# Title Page

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



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**Conflict of interest statement**

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## Abstract (250 words, structured)

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

**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 three weeks in combination with trastuzumab were evaluated in 18 HER2-positive cancer patients. The primary objective was to assess safety and tolerability of BI-1607 by determining dose-limiting toxicities, maximum tolerated dose (MTD) 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 1 patient (5.6 %) at 900 mg; the MTD was not reached. Treatment-emergent adverse events grade ≥3 occurred in 5 patients (28 %), including exanthema, increase in liver enzymes, urticaria, acute kidney injury, and aggravated condition. Overall best response was stable disease, observed in 7 out of the 9 evaluable patients (78 %). BI-1607 exhibits linear pharmacokinetics for doses above 500 mg, and full receptor saturation throughout the 21 days at 700 mg. No ADAs were observed.

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

## Translational Relevance (120-150 words)

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 FcγRs. This enhances the effector functions, e.g., antibody-dependent cellular phagocytosis (ADCP) 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.

# Introduction

The Fc gamma receptors (FcγR) are key regulators of the activity of IgG-type antibodies. Several activating (FcγRI, FcγRIIA, and FcγRIIIA) and a single inhibitory (FcγRIIB) act in concert to regulate FcγR-expressing effector cell responses during therapy. Activating FcγRs, like co-stimulatory T cell checkpoints, promote immune effector cell activation. Conversely, FcγRIIB, the inhibitory receptor, blocks activation (1). Direct targeting monoclonal antibodies (mAbs) such as trastuzumab, also termed cytotoxic mAbs, bind to target cell surface receptors and mark the cell for innate immune destruction. This mechanism is dependent on binding to FcγRs 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, human epidermal growth factor receptor 2 (HER2), and the melanoma antigen gp75 (3). Several mAbs used in cancer therapy require functional activating FcγRs for therapeutic activity, and conversely, the high expression of FcγRIIB reduces mAb efficacy (3). Indeed, 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-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) (5). Similarly, the anti-EGFR antibody cetuximab is known to stimulate ADCC, and polymorphisms in *FcγR2A* and *FcγR3A* 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 immune suppressive functions on effector cells present inside the tumor microenvironment (TME), we generated an Fc:FcγR-muted anti-FcγRIIB antibody (BI-1607, 6G11 IgG1N297Q) based on a (8) highly FcγRIIB-specific antibody clone (4) (Figure 1A-B). 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. Further, in this first-in-human 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 those 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 (scFv) library (Lib 2000) and was converted into an a-glycosylated 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 due to 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, FcγRIIIA, cynomolgus FcγRIIB, or mouse FcγRIIB were used for coating. Bound BI-1607 or BI-1206 were detected using anti-hIgG (H+L)-HRP. 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 using 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 i.v. with 10 mg/kg BI-1607 or BI-1206. Leukocyte analysis by flow cytometry was conducted prior to 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) ($1x10^6$ cells/mouse). Tumor growth was monitored (measured by a caliper), and when tumors reached approximately 7x7 mm, mice were randomized and treated intraperitoneally (i.p.) with either isotype control (mIgG2a 10mg/kg), anti-

HER2 (mIgG2a 1 or 10mg/kg), mBI-1607 (8) (mIgG1N297A 20 mg/kg), or a combination of mBI-1607 (20mg/kg) and anti-HER2 (1 or 10mg/kg). The mice were dosed three times (2-3 days apart) and the 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 one 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. Further, the tumor solution was filtered through a cell strainer to obtain a single-cell solution. The cell solution was blocked with IVIG prior to 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 (i.v.) with the gp75-expressing mouse melanoma cell line B16F10 (RRID:CVCL_0159 $5x10^4$ cells/mouse). Four days post tumor injection, mice were randomized and treated i.p. with either isotype control (mIgG2a 10 mg/kg), anti-gp75 (TA99 mIgG2a RRID:AB10949462 10 mg/kg), mBI-1607 (20 mg/kg) or a combination of TA99 (10 mg/kg) and mBI-1607 (20 mg/kg). The treatment was given 5 times with 2-3 days apart, and the mBI-1607 was always given 20 minutes ahead of the anti-HER2 mAb. Three weeks post-tumor injection 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 aged ≥18 years and had HER2 positive (10) locally advanced unresectable or metastatic solid tumors whose tumor had progressed after standard anticancer treatment, including prior lines of trastuzumab and at least one line of trastuzumab antibody-drug conjugate (ADC) (e.g., trastuzumab-emtansine (TDM-1) [Creative Biolabs Cat# 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 1 measurable disease lesion as defined by RECIST v1.1 criteria, left ventricular ejection fraction ≥50%, a life expectancy of ≥12 weeks and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and adequate organ function as confirmed by laboratory values. Key exclusion criteria included: 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).

