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July 19, 2019

NCCN Guidelines Review Panel: Breast Cancer

On behalf of Amgen Inc., I respectfully request the NCCN panel members to review the recently published data on KANJINTI™ (trastuzumab-anns). Based on the totality of evidence supporting demonstration of clinical comparability, Amgen has received US Food and Drug Administration (FDA) approval of KANJINTI™ as a biosimilar to US-licensed HERCEPTIN® (trastuzumab).

Specific Changes: Please consider the addition of trastuzumab-anns (420 mg in a multiple-dose vial) as an appropriate substitute for trastuzumab within the NCCN Guidelines, NCCN Patient Guidelines, and the associated “NCCN Drugs and Biologics Compendium™” for the following cancer types per the FDA-approved KANJINTI™ label: adjuvant breast cancer, metastatic breast cancer, metastatic gastric cancer or gastroesophageal junction adenocarcinoma.

Rationale: Biosimilars offer the potential to expand treatment options and mitigate cost barriers for payers. Trastuzumab-anns development program aligns with the statutory requirements outlined in the draft FDA guidance for establishing biosimilarity to the reference product. The totality of evidence submitted includes comparisons of extensive structural and functional product characterization, animal data, pharmacokinetic (PK) and pharmacodynamic data (PD), immunogenicity, safety, and efficacy – supporting that trastuzumab-anns is highly similar to trastuzumab and that there are no clinically meaningful differences between the products.

- The extensive analytical characterization and comparison of the structural and functional properties of trastuzumab-anns to trastuzumab demonstrated that they are highly similar. In *in vivo* studies trastuzumab-anns and trastuzumab treatment groups were found to have similar and dose-dependent antitumor activity in xenograft models using BT-474 (breast tumor) and NCI-N87 (gastric tumor) cells.
- The PK profile of trastuzumab-anns was similar to that of trastuzumab in healthy subjects following a single dose and in patients with HER2-positive early breast cancer (eBC) following multiple dosing. Comparative clinical data in patients with eBC demonstrated clinical similarity (PK, efficacy, safety, immunogenicity) between trastuzumab-anns and trastuzumab:
 - Sensitive endpoints and patient populations were chosen to identify any potential clinically meaningful differences with the reference biologic, which may differ from those of the pivotal clinical studies for the reference product.
 - Conducting biosimilar studies in a sensitive patient population provides scientific evidence supporting extrapolation to less sensitive and homogenous populations.

- Study design includes both a neoadjuvant and adjuvant treatment phase. To explore the potential clinical effects on safety and immunogenicity, the study included a single-transition from trastuzumab to trastuzumab-anns, which mimics real world clinical use of biosimilars.
- Uses pathologic complete response (pCR) as the recognized endpoint for neoadjuvant studies in breast cancer, which has been found to correlate with overall survival, and is considered a more robust endpoint for evaluation of clinical comparability vs the evaluation of objective response rate in the metastatic setting.

Based on the robust scientific data package submitted to the FDA, justification was provided to support extrapolation to approved indication(s) of Herceptin®:

- The mechanism of action of trastuzumab, regardless of tumor type or location, is the binding to HER2 receptor and inhibits proliferation of tumor cells that overexpress human epidermal growth factor receptor 2 (HER2) receptors.
- Comparative PK data, combined with the knowledge of the PK profiles of trastuzumab-anns in *in vivo* and *in vitro* assays, indicate that trastuzumab-anns will retain a PK profile similar to trastuzumab for all available FDA-approved indications.
- In the clinical comparability study, the overall incidence of adverse events (AEs) was comparable between the treatment groups. Neoadjuvant and adjuvant phases of treatment in eBC are sensitive and representative of the safety risks in metastatic breast cancer and metastatic gastric cancer.
- Immunogenicity was similar in the clinical comparability study of trastuzumab-anns, trastuzumab/trastuzumab, and trastuzumab/trastuzumab-anns treatment groups; two (1%), two (1%), and four (2%) patients, respectively, tested positive for binding antibody at any time during the study. With no patients developing neutralizing antibodies.

A summary of the totality of evidence and sufficient scientific justification for extrapolation is provided in the enclosed KANJINTI™ (trastuzumab-anns) Clinical Fact Sheet.

FDA Status: KANJINTI™ (trastuzumab-anns) is FDA-approved, and indicated for:

- Adjuvant treatment of HER2-overexpressing node-positive or node-negative (ER/PR-negative or with one high-risk feature) breast cancer:
 - As part of a treatment regimen containing doxorubicin, cyclophosphamide and either paclitaxel or docetaxel
 - As part of treatment with docetaxel and carboplatin
 - As a single agent following multi-modality anthracycline-based therapy
- In combination with paclitaxel for the first line treatment of HER2-overexpressing metastatic breast cancer
- As a single agent for treatment of HER2-overexpressing breast cancer in patients who have received one or more chemotherapy regimens for metastatic disease
- In combination with cisplatin and capecitabine or 5-fluorouracil, for the treatment of patients with HER2 overexpressing metastatic gastric or gastroesophageal junction adenocarcinoma, who have not received prior treatment for metastatic disease.

Enclosed, please find the following:

- KANJINTI™ (trastuzumab-anns) Clinical Fact Sheet
- von Minckwitz G, Colleoni M, Kolberg HC, et al. Efficacy and safety of ABP 980 compared with reference trastuzumab in women with HER2-positive early breast cancer (LILAC study): a randomised, double-blind, phase 3 trial. *Lancet Oncol.* 2018; 19(7):987-998.
- Hutterer KM, et al. Assessing Analytical and Functional Similarity of Proposed Amgen Biosimilar ABP 980 to Trastuzumab. *BioDrugs* (2019). <https://doi.org/10.1007/s40259-019-00350-9>
- Hanes V, Chow V, Zhang N, et al. A randomized, single-blind, single-dose study evaluating the pharmacokinetic equivalence of proposed biosimilar and trastuzumab in healthy male subjects. *Cancer Chemother Pharmacol.* 2017; 79(5):881-888.

Amgen is providing you with the attached reprint. Please note that if you are a covered recipient as defined by the Affordable Care Act (ACA), Amgen's cost to obtain such reprint may need to be disclosed and reported in accordance with the requirements under the ACA, state law, and related disclosure obligations by Amgen. If you are a non-covered recipient requesting information on behalf of or for the benefit of a covered recipient (physician or teaching hospital), the same requirements may apply.

Should you have any questions or require additional materials, please feel free to contact me directly at +1 (805) 313-4438. Thank you in advance for your prompt attention to this matter and I look forward to your response.

