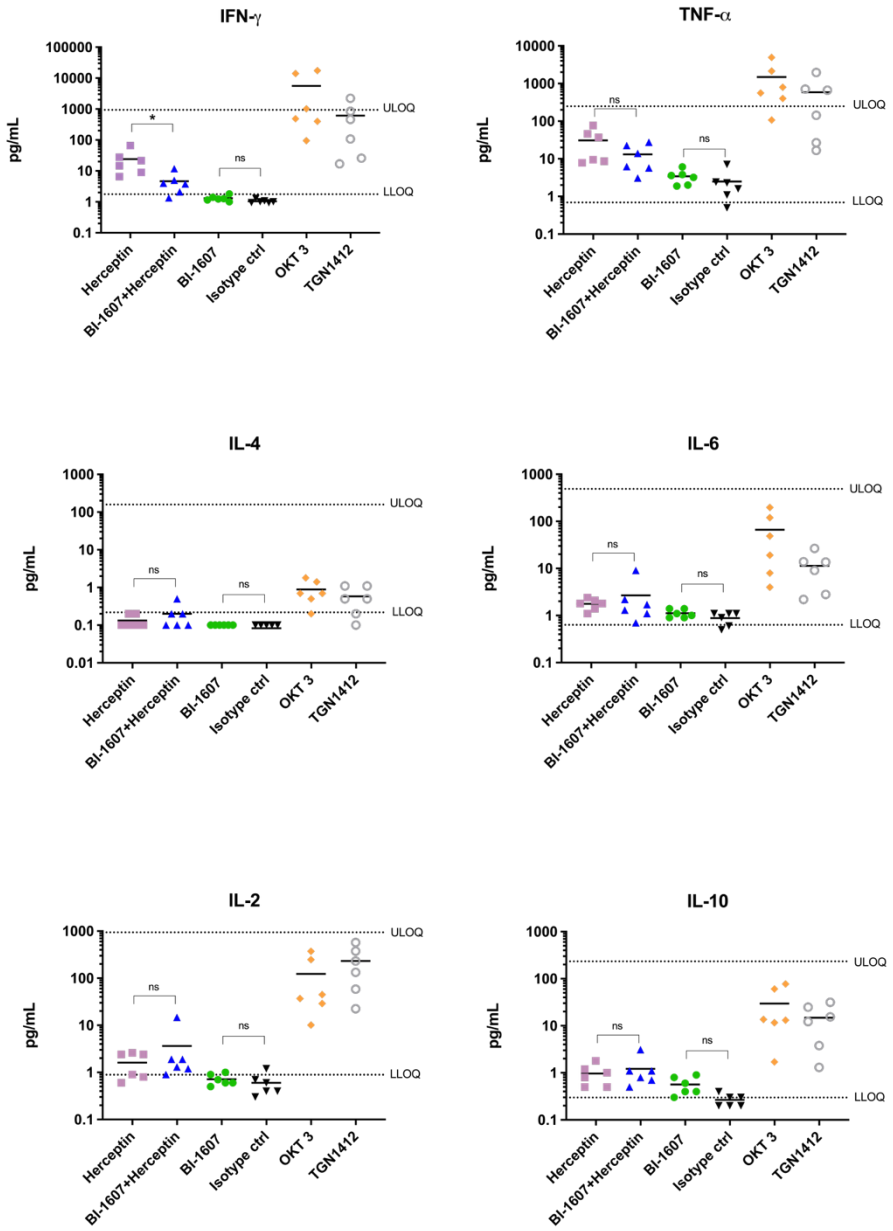


Figure S5: Cytokine release in high density cell culture. 1x10^5 PBMCs resuspended in fresh serum free CTL-Test media were stimulated with 10 µg/mL antibody added in solution to the cells for 24 hours at 37°C in 5% CO_2. The cytokine levels in the culture supernatant were measured using a 7-plex customized MSD plate. A paired one-way ANOVA with Tukey's multiple comparison was used to determine significance; * = $p<0.05$; ns = not significant. Lines indicate mean values for each cytokine; each dot is indicative of an individual healthy donor (n=6).

Abbreviations: OKT 3=anti-CD3 Ab; TGN1412=anti-CD28 Ab; ULOQ=upper limit of quantification; LLOQ=lower limit of quantification.