The disease was measurable as per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, adequate hematologic and organ function, and a life expectancy of ≥ 12 weeks. 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 5 half-lives of the respective drug before the first dose of BI-1607.

## Study design and treatment

This was a first-in-human (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 i.v. every three weeks (Q3W) in combination with trastuzumab in patients with HER2-positive locally advanced unresectable or metastatic solid tumors, to characterize the dose-limiting toxicities (DLT), to determine the maximum tolerated dose (MTD) or maximum administered dose (MAD), and to determine the recommended phase 2 dose (RP2D) 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 immunological 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 MTD and to reduce the risk of overdosing (11). The starting dose of 75 mg was based on an aggregate of preclinical pharmacokinetic 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 (MABEL)

principles (12), a starting dose targeting a 50 % RO 24 h after administration was selected. The selected dose of 75 mg (1 mg/kg in 75 kg subject) provides a 250-fold safety margin compared to well-tolerated dose of 250 mg/kg in mice established in a GLP toxicological 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 (IRRs), 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 IRRs, BI-1607 was administered as a slow IV infusion starting at a rate of 30 mg/h and sequentially increasing the infusion rate.

Trastuzumab was also administered Q3W at an 8 mg/kg dose for cycle 1 and 6 mg/kg for Cycle 2 onwards, 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 National Cancer Institute Common Terminology Criteria for AEs version 5.0 (CTCAE v5.0), were documented until 30 days after the last dose of study treatment. Occurrence of DLTs 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 computed tomography/positron emission or magnetic resonance imaging scan at screening, weeks 6, 12, 18, 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 v1.1 (13). For response assessment, all patients who received at least 1 dose of BI-1607 and trastuzumab and had at least one evaluable post-baseline disease assessment (i.e. at least one valid post-dose assessment collected at least 42 days after 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 cycle 1 and 4. A validated ELISA with a lower limit of quantification (LLOQ) 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 (ADAs) to BI-1607 using pre- and post-treatment samples of infusion cycles 1-4 and cycles 6, 8, 12, and 16. Cytokine and chemokine levels were assayed in plasma collected in cycle 1 (predose, end of infusion, 4 and 24 hr post-infusion)

using MesoScale Discovery catalog kits K15049 and K15067. FcγRIIB receptor occupancy (RO) was evaluated on CD19+ B cells in whole blood collected in the same manner as serum for PK assessment in cycles 1-4 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 predose in cycle 1. The polymorphisms *FcγR2A*-H131R, *FcγR2B*-I232T, *FcγR2C*, and *FcγR3A*-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 post dose measurement was available in addition to baseline.

## Statistical analyses

PK and RO assessments were analyzed using non-linear mixed effects modeling in NONMEM 7.5. (RRID:SCR_016986) (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 (BQLs) were included and treated as censored information using the M3 method (15). Interindividual variability (IIV) was modeled exponentially, except for bounded parameters where additive models in the logit domain were used. Non-diagonal 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). 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 (QTcF). A linear mixed effects (LME) model approach was applied to explore a potential positive correlation between time-matched PK samples and ECG (17).