Sincerely,



Raymond S. Wong, PharmD, MBA
Medical Director, Oncology Medical Affairs

all samples to determine whether the inhibition of drug activity was due to neutralising antibodies to ABP 980. A post-treatment sample was defined as positive for neutralising antibodies if it was simultaneously positive for binding antibodies and neutralising activity.

We recorded the numbers and percentages of patients in each treatment group who had pre-existing or developed binding and neutralising binding antibodies against investigational products. Pre-existing antibody incidence was defined as the number of patients with positive antibody results at the time of or before the first dose of investigational product divided by the number of patients with an immunoassay result on or before the first dose. We defined patients who developed antibodies as the number of patients with a negative antibody result or no result available at or before baseline and a positive antibody result at any time after the first dose of investigational product divided by the number of patients with at least one immunoassay result after baseline. A transient antibody result was defined as a positive result after baseline with a negative result at the patient's last time tested within the study period.

Outcomes

The co-primary efficacy endpoints were risk difference and risk ratio (RR) of pathological complete response, defined as the absence of invasive tumour cells in the breast tissue and in axillary lymph nodes regardless of ductal carcinoma in situ (as defined by the FDA).¹⁵ The primary analysis was based on local laboratory findings in patients with assessable tumour samples. We did sensitivity analyses based on central pathology findings to reduce variability between pathologists at the local level. Efficacy results are reported for the neoadjuvant phase (ABP 980 and trastuzumab groups).

Secondary efficacy endpoints were risk differences and RRs for pathological complete response in breast tissue (absence of invasive tumour cells, regardless of residual ductal carcinoma in situ); risk differences and RRs for pathological complete response in breast tissue and axillary lymph nodes in the absence of ductal carcinoma in situ (defined as the absence of invasive tumour cells in breast tissue and axillary lymph nodes and absence of ductal carcinoma). These results will be reported separately.

Safety assessments reported in this Article are the incidence of treatment-emergent adverse events, changes in LVEF, exposure to investigational product and paclitaxel, and formation of antibodies against an investigational product (immunogenicity). Safety results are presented for the neoadjuvant phase (ABP 980 and trastuzumab groups) and adjuvant phase (ABP 980, trastuzumab, and switching groups). Other safety outcomes that will be reported elsewhere were on-study event-free survival, overall survival, pharmacokinetics, concomitant medications, laboratory tests (including serum chemistry and haematology), vital signs, and physical examination.

Subgroup analyses done in prespecified groups for the neoadjuvant phase, adjuvant phase, and entire study. These included age group, race, T stage, axillary lymph node involvement, hormone receptor status, paclitaxel dosing schedule, and geographical region, and will be reported separately.

Statistical analysis

The primary efficacy hypothesis was that ABP 980 would be equivalent to trastuzumab when each was given in combination with standard-of-care neoadjuvant cancer treatment (paclitaxel). The planned sample size was 808 to ensure that 768 patients (384 in each group) were randomly assigned treatment. We calculated that this number would achieve 90% power to show equivalence when assessed by RR for pathological complete response with 5% dropout during run-in chemotherapy phase. This sample size was also calculated to provide at least 90% power to show equivalence when assessed by risk difference between groups for pathological complete response with margins of -13% and 13% and a two-sided 0.05 significance level. We assumed that the proportion of patients who would achieve a pathological complete response would be approximately 42.5% in the ABP 980 and trastuzumab groups.¹⁶

We initially used a sequential testing method to test similarity between ABP 980 and trastuzumab by comparing the two-sided 90% CI for risk difference between the ABP 980 and trastuzumab groups with statistical margins of -13% and 13%. If the test on the risk difference was successful, similarity was then tested by RR of pathological complete response at a two-sided significance level of 0.05 by comparing the two-sided 90% CI between the ABP 980 and trastuzumab groups with statistical margins of 0.759 and 1.318.

The population assessable for pathological complete response was defined as all randomised patients who received any amount of investigational product, underwent surgery, and had an available pathological complete response assessment from the local laboratory. The safety analysis population consisted of all patients who were randomised and received any amount of investigational product. We did sensitivity analyses in the intention-to-treat and per-protocol populations (data not shown). The intention-to-treat population included all patients randomly assigned to a study group, regardless of whether they received any investigational product. The per-protocol population included all patients who were randomised, had local laboratory pathological complete response results, and had no protocol deviations that prevented assessment of the primary objective.

All statistical analyses were done with SAS version 9.1.3 or later. This study is registered with ClinicalTrials.gov, number NCT01901146, and Eudra, number CT 2012-004319-29.