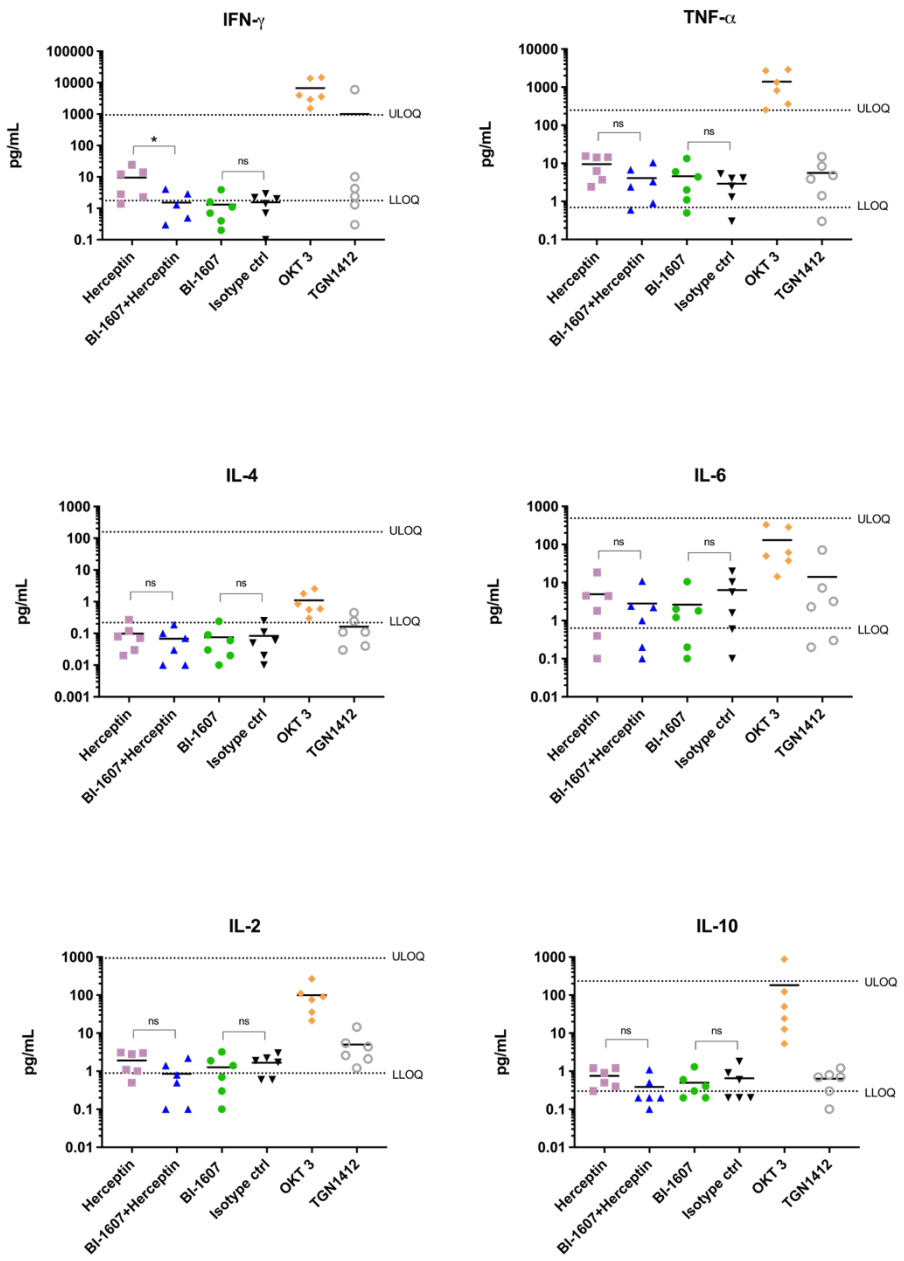


Figure S6: Cytokine release with plate bound antibodies. 1x10^5 PBMCs resuspended in fresh cell culture media containing fetal bovine serum were stimulated with plate bound antibody for 48 hours at 37°C in 5% CO2. The cytokine levels in the culture supernatant were measured using a 7-plex customized MSD plate. A paired one-way ANOVA with Tukey's multiple comparison was used to determine significance; * = $p<0.05$; ns = not significant. Lines indicate mean values for each cytokine; each dot is indicative of an individual healthy donor (n=6).
Abbreviations: OKT 3=anti-CD3 Ab; TGN1412=anti-CD28 Ab; ULOQ=upper limit of quantification; LLOQ=lower limit of quantification.