## Data availability

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

# 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). Here, BI-1607's highly specific binding to selected human, murine, and cynomolgus FcγR proteins was verified by ELISA (Figure 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 wildtype 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 (Figure 1D-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 to that of an isotype control antibody and to anti-HER2 as single treatment in the TUBO breast cancer model. Combination with mBI-1607 delayed tumor growth and increased survival compared to anti-HER2 single agent treatment (Figure 1F and 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 to single anti-HER2 treatment (Figure 1G). In addition, an increase in monocytes, NK-cells, and B-cells infiltration was seen in this group (Figure S2) . In the B16F10 melanoma model, mBI-1607 improved the anti-tumor 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, compared to isotype control or to TA99 single agent treatments (Figure 1H).

## Patient characteristics and disposition

A total of 18 participants were included in the dose escalation cohorts (75, 225, 500, 900, and 700 mg Q3W) with a median age of 63 years (range 46-77), and 4 (22%) were male (**Table** 1). The most common tumor types included breast (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 Q3W 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 (RDR) for further exploration in subsequent Phase 2 studies.

Of the 18 subjects in the Safety Population, 17 subjects (94.4%) experienced at least 1 treatment-related adverse event (TEAE) with no dose-dependent trends observed (Table 2). The full list of TEAEs by system organ class stratified by dose level is provided in Table S2. Nonetheless, most subjects experienced TEAEs with a maximum grade of Grade 1 (5 subjects; 27.8 %) or Grade 2 (7 subjects; 38.9 %). Grade ≥3 TEAEs occurred in 5 patients (28 %), with only 3 treatment-emergent serious adverse events (SAE), all unrelated to the study drugs, being reported in a total of 2 patients, namely, cholangitis, acute kidney injury and condition aggravated.

Premedication for Infusion-related reaction (IRR) was allowed; however, it was recommended only if medically indicated. IRRs were reported in 8 subjects (44%), including erythema, infusion site pruritus, infusion site urticaria, rash, rash macular, rash maculo-papular and urticaria, and all were related to the study drugs. IRRs were managed with corticosteroids IV treatment and infusion rate reduction which resulted to resume drug administration for all patients except for patient with grade 3 urticaria, which was described as a DLT.

However, only two IRRs grade 3 were recorded in 2 patients in the 900 mg cohort. Concentration-QT analysis revealed no statistically significant positive correlation between time-matched PK measurements and heart rate or QTcF captured from triplicate ECGs (Figure 2).

No significant depletion of CD19+ B cells was seen in the clinical study, as expected with BI-1607 being engineered for impaired FcγR binding (Figure S3).

## Antitumor activity

Out of the 18 treated patients, 9 met the criteria for response evaluation (see method section). Tumor progression in target lesions was observed in 2 patients, both from the low dose cohort groups (Figure 3). Although none achieved an objective response, 7 patients (78 %) exhibited stable disease, with the longest duration of 6 months. Regarding the remaining 9 patients, 3 patients drop the study prior response assessments, and 6 patients exhibited progressive disease, although prior the 42 days

landmark, and thus could not be included into the response population but are here reported for completeness.

## PK and receptor occupancy

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

A two-compartment model with Michaelis-Menten elimination provided an adequate description of the non-linear PK behavior, with reasonable parameter estimates and precision (Figure S4). Accounting for additional complexities, such as target-mediated disposition, did not improve model performance ($p>0.05$). A value of 6100 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 pharmacokinetics at clinically relevant doses.

Regarding FcγRIIB RO, data was well described with a direct saturable model driven by serum BI-1607 concentrations, reporting maximum receptor occupancy of 80.8 % and a low value of $C_{50}$ (concentration at which 50 % of maximum receptor occupancy is attained) of 637 ng/mL. Variability was only supported by the linear clearance term (CL), the volume of the central compartment (V1), and the maximum receptor occupancy ($E_{MAX}$). 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 ADAs against BI-1607 were detected.