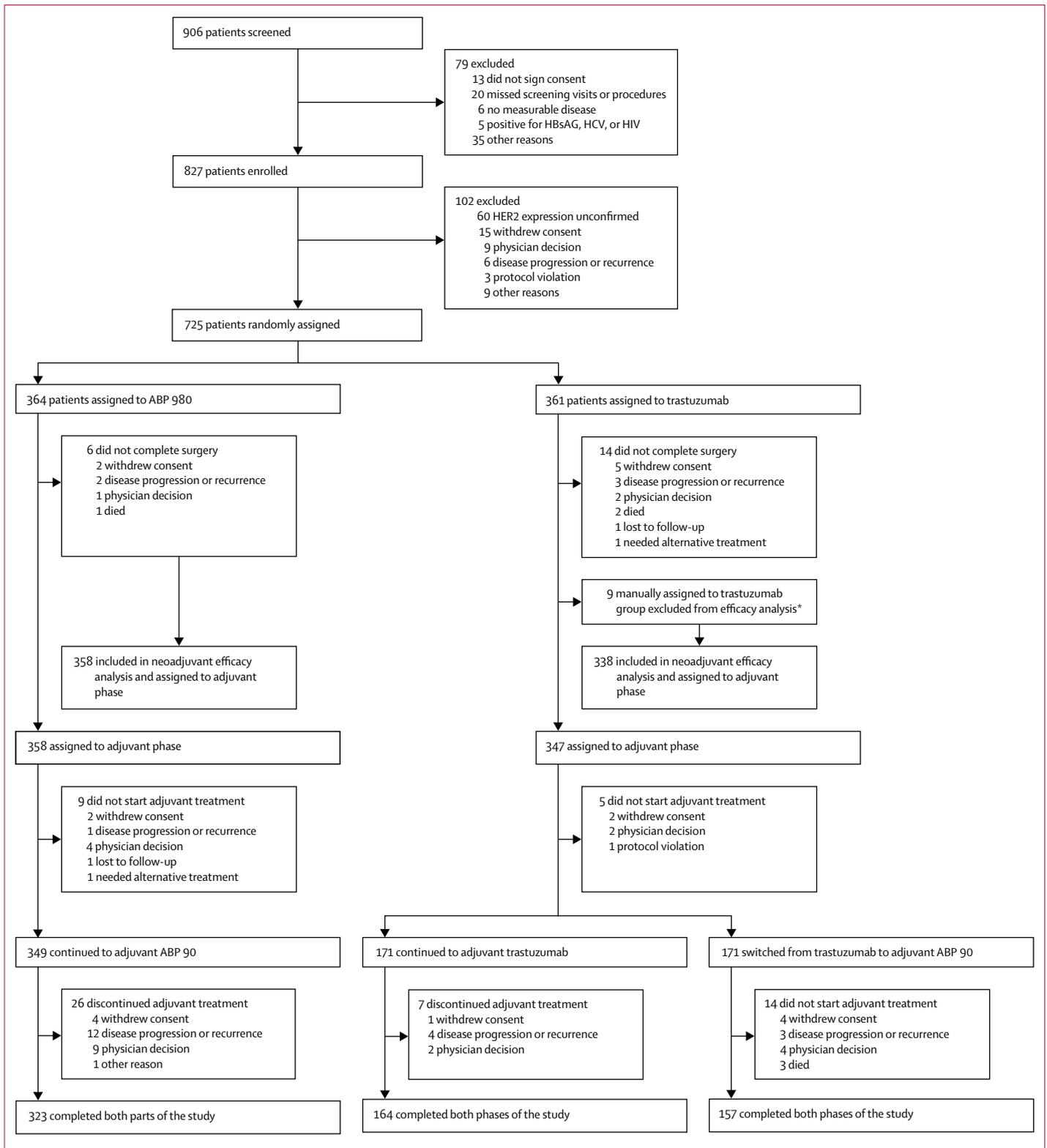


Figure 1: Trial profile

HBsAg=hepatitis B surface antigen. HCV=hepatitis C virus. *Nine patients were assigned to the trastuzumab group because of a delay in manufacturing of ABP 980 at the start of the study. These patients were excluded from the primary efficacy analysis but included in the final safety analysis.

Role of the funding source

The funder had a role in study design, data analysis, data interpretation, and writing of the report, and had access to the raw data, but had no role in data collection. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We enrolled patients between April 29, 2013, and Sept 29, 2015. The data cutoff for the primary analysis was May 5, 2016, and the database lock for the final analysis was March 29, 2017. Of 906 patients screened, 79 were excluded (figure 1). 827 patients were enrolled and 725 were randomised (figure 1). The treatment groups were well balanced in terms of baseline characteristics (table 1). The baseline distribution of sentinel lymph node biopsies was balanced the two groups (39 [11%] patients in the ABP 980 group and 29 [9%] in the trastuzumab group). Lymph node surgery was not done in 13 patients after neoadjuvant treatment because they had negative or only up to two positive sentinel nodes; these patients were equally distributed between the two treatment groups of the neoadjuvant phase (six [2%] of 358 patients in the ABP 980 group vs seven [2%] of 338 in the trastuzumab group). Patients' exposure to investigational products is shown in table 2. Exposure to paclitaxel during the neoadjuvant phase was similar in the ABP 980 and trastuzumab groups. Paclitaxel was administered only in the neoadjuvant phase. The mean cumulative dose for patients receiving paclitaxel every 3 weeks was 686·0 (SD 65·2) mg/m² in the ABP 980 group and 679·0 (83·0) mg/m² in the trastuzumab group. For patients who received paclitaxel weekly, the mean cumulative dose was 913·0 (SD 131·2) mg/m² in the ABP 980 group and 906·0 (132·8) mg/m² in the trastuzumab group. Median follow-up was 12 months (IQR 1·04–1·08) in patients who only received ABP 980, 12 months (1·04–1·07) in those who only received trastuzumab, and 12 months (1·04–1·08) in the patients who switched from trastuzumab to ABP 980 in the adjuvant phase.

All patients who underwent surgery were assessable for the primary endpoint of pathological complete response (696 patients in total; 358 of whom received ABP 980 and 338 who received trastuzumab). 172 (48%, 95% CI 43–53) of 358 patients who received neoadjuvant ABP 980 and 137 (41%, 35–46) of 338 patients who received neoadjuvant trastuzumab achieved a pathological complete response in breast tissue and axillary nodes based on local laboratory assessments. The risk difference (ABP 980 minus trastuzumab) of pathological complete response was 7·3% (90% CI 1·2–13·4). The RR (ABP 980 vs trastuzumab) of pathological complete response was 1·188 (90% CI 1·033–1·366). The primary endpoint, however, was not met, because the upper boundaries of the 90% CIs for risk difference and RR exceeded the predefined equivalence margins (figure 2).

	ABP 980 (n=364)	Trastuzumab (n=190)	Switched from adjuvant trastuzumab to ABP 980 (n=171)
Age (years)	53·0 (46·0–60·0)	53·0 (45·0–60·0)	53·0 (44·0–62·0)
Ethnicity			
White	331 (91%)	175 (92%)	158 (92%)
Black or African American	10 (3%)	2 (1%)	2 (1%)
Other	23 (6%)	13 (7%)	11 (6%)
Weight (kg)	70·6 (61·60–81·00)	70·2 (62·00–79·00)	73·3 (62·20–81·30)
Geographical region			
Eastern Europe	271 (75%)	141 (74%)	132 (77%)
Western Europe	43 (12%)	24 (13%)	22 (13%)
Other	50 (14%)	25 (13%)	17 (10%)
ECOG performance status score			
0	298 (82%)	163 (86%)	149 (87%)
1	66 (18%)	27 (14%)	22 (13%)
Tumour stage			
<T4	282 (78%)	147 (77%)	134 (78%)
T4	82 (23%)	43 (23%)	37 (22%)
Axilla lymph node involvement			
Yes	277 (76%)	136 (72%)	130 (76%)
No	87 (24%)	54 (28%)	41 (24%)
Hormone receptor status			
Positive for ER, PR, or both	265 (73%)	140 (74%)	128 (75%)
Negative for ER and PR	99 (27%)	50 (26%)	43 (25%)
Histological grade			
1	8 (2%)	1 (1%)	0
2	174 (48%)	93 (49%)	80 (47%)
3	120 (33%)	67 (35%)	65 (38%)
Unknown	62 (17%)	29 (15%)	26 (15%)
Left ventricular ejection fraction (%)	65 (61·0–68·0)	65 (60·0–68·0)	65 (60·0–68·0)

Data are median (IQR) or n (%). Percentage values might not total 100% because of rounding. ECOG=Eastern Cooperative Oncology Group. ER=oestrogen receptor. PR=progesterone receptor.