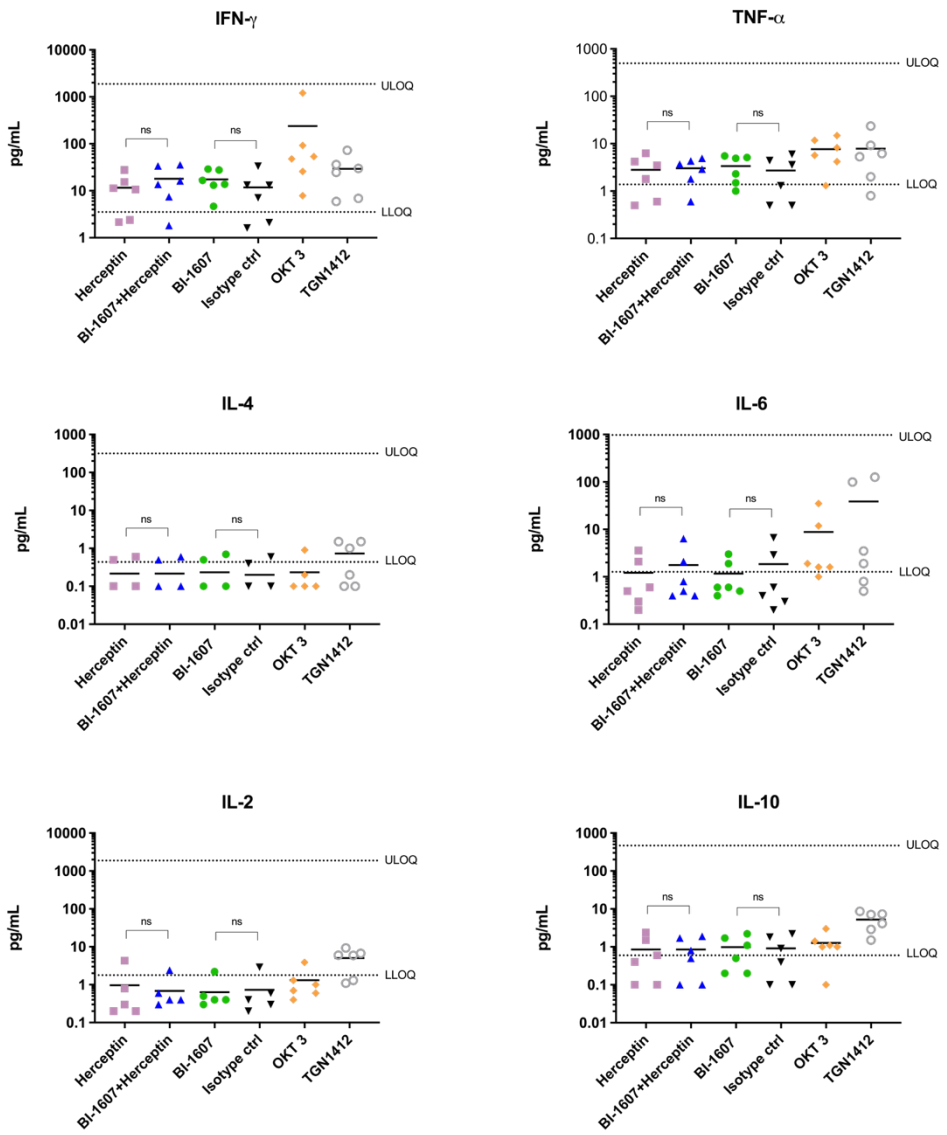


Figure S7: Cytokine release in whole blood. Whole blood was stimulated with 5 µg/mL antibody added in solution to the cells for 48 hours at 37°C in 5% CO_2. The cytokine levels in the culture supernatant were measured using a 7-plex customized MSD plate. A paired one-way ANOVA with Tukey's multiple comparison was used to determine significance; ns = not significant. Lines indicate mean values for each cytokine; each dot is indicative of an individual healthy donor (n=6).
Abbreviations: OKT 3=anti-CD3 Ab; TGN1412=anti-CD28 Ab; ULOQ=upper limit of quantification; LLOQ=lower limit of quantification.