Cytokines were analyzed in the peripheral blood of a subset of patients up to 24 hours post infusion, and no analytes were affected by BI-1607/trastuzumab infusion. Cytokines analyzed included IFN-γ, IL-10, IL-6, IL-8, MIP-1β, TNF-α, IL-1β, IL-23, IL-12p70, IL-2, IL-4, TARC and VEGF. The absence of cytokines has previously been confirmed in several *in vitro* assays of BI-1607 and peripheral blood mononuclear cells (PBMCs) (Figure S5-S8).

The effect of *FcγR2A*, *FcγR2B*, *FcγR2C*, and *FcγR3A* 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, results are at this time inconclusive.

## Discussion

The efficacy of tumor targeting and immunomodulatory mAbs 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) (18,19). Accordingly, Fc-engineering to enhance binding to activating FcγRs 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 monoclonal antibody to human FcγRIIB, is well tolerated across doses ranging from 75 mg up to 900 mg Q3W, 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. Firstly, regarding efficacy, the inhibitory FcγRIIB is strongly upregulated on tumor and effector cells in resistant niches, e.g., in the bone marrow of leukemia patients (21) and the solid cancer tumor microenvironment (22), promoting resistance to antibody therapy (22-24). The activating FcγRs, 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 FcγRs, i.e., FcγRI, FcγRIIA, or FcγRIIIA are expressed in a particular patient or a patient's tumor (2).

Secondly, 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 FcγRs, including FcγRIIIA -targeted by most antibody engineering approaches, e.g., by afucosylation or amino acid substitution-, which are abundantly expressed on pro-inflammatory immune effector cells in the blood (22). Since the engagement of activating FcγRs on blood effector cells is associated with decreased tolerability and occurrence of IRRs (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 all other tumor-targeting mAbs whose activity relies on ADCP. 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, while not exhibiting anti-tumor efficacy of its own, does enhance anti-HER2 efficacy. Interestingly, our preclinical data

demonstrated 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.

While the introduction of trastuzumab has dramatically changed outcomes in patients with HER2-positive cancer, primary 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 cancer patients 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 AEs observed were mild or moderate (CTCAE grade 1 or 2). IRRs 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 on study, and IRRs were not observed in subsequent cycles. In most cases, dexamethasone was added as pre-medication following the occurrence of an IRR. Three subjects (17 %) had SAE reported, all of them unrelated to the study treatment. Four deaths were reported due to disease progression, condition aggravated, 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 (reviewed in (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 where 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 mAbs 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 higher A:I ratio but which retains FcγRIIB-binding (4), was approved in combination with chemotherapy for the treatment of metastatic breast cancer in patients 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, besides wildtype IgG Fc antibodies like trastuzumab and zanidatamab, can also enhance Fc-enhanced 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 hematological malignancies, such as rituximab or obinutuzumab could also benefit from FcγRIIB blockade. This hypothesis is currently being tested in the ongoing clinical study (NCT03571568) where BI-1206, a fully competent IgG1 monoclonal directed against FcγRIIB is being combined with rituximab for the treatment of NHL.

Concentration-QT analysis showed no contribution on QT-time caused by BI-1607. Population PK/PD analysis was performed to quantitatively describe the observed non-linear pharmacokinetics, common in monoclonal antibodies 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 receptor occupancy is attained. This result suggests that the circulating B cells might not be the main driver of drug non-linear 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 receptor occupancy, 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 (Figure S9). Given this direct relationship and the low $C_{50}$ value (637 ng/mL), maximum receptor occupancy is practically attained instantaneously after i.v. infusion for all doses explored. Thus, sequential administration of drugs (being BI-1607 the first one given) is sufficient, and there is no need for an earlier BI-1607 drug administration. 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. Indeed, two breast cancer patients receiving 500 mg or 900 mg doses and who had previously received 7 and 11 prior lines of treatment - that included Trastuzumab (3 and 7 treatment lines, respectively), TDM-1 (1 and 2 treatment lines, respectively) and Trastuzumab- Deruxtecan (1 treatment line each)- had stable disease for over 7 and 9 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+ 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 first-in-human 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. 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.