Table 1: Baseline characteristics of safety population

In the sensitivity analyses based on central pathology review of tumour samples, 162 (48%, 95% CI 42–53) of 339 patients in the ABP 980 group and 138 (42%, 36–47) of 330 in the trastuzumab group showed pathological complete response in breast tissue and axillary nodes. The risk difference between groups and RR of ABP 980 versus trastuzumab were within the predefined equivalence margins (figure 2).

The overall incidence of adverse events in the two treatment groups during both the neoadjuvant and adjuvant phases was similar (tables 3, 4, appendix pp 3–7). In the neoadjuvant phase, 19 (5%) of 364 patients in the ABP 980 group and 23 (6%) of 361 in the trastuzumab group had adverse events that led to dose delays of investigational products, three (1%) and two (1%), respectively, had events that led to discontinuation of treatment, and four (1%) and two (1%), respectively, had events that led to withdrawal from the study. In the adjuvant phase, 16 (5%) of 349 patients in the

	Neoadjuvant treatment		Adjuvant treatment		
	ABP 980 (n=364)	Trastuzumab (n=361)	ABP 980 (n=349)	Trastuzumab (n=171)	Switched from adjuvant trastuzumab to ABP 980 (n=171)
Total number of doses of investigational product administered					
Neoadjuvant					
1-3	7 (2%)	9 (3%)	0	0	0
4	357 (98%)	352 (98%)	0	0	0
Adjuvant					
1-10	0	0	41 (12%)	12 (7%)	17 (10%)
11-13	0	0	308 (88%)	159 (93%)	154 (90%)
Weight-based average dose (mg/kg)*	6.5 (6.5-6.5)	6.5 (6.5-6.5)	6.2 (6.17-6.18)	6.2 (6.15-6.17)	6.2 (6.15-6.18)
Weight-based cumulative dose (mg/kg)*	26.0 (26.0-26.0)	26.0 (26.0-26.0)	74.0 (68.0-76.0)	74.0 (74.0-80.0)	74.0 (70.0-80.0)
Total cumulative dose (mg)†	1820.0 (1605.5-2106.0)	1830.0 (1612.0-2080.0)	5106.0 (4399.6-5920.0)	5200.0 (4440.0-5920.0)	5208.0 (4514.00-6142.00)

Data are number (%) or median (IQR). Percentage values might not total 100% because of rounding. *For visits where partial loading or reloading doses were indicated on the electronic case report form, 4 mg/kg was given, and for visits where maintenance doses were indicated on the form, 3 mg/kg was used. †Calculated with use of the patient's weight at screening.

Table 2: Investigational product exposure in the safety analysis population

ABP 980 group, six (4%) of 171 in the trastuzumab group, and eight (5%) of 171 in the switching group had adverse events that led to dose delay of investigational products, seven (2%), three (2%), and four (2%), respectively, had events that led to treatment discontinuation, and seven (2%), two (1%), and two (1%), respectively, had events that led to withdrawal from the study.

Grade 3 or worse adverse events during the neoadjuvant phase occurred in 54 (15%) of 364 patients in the ABP 980 group and 51 (14%) of 361 patients in the trastuzumab group, of which the most frequent grade 3 or worse event of interest was neutropenia, occurring in 21 (6%) patients in both groups. In the adjuvant phase, grade 3 or worse adverse events occurred in 30 (9%) of 349 continuing ABP 980, 11 (6%) of 171 continuing trastuzumab, and 13 (8%) of 171 who switched from trastuzumab to ABP 980; the most frequent grade 3 or worse events of interest were infections and infestations (four [1%], two [1%], and two [1%]), neutropenia (three [1%], two [1%], and one [1%]), and infusion reactions (two [1%], two [1%], and three [2%]).

We recorded no differences in the incidence of events of interest between treatment groups in the neoadjuvant or adjuvant phases (tables 5, 6). Overall, the incidence of adverse events of interest was lower in the adjuvant phase than in the neoadjuvant phase (tables 5, 6). In patients who initially received neoadjuvant trastuzumab, the incidence of adverse events of interest did not differ between patients who continued receiving trastuzumab in the adjuvant phase and those who switched to ABP 980 in the adjuvant phase (table 6).

A complete list of treatment-emergent serious adverse events is provided in the appendix (pp 8-10). In the neoadjuvant phase, serious adverse events occurred in

18 (5%) of 364 patients in the ABP 980 group and five (1%) of 361 in the trastuzumab group. The most common were infections and infestations. Three (<1%) of 364 patients in the ABP 980 group and two (<1%) of 361 patients in the trastuzumab group had serious adverse events that were judged to be related to the investigational products. In the adjuvant phase, 18 (5%) of 349 patients in the ABP 980 group, six (4%) of 171 in the trastuzumab group, and six (4%) of 171 in the switching group had serious adverse events. One (<1%) of 171 patients in the switching group had a serious adverse event (ventricular extrasystoles) that was judged to be related to the investigational product. Six patients in the ABP 980 treatment group and one in the trastuzumab group had serious adverse events from accidents or surgery that were deemed to be unrelated to the investigational products. The most common serious treatment-emergent adverse events during the adjuvant therapy phase were gastrointestinal disorders, injury, poisoning, and procedural complications, and infections and infestations (appendix pp 8-10).

Six patients died during the study, among whom four died before or more than 30 days after treatment with an investigational product. Two patients died from adverse events not judged to be related to the investigational products. One patient in the ABP 980 group died from pneumonia during the neoadjuvant phase and the other, in the switching group, died from septic shock in the adjuvant phase.

Overall, the incidence of adverse events was lower in the adjuvant phase, when there was no run-in chemotherapy, than in the neoadjuvant phase, which was preceded by chemotherapy (tables 3, 4). Switching patients from trastuzumab to ABP 980 did not affect safety; the incidence of adverse events in the switching