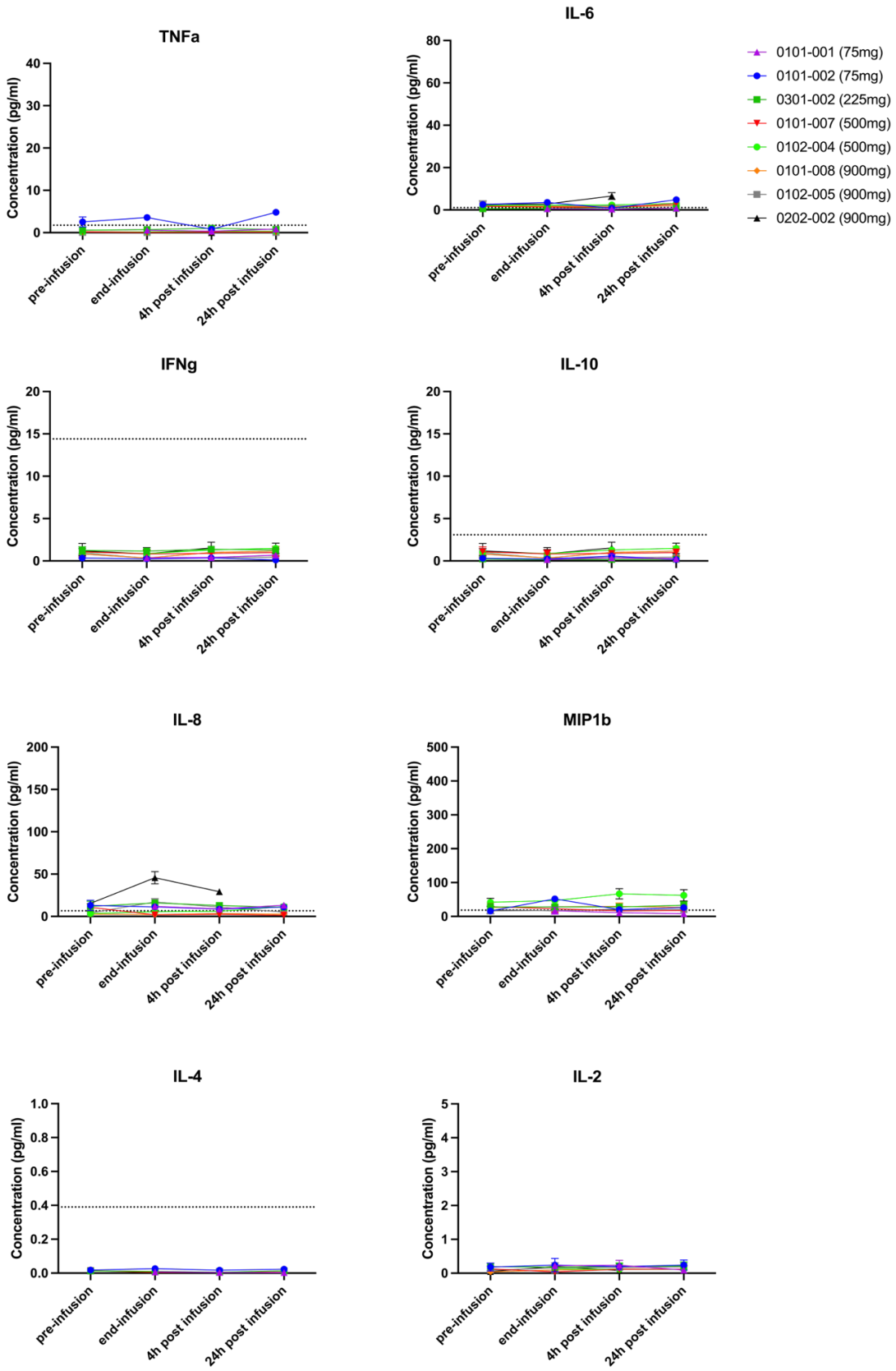


Figure S8: Cytokines measured pre and post IV BI-1607 infusion. Dotted line represents upper limit normal range (according to manufactures instructions based on 27 individuals (MesoScale Discovery #K15049) or based on 10 healthy donors tested in-house with MesoScale Discovery kit #K15049).

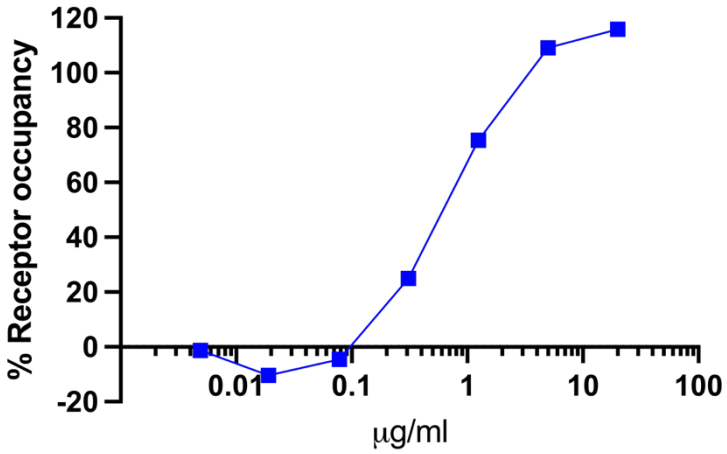


Figure S9: In vitro receptor occupancy (RO) on whole blood. Blood from healthy donors was spiked with known concentrations of BI-1607 and incubated 18-24h hours at room temperature. A mouse anti-hCD32b IgG2b and 005-C05 (an anti-CD32b antibody binding same epitope as BI-1607 but with lower affinity) were added to separate samples to detect total and free amounts of CD32b receptor, respectively, and incubated 30 minutes at room temperature. The samples were lysed and washed prior to flow cytometry. The graph shows calculated RO percentage for CD19+ cells expressing CD32b.