## Acknowledgments

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# Tables

***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 (75 mg) | 57/F | Breast / 2002 | 21 | 16 | 1 | TRAS+TUCA | 2 | PD |
| | 53/F | Breast / 2018 | 6 | 3 | 2 | TDX | 2 | NE (PD)** |
| | 63/F | Uterus/ 2018 | 6 | 1 | -- | BEV | 2 | NE (PD)** |
| Cohort 2 (225 mg) | 72/F | Breast / 2011 | 10 | 5 | 1 | TDX | 2 | PD |
| | 70/F | Uterus/ 2020 | 3 | 2 | -- | TRAS+DUO | 2 | NE (PD)** |
| | 65/F | Breast /2011 | 4 | 2 | 1 | TDX | 6 | SD |
| Cohort 3 (500 mg) | 53/F | Breast / 2007 | 7 | 3 | 1 | TDX+ZO+LET | 7 | SD |
| | 55/M | Paget's disease extramammary/ 2021 | 2 | 1 | -- | TRAS+PAC | 2 | NE (PD)** |
| | 62/M | Esophagus / 2023 | 2 | 1 | -- | DOC+FLU+FOL+OXA | 1 | NA |
| Cohort 4 (900 mg) | 46/F* | Breast / 2016 | 3 | 2 | 1 | TDX | 1 | NA |
| | 77/F | GEJ / 2020 | 4 | 1 | 1 | TDX | 3 | SD |
| | 48/F | Breast / 2013 | 11 | 7 | 1 | TRAS+DOC | 9 | SD |
| Cohort 5 (700 mg) | 69/M | Stomach / 2018 | 3 | 2 | -- | RAM+PAC | 2 | NE (PD)** |
| | 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 | Stomach2019 | 3 | 1 | 1 | Clinical Trial ADC | 2 | NE (PD)** |

*Patient received 380 mg dose

**Patient did not meet the criteria for response evaluation due to progressive disease prior to the 42 days landmark

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: Non available; NE: Non evaluable; OXA: Oxaliplatin; PAC: Paclitaxel; PD: Progressive disease; RAM: Ramorizumab; SD: stable disease; TDM-1: Trastuzumab emtansine; TDX: Trastuzumab deruxtecan; TRAS: trastuzumab; TUCA: tucatinib; ZO: Zometa

***Table** 2. Treatment-emergent adverse events (TEAE) in all treated patients (n=18)*

| **Category** | **BI-1607** | | | | | |
|---|---|---|---|---|---|---|
| | Dose cohort | | | | | |
| | 75 mg | 225 mg | 500 mg | 900 mg | 700 mg | Total |
| Number of subjects | 3 | 3 | 3 | 3 | 6 | 18 |
| Subjects with at least 1 TEAE any grade | 3 (100%) | 3 (100%) | 3 (100%) | 3(100%) | 5 (83%) | 17 (94%) |
| Subjects with ≥Grade 3 TEAEs | 0 | 0 | 1 (33%) | 2 (66%) | 2 (33%) | 5 (28%) |
| Subjects with TEAEs related to BI-1607 | 2 (66%) | 2 (66%) | 3 (100%) | 3 (100%) | 3(50%) | 13 (72%) |
| Subjects with TEAEs related to trastuzumab | 2 (66%) | 3 (100%) | 3(100%) | 0 | 2 (33%) | 10 (56%) |
| Subjects with ≥Grade 3 TEAEs related to study BI-1607 | 0 | 0 | 0 | 2 (66%) | 1 (17%) | 3 (17%) |
| Subjects with ≥Grade 3 TEAEs related to trastuzumab | 0 | 0 | 0 | 0 | 1 (17%) | 1 (6%) |
| Subjects with serious TEAEs related to study treatment | 0 | 0 | 0 | 0 | 0 | 0 |
| Subjects with treatment related AEs that led to discontinuation | 0 | 0 | 0 | 1 (33%) | 0 | 1 (6%) |
| **TEAEs 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%) |

ALT: Alanine transaminase; AST: Aspartate transaminase; IRR: Infusion related reactions.