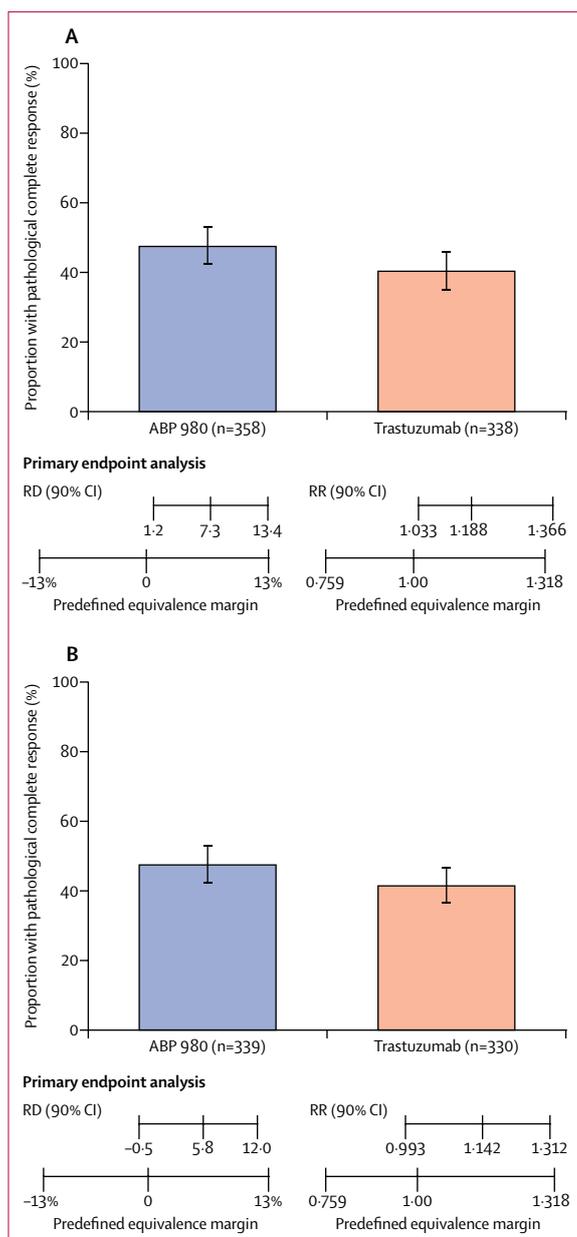


Figure 2: Proportions of patients with pathological complete responses (A) Local laboratory review. (B) Central laboratory review. Data are percentages and the error bars represent 95% CIs. RD=risk difference. RR=risk ratio.

group was consistent with that reported in patients who continued receiving trastuzumab in the adjuvant phase (appendix pp 3–10).

The incidence of LVEF decline from the value after chemotherapy run-in and before randomisation by at least 10 percentage points and to less than 50% ranged from 1.8% to 3.5% across the treatment groups (appendix p 11), and the median LVEF values did not change in any treatment group over the full course of the study (data not shown). The trastuzumab and switching groups had similar LVEF results (appendix p 11).

	ABP 980 (n=364)			Trastuzumab (n=361)		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
Arthralgia	63 (17%)	1 (<1%)	0	55 (15%)	0	0
Asthenia	53 (15%)	1 (<1%)	0	59 (16%)	0	0
Neuropathy peripheral	48 (13%)	3 (1%)	0	36 (10%)	7 (2%)	0
Anaemia	38 (10%)	2 (1%)	0	35 (10%)	3 (1%)	0
Neutropenia	37 (10%)	12 (3%)	4 (1%)	25 (7%)	14 (4%)	6 (2%)

The table shows grade 1–2 events that occurred in $\geq 10\%$ of patients in any group and grade 3 and 4 adverse events that occurred in $>2\%$ of patients in any group; none of the events were grade 5. A complete list of adverse events is available in the appendix (pp 3–7). Adverse events were classified with Medical Dictionary for Regulatory Activities version 19.0 codes. Only treatment-emergent adverse events are summarised. Patients are included only once, even if they had multiple events in a category.

Table 3: Adverse events during neoadjuvant treatment in the safety analysis population

Of the seven patients who had cardiac failure adverse events during the neoadjuvant phase (six [2%] of 364 patients in the ABP 980 group and one [$<1\%$] of 361 in the trastuzumab group), none experienced cardiac failure coincident with LVEF decline of at least 10 percentage points and to less than 50%. All cardiac failure events were grade 1 or 2, and patients completed all planned doses of investigational product with no worsening of the cardiac failure event. During the adjuvant phase, two (1%) of 349 patients in the ABP 980 group, one (1%) of 171 in the trastuzumab group, and one (1%) of 171 in the switching group had cardiac failure events. One patient in the switching group had a grade 3 cardiac failure event and all others were grade 1 or 2. One patient in the trastuzumab group had a cardiac failure event that was coincident with LVEF decline of at least 10 percentage points and to less than 50%. No patients discontinued investigational products due to cardiac failure in the adjuvant phase.

Two patients in the ABP 980 group and two in the trastuzumab group developed binding antibodies during the neoadjuvant phase. Neither of these patients tested positive for neutralising antibodies.

During the course of the entire study, eight patients (two [1%] in the ABP 980 group, two [1%] in the trastuzumab group, and four [2%] in the switching group) tested positive for binding antibodies at any time during the study (appendix p 12). No patients tested positive for neutralising antibodies. Two (1%) patients in the ABP 980 group, one (1%) in the trastuzumab group, and two (1%) in the switching group who were negative for binding antibodies at baseline later had positive results, all of which were transient (ie, results were negative at the last time the patient was tested). None of these patients tested positive for neutralising antibodies after baseline.

Discussion

We designed this equivalence study to compare the effects of the biosimilar ABP 980 with those of reference product trastuzumab on pathological complete response in women with HER2-positive early breast cancer in the

	ABP 980 (n=349)			Trastuzumab (n=171)			Switched from adjuvant trastuzumab to ABP 980 (n=171)		
	Grade 1-2	Grade 3	Grade 4	Grade 1-2	Grade 3	Grade 4	Grade 1-2	Grade 3	Grade 4
Neutropenia	22 (6%)	2 (1%)	1 (<1%)	10 (6%)	0	0	5 (3%)	1 (1%)	0
Arthralgia	20 (6%)	0	0	9 (5%)	0	0	9 (5%)	0	0
Asthenia	17 (5%)	1 (<1%)	0	7 (4%)	0	0	10 (6%)	0	0
Anaemia	17 (5%)	0	0	7 (4%)	0	0	10 (6%)	0	0
Neuropathy peripheral	8 (2%)	0	0	3 (2%)	0	0	2 (1%)	0	0

The table shows grade 1-2 adverse events that occurred in >10% of patients in any group and grade 3 and 4 adverse events that occurred in >2% of patients in any group; none of the events were grade 5. A complete list of adverse events is provided in the appendix (pp 3-7). Adverse events were classified with Medical Dictionary for Regulatory Activities version 19.0 codes. Only treatment-emergent adverse events are summarised. Patients are only included once, even if they had multiple events in a category.