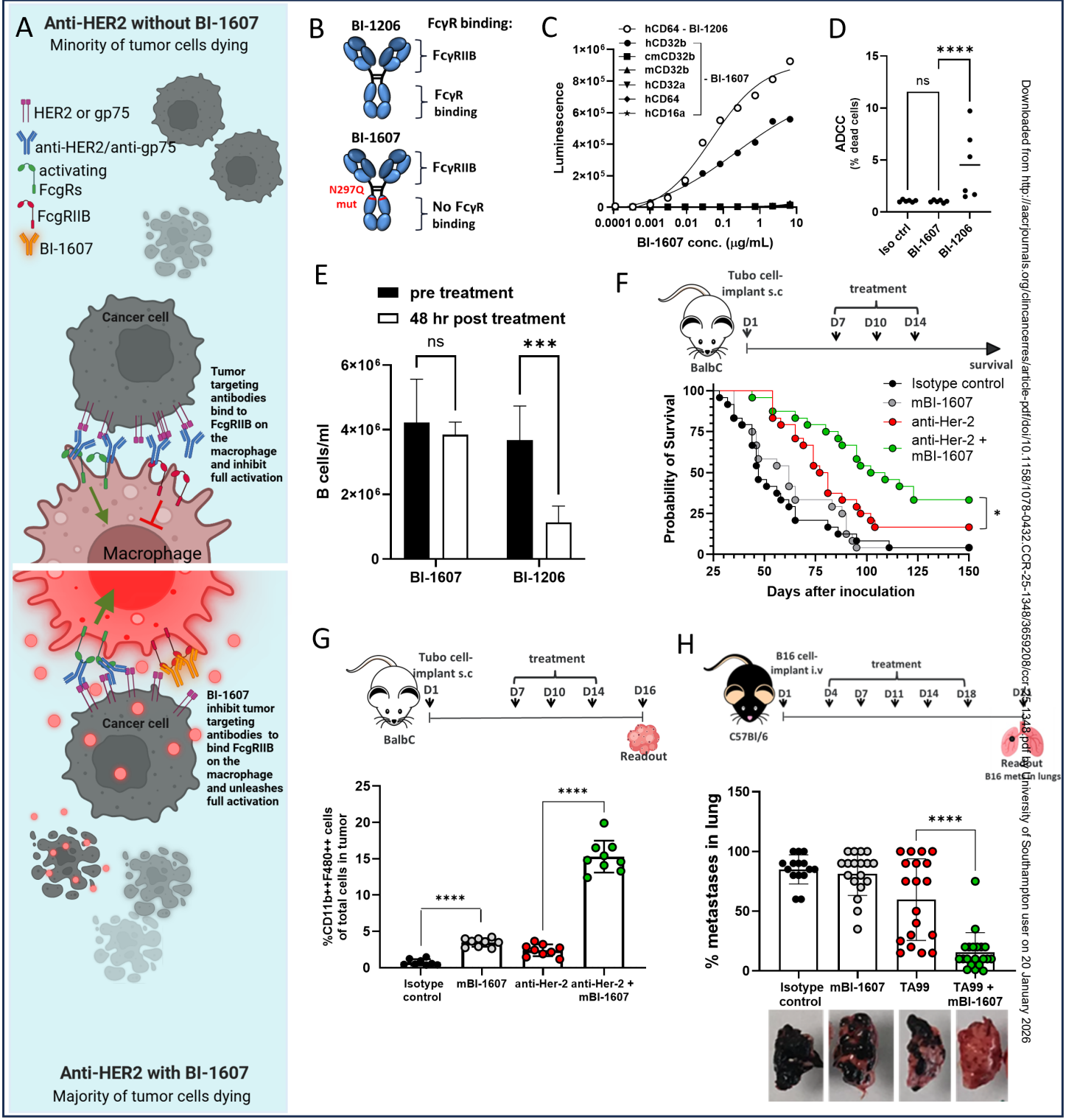
# Figure Legend

**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 FcγRs, thereby enhancing effector functions of this antibody, such as antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). (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 Fcγ-receptors. (C) BI-1607 binding to human FcγRIIB, CD32a, CD64, CD16a, cynomolgus FcγRIIB and murine FcγRII. BI-1206 (the WT format of BI-1607 without mutation N297Q [thus has a functional Fc domain]) was used as 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 CLL cells. Each dot represents 1 CLL patient. ****$p \leq 0.0001$ as assessed by paired student's t test. (E) B cell levels in hFcγRIIB transgenic mice prior to and 48 hours after treatment with BI-1607 or BI-1206. (F) Anti-HER2 in combination with mBI-1607 demonstrated delayed tumor growth and increased survival compared to 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 to single anti-HER2 treatment. (H) In the B16F10 melanoma model, the mBI-1607 improved the anti-tumor 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.

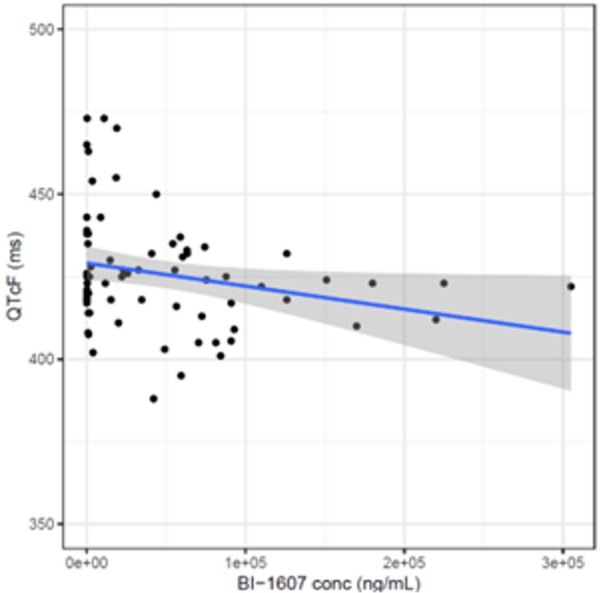
**Figure 2. QTcF and heart rate vs BI-1607 serum concentration**. Black dots represent data, blue line LME model fit and grey shaded area the 95% confidence interval of model.

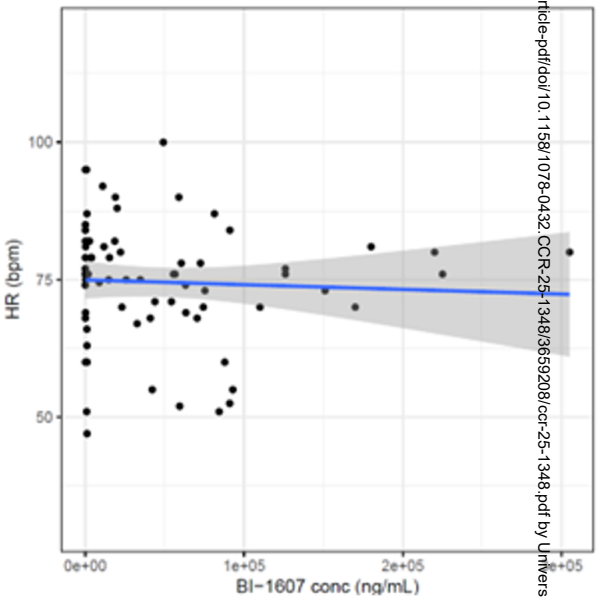
**Figure 3. Tumor response.** (A) Waterfall plot of the best overall responses in the evaluable patient population with respect to the tumor size. Patients with at least one valid post-dose assessment (CT/PET or MRI scan) at day 42 or later were included in the evaluable patient population (n=9). * indicate a patient with the best overall response PD 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.

**Figure 4. Time course of serum BI-1607 concentrations and CD32 receptor occupancy.** Colored lines represent the median of the raw data at each dose level and cycle. Dashed solid line in the upper panels represents the lower limit of quantification (LLOQ). Note, first data below LLOQ is set to LLOQ/2 and the rest are set to 0.