Table 4: Adverse events during adjuvant treatment in the safety analysis population

	ABP 980 (n=364)				Trastuzumab (n=361)			
	Grade 1-2	Grade 3	Grade 4	Grade 5	Grade 1-2	Grade 3	Grade 4	Grade 5
Infusion reactions	73 (20%)	7 (2%)	0	0	61 (17%)	7 (2%)	0	0
Neutropenia	48 (13%)	16 (4%)	5 (1%)	0	36 (10%)	15 (4%)	6 (2%)	0
Infections and infestations	44 (12%)	4 (1%)	2 (1%)	1 (<1%)	53 (15%)	1 (<1%)	1 (<1%)	0
Hypersensitivity	22 (6%)	2 (1%)	0	0	17 (5%)	2 (1%)	0	0
Cardiac failure	6 (2%)	0	0	0	1 (<1%)	0	0	0
Pulmonary toxicity	1 (<1%)	0	0	0	1 (<1%)	0	0	0

Adverse events were classified with Medical Dictionary for Regulatory Activities version 19.0 codes. Only treatment-emergent adverse events of interest are summarised. Patients are only included once, even if they had multiple events in a category.

Table 5: Adverse events of interest during neoadjuvant treatment in the safety analysis population

	ABP 980 (n=349)				Trastuzumab (n=171)				Switched from adjuvant trastuzumab to ABP 980 (n=171)			
	Grade 1-2	Grade 3	Grade 4	Grade 5	Grade 1-2	Grade 3	Grade 4	Grade 5	Grade 1-2	Grade 3	Grade 4	Grade 5
Infusion reactions	26 (8%)	2 (1%)	0	0	12 (7%)	2 (1%)	0	0	17 (10%)	2 (1%)	1 (1%)	0
Neutropenia	35 (10%)	2 (1%)	1 (<1%)	0	14 (8%)	2 (1%)	0	0	12 (7%)	1 (1%)	0	0
Infections and infestations	50 (14%)	4 (1%)	0	0	15 (9%)	2 (1%)	0	0	21 (12%)	1 (1%)	0	1 (1%)
Hypersensitivity	11 (3%)	0	0	0	7 (4%)	0	0	0	8 (5%)	0	0	0
Cardiac failure	2 (1%)	0	0	0	1 (1%)	0	0	0	1 (1%)	0	0	0
Pulmonary toxicity	4 (1%)	0	0	0	1 (1%)	1 (1%)	0	0	0	1 (1%)	0	0

Adverse events were classified with Medical Dictionary for Regulatory Activities version 19.0 codes. Only treatment-emergent adverse events of interest are summarised. Patients are only included once, even if they had multiple events in a category.

Table 6: Adverse events of interest during adjuvant treatment in the safety analysis population

neoadjuvant setting. Although the primary efficacy endpoint of our study was not met because, based on local laboratory review of tumour samples, the upper bounds of the 90% CIs for RR and risk difference exceeded the predefined equivalence margins, our sensitivity analyses based on central laboratory evaluation of tumour samples indicated similar efficacy of the two drugs, with both risk estimates contained within the predefined equivalence margins. ABP 980 and trastuzumab had similar safety outcomes in both the neoadjuvant and adjuvant phases of the study. The incidence of serious adverse events was slightly higher in the ABP 980 group than in the trastuzumab group during

the neoadjuvant phase, including a higher number of infectious adverse events, but many adverse events were probably confounded by concomitant paclitaxel or were surgical complications or trauma unrelated to the investigational products. The numbers of patients with serious adverse events in the neoadjuvant phase judged to be related to investigational products were similar in the two groups. Similarly, most of the serious adverse events in the adjuvant phase were unrelated to investigational products, and only one patient in the switching group had a serious event that was associated with treatment. Overall, therefore, the safety profiles of ABP 980 and trastuzumab for adverse events, serious

adverse events, and events of interest were similar. The frequencies, types, and severities of adverse events were consistent with the historical safety profile of trastuzumab.^{1,2}

We chose women with early-stage breast cancer as the study population for this trial because this population is more homogeneous than patients who have metastatic disease, and, therefore, is more suitable for an equivalence study.^{15,17} We selected pathological complete response as the primary efficacy endpoint to be consistent with previous studies of trastuzumab and because it is a clinically meaningful and validated endpoint that is directly associated with increased event-free survival.¹⁸ The proportions of patients in the ABP 980 and the trastuzumab groups were consistent with those previously reported for trastuzumab,^{19–23} but, despite clinically similar efficacy, in the local review of tumours the risk difference and RR for pathological complete response between the two groups slightly exceeded the upper statistical margins for equivalence. In the central review of tumour samples, however, the point estimates for risk difference and RR were lower and fell within the similarity margins.

A potential limitation of the study is that we did not assess clinical response of breast cancer to the neoadjuvant treatment; clinical tumour response is highly variable and there is no validated standard method to differentiate between two very similar products. Histopathological assessment of pathological complete response remains the standard method to investigate whether breast cancer patients have residual disease after receiving neoadjuvant treatment. The choice of locally reviewed pathological complete response as the primary endpoint is another potential limitation of this study. Central assessment is generally more conservative and reduces variability, which provides greater confidence in the results. We chose to base the primary endpoint on local review of tumour samples partly because of concerns about potential logistical difficulties associated with transfer of tissue across the four different regions in which the study was done (eg, ensuring integrity of the samples is maintained during international transport). Use of local laboratories increased the likelihood that we would have sufficient tissue from patients to make meaningful comparisons of treatment effects, despite the risk of higher variability. We found, however, that transport of samples for central review was feasible and did prespecified sensitivity analyses of the central findings to address the issue of pathologist variability at the local level. In most cases, the amount and integrity of the samples that were transported to the central laboratory were adequate to assess pathological complete response. To our knowledge, this study is the first to show that including central pathology review of pathological complete response is feasible in a large, international, multicentre clinical trial.

Treatment with trastuzumab has been associated with an increased risk of cardiac toxicity, possibly due to

previous exposure to anthracyclines.²⁴ Therefore, we carefully assessed LVEF and cardiac adverse events. We found no change in median LVEF values over the course of the study, and decreases in LVEF were seen in few patients, with the frequencies being similar across treatment groups. The frequency of cardiac disorders was low throughout the study and none resulted in discontinuation of investigational product. Only seven patients had cardiac failure in the neoadjuvant phase, and all events were grade 1 or 2. Moreover, all seven patients received the planned doses of investigational products, which suggests resolution or no worsening of cardiac failure. Furthermore, LVEF decline and a cardiac failure adverse event coincided in only one patient in the adjuvant phase, which suggests very low cardiac toxicity in this study.

To our knowledge, this is the first study of a trastuzumab biosimilar encompassing a single-switch design from the reference product to a biosimilar, which allowed us to assess the clinical safety and immunogenicity of this approach to treatment. Safety and immunogenicity were similar in patients who were switched and in those who continued to receive trastuzumab as adjuvant therapy.