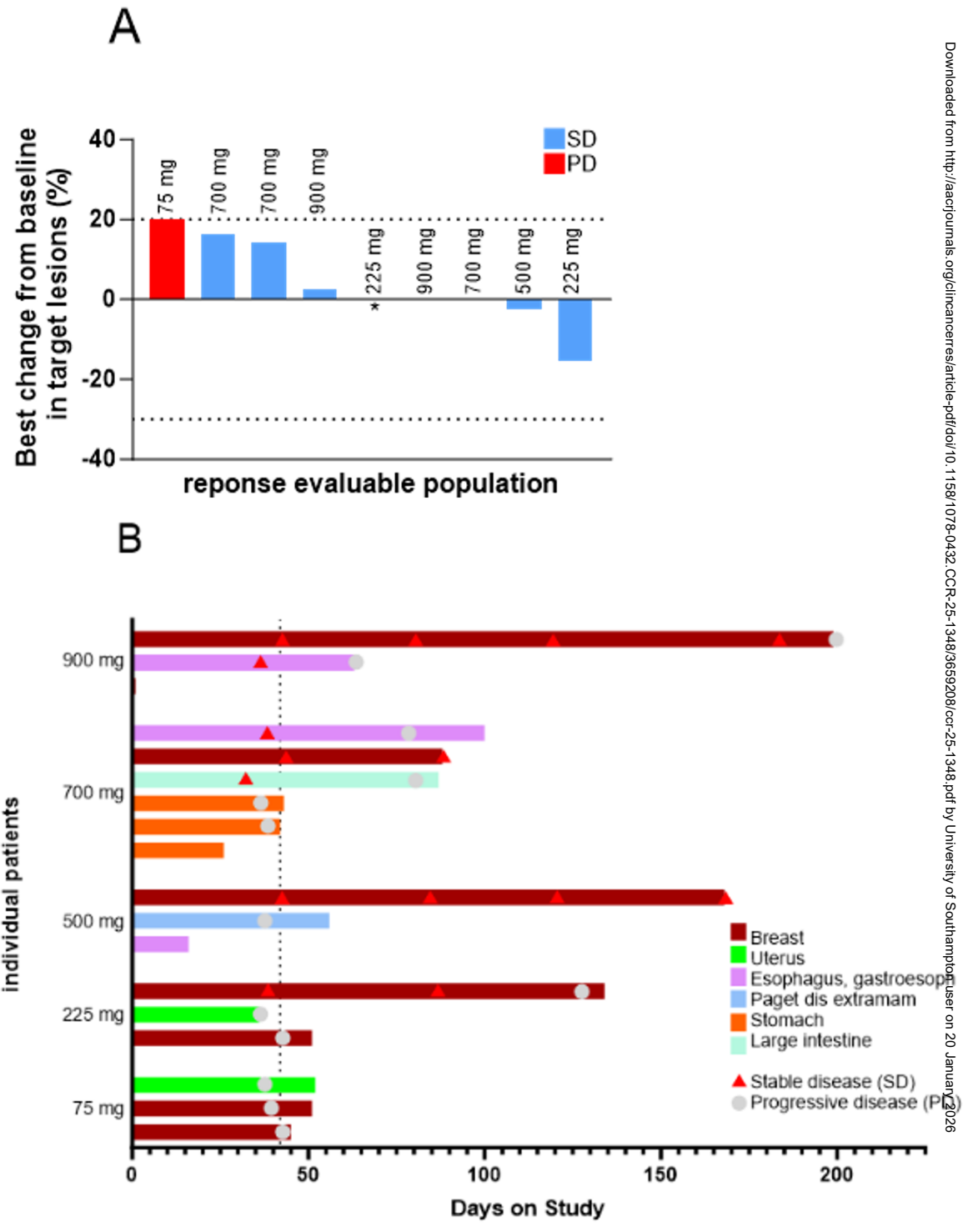


# Figure 2



# Figure 3



# Figure 4