Safety, efficacy, and clinical outcomes did not differ for the biosimilar ABP 980 and trastuzumab reference product in women with HER2-positive early breast cancer. The frequencies, types, and severities of adverse events, including cardiac events, did not differ between treatment groups and were consistent with the known safety profile of trastuzumab. Immunogenicity was low for both drugs. Similarities persisted in the neoadjuvant and adjuvant phases, and switching from trastuzumab to ABP 980 did not lead to any new or unexpected safety signals. Overall, our results add to the evidence from analytical, functional, and pharmacokinetic studies supporting the clinical similarity of ABP 980 and trastuzumab.

Contributors

GvM, NZ, and VH conceived and designed the study and analysed the data. MC, H-CK, SM, PS, and ZT acquired patients' data. All authors reviewed the study results and interpreted the data, contributed substantially to development of the manuscript, and reviewed and approved the final version for submission.

Declaration of interests

GvM is a consultant for Amgen. MC is a consultant for AstraZeneca, Celldex, Novartis, OBI Pharma, Pfizer, Pierre Fabre, and Puma Biotechnology. H-CK is a consultant for Amgen, Carl Zeiss Meditec, Genomic Health, GSK, Janssen, LIV Pharma, Novartis, Pfizer, Roche, SurgVision, TEVA, and Theracision. NZ and VH are employees and stockholders of Amgen. The other authors declare no competing interests.

Acknowledgments

The study was funded by Amgen. We thank the patients and all those who participated in the study. Medical writing support to develop drafts, tables, and figures in consultation with the authors, copyediting, fact checking, and referencing) was funded by Amgen and provided by Pasquale Iannuzzelli, Innovation Communications Group, under the direction of Monica Ramchandani, Amgen.

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Assessing Analytical and Functional Similarity of Proposed Amgen Biosimilar ABP 980 to Trastuzumab

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Published online: 10 April 2019
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Abstract

Background ABP 980 has been developed as a biosimilar to Herceptin[®] (trastuzumab). Comprehensive analytical characterization incorporating orthogonal analytical techniques was used to compare ABP 980 to trastuzumab reference products sourced from the United States (US) and the European Union (EU).

Methods Physicochemical property comparisons included the following: primary structure related to amino acid sequence and post-translational modifications, including glycans; higher-order structure; product-related substances and impurities, including size and charge variants; subvisible and submicron particles, and protein content. In addition, functional similarity was assessed for Fab-mediated, Fc-mediated, and combined Fab- and Fc-mediated activities.

Results ABP 980 has the same amino acid sequence as and similar post-translational modification profiles to trastuzumab (US) and trastuzumab (EU). Importantly, ABP 980 was found to be highly similar to trastuzumab for all functional activities related to the mechanism(s) of action. Higher-order structure, product-related substances and impurities, particles and aggregates were also highly similar between ABP 980 and trastuzumab. Where minor differences were noted, they were evaluated and found unlikely to impact clinical performance. The totality of evidence, including the pharmacokinetic clinical similarity of ABP 980, further supports that ABP 980 is highly similar to trastuzumab.

Conclusion Based on the comprehensive analytical similarity assessment, ABP 980 is analytically highly similar to the reference product, trastuzumab.

Key Points

ABP 980 was found to be highly similar to trastuzumab for all functional activities related to the mechanism(s) of action.

Higher-order structure, product-related substances and impurities, particles and aggregate were highly similar between ABP 980 and trastuzumab.

1 Introduction

Herceptin[®] (trastuzumab) is approved for use in the United States (US), European Union (EU), and much of the rest of the world for treatment of metastatic breast cancer, early breast

cancer, and metastatic gastric cancer [1, 2], and is the standard of care for patients with human epidermal growth factor receptor 2 (HER2)-overexpressing breast cancer [3–5]. Trastuzumab binds to the extracellular domain (ECD) of HER2, blocking receptor activation and subsequent proliferation of cells that express HER2, and induces antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) on HER2-expressing cells [6]. ABP 980 is being developed by Amgen Inc. as a biosimilar to trastuzumab. ABP 980 and trastuzumab are immunoglobulin G type 1 (IgG1) monoclonal antibodies (mAbs) expressed in Chinese hamster ovary (CHO) cells.

Biosimilars are produced in different cell lines (i.e., from a different transfection event) and have different manufacturing processes to the reference product. Therefore, minor structural and functional differences between biosimilars and the reference product are unavoidable due to their complex molecular structure and the unique, often proprietary, manufacturing processes involved in their production. Consequently, it is not possible to produce biosimilar molecules that are identical to the reference product [7]. Nevertheless, each biosimilar must demonstrate similarity to the reference product. To meet this challenge, the US Food and Drug Administration (FDA) and

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European Medicines Agency (EMA) have published guidance for the stepwise development of biosimilars. The foundation of the stepwise approach is demonstrating structural and functional similarity [8]. Sensitive analytical methods capable of detecting structural and functional differences are the foundation of the stepwise biosimilar evaluation process, and any differences that are identified are further evaluated to confirm they do not affect clinical efficacy and safety [9–11]. Additionally, ABP 980 has been shown to be similar to trastuzumab in non-clinical xenograft models and a phase 1 pharmacokinetic (PK) study in healthy volunteers [12, 13]. Further, results from a phase 3 clinical study have confirmed similar efficacy and safety between ABP 980 and trastuzumab when treating patients with early breast cancer [14].

A comprehensive analytical and functional assessment of the proposed biosimilar ABP 980 was conducted to determine its similarity to FDA-licensed trastuzumab (trastuzumab [US]) and EU-authorized trastuzumab (trastuzumab [EU]). This analysis included lots manufactured from all drug substance lots used in the clinical trials. ABP 980 lots were compared to 23 lots of trastuzumab (US) and 33 lots of trastuzumab (EU), which were procured over a period of approximately 5 years. The methods used for the analytical similarity assessment were selected based on knowledge regarding the structure and function of ABP 980 and trastuzumab, focusing on those characteristics critical to biological activity, including HER2 binding, potency as measured by ligand-independent inhibition of proliferation, and ADCC. The similarity assessment strategy was designed to comprehensively assess structure and functional attributes compared to pre-defined assessment criteria, to ensure that any differences between ABP 980 and trastuzumab were identified and evaluated in line with US and EU regulatory guidelines. The results presented here are numeric results, along with representative profiles, demonstrating the similarity of ABP 980 to the reference product, trastuzumab.

2 Materials and Methods

ABP 980 was developed by Amgen as a biosimilar to Herceptin® (trastuzumab). Multiple lots of trastuzumab from US and EU regions were procured over a period of approximately 5 years. The expiration dates for the trastuzumab lots ranged from February 2015 to March 2020. All analyses were performed at Amgen except where noted. The lots included for the similarity assessment of each parameter were based on the impact of the process on that parameter. For parameters primarily influenced by the drug substance manufacturing process (e.g., glycan profile, biological functions), only a single drug product lot for each independent drug substance was included. When a drug product lot was not available, the drug substance lot was included for these parameters. For parameters primarily influenced by the drug product manufacturing

process (e.g., protein content, reconstitution time), data from all available drug product lots were included.

2.1 Product Strength: Protein Content and Reconstituted Protein Concentration

Samples were reconstituted then gravimetrically diluted, and the protein concentration of each vial was determined using the ultraviolet (UV) absorbance, the dilution factor, and the theoretical protein extinction coefficient [15]. The product volume of each vial was determined gravimetrically, correcting for density. The protein content of each vial was the product of the protein concentration and the product volume.

2.2 Peptide Mapping

A single replicate of each sample was reduced with dithiothreitol (DTT) in denaturant and alkylated with sodium iodoacetate. Excess reagents were removed by gel-filtration desalting spin columns. Subsequently, samples were digested with trypsin for 35 min at 37 °C. The resulting peptides were separated by reversed-phase (RP) ultra high-performance liquid chromatography (UHPLC) in a trifluoroacetic acid (TFA)/acetonitrile (ACN) gradient, monitored by UV at 214 nm and mass spectrometry (MS).

2.3 Whole Mass

The intact molecular mass of ABP 980 and trastuzumab (EU) was determined by electrospray ionization–time of flight–mass spectrometer (ESI–TOF–MS) analysis. Single replicates of samples were separated from buffer components and introduced to the mass spectrometer by high-performance liquid chromatography (HPLC). The resulting ion spectra were deconvoluted to produce molecular mass profiles. The theoretical mass calculations assume that all of the disulfide bonds are intact and there are no C-terminal lysine residues on the heavy chain (HC) of trastuzumab.

2.4 N-Glycan Mapping

The N-linked glycan profiles of samples were evaluated by hydrophilic interaction liquid chromatography (HILIC) UHPLC glycan map analysis. N-linked glycans were released from the protein backbone using peptide-N-glycosidase F (PNGase F) of a single replicate. The released glycans were derivatized with the fluorescent label 2-aminobenzoic acid at the reducing terminal N-acetylglucosamine (GlcNAc). Fluorescently labeled oligosaccharides were separated by HILIC. Bound oligosaccharides were eluted,

and relative percent peak areas of the oligosaccharides were calculated.

2.5 Size Variants by Size-Exclusion Ultra high-Performance Liquid Chromatography (SE-UHPLC)

Size-exclusion ultra high-performance liquid chromatography (SE-UHPLC) was used to quantify levels of individual size variants, such as high molecular weight (HMW) species, low molecular weight (LMW) species, and main peak. The single replicate samples were loaded onto an SE-UHPLC column, separated isocratically, and the eluent was monitored by UV absorbance.

2.6 Secondary Structure by Fourier-Transformed Infrared Spectroscopy (FTIR)

Secondary structure was assessed by Fourier-transformed infrared spectroscopy (FTIR). Single replicate FTIR measurements were made at room temperature using a Bruker Vertex 70 FTIR spectrometer (Bruker Corporation, Billerica, MA). The spectrum of the buffer blank was recorded under identical conditions and was subtracted from the protein spectrum. The second derivative spectrum was calculated using a 9-point smoothing of the original spectrum. Spectral similarity was quantitatively determined using the Thermo OMNIC software QC compare function (Thermo Scientific).

2.7 Tertiary Structure by Near Ultraviolet-Circular Dichroism (UV CD) Spectroscopy

Tertiary structure was assessed by near ultraviolet-circular dichroism (UV CD) spectroscopy. Single replicate near UV CD measurements were made on an Applied Photophysics Chirascan spectropolarimeter (Applied Photophysics Ltd, Leatherhead, United Kingdom) at ambient temperature using cuvettes with a path length of 1 cm. All drug product samples were diluted to approximately 0.7 mg/mL with product buffer prior to measurements. The resulting spectra were corrected for protein concentration and contributions from buffer, and reported as circular dichroism ellipticity. Spectral similarity was quantitatively determined using the Thermo OMNIC software QC compare function (Thermo Scientific).

2.8 Thermal Stability by Differential Scanning Calorimetry (DSC)

Thermal stability was assessed by differential scanning calorimetry (DSC). DSC measurements were made in

triplicate using a MicroCal VP-Capillary DSC system (GE Healthcare Bio-Sciences, Pittsburgh, PA). All samples were diluted to approximately 1 mg/mL with product buffer prior to measurements.

2.9 Subvisible Particles by Light Obscuration (LO) and Micro-Flow Imaging (MFI)

Subvisible particles were assessed by light obscuration (LO). Single replicate LO measurements were made with an HIAC 9703 + liquid particle counting system (Beckman Coulter Life Sciences; Brea, CA) equipped with an HRLD 150 sensor. For each sample test, the particle concentration results were reported as cumulative particle counts/mL.

Subvisible particles were further assessed by micro-flow imaging (MFI). Single replicate MFI measurements were made with an MFI 5200 system equipped with a 100 μm /1.6 mm/SP3 flow cell (ProteinSimple, San Jose, CA). For each sample test, particle concentration results were reported as cumulative particle counts/mL of $\geq 5 \mu\text{m}$ non-spherical particles with an aspect ratio of < 0.85 .

2.10 Aggregates by Sedimentation Velocity Analytical Ultracentrifugation (SV-AUC)

Aggregates were analyzed by sedimentation velocity analytical ultracentrifugation (SV-AUC). Triplicate SV-AUC measurements were made at 45,000 rpm following UV absorbance at 280 nm. All product samples were diluted to approximately 0.5 mg/mL with product buffer before measurements. Scans were collected at 20 °C without delay between them. Data were analyzed allowing the frictional ratio, time invariant noise, and meniscus values to float during the non-linear least squares fit.

2.11 Aggregates by Size-Exclusion High-Performance Liquid Chromatography (SE-HPLC) coupled with Light Scattering (LS) Detection

Aggregates were analyzed by size-exclusion high-performance liquid chromatography–light scattering (SE-HPLC–LS). Triplicate samples without dilution were injected into the HPLC system, and the molecular weights were calculated for monomer and HMW species.

2.12 Fc Neonatal Receptor (FcRn) Binding

A two-step AlphaScreen[®] (Perkin Elmer, Shelton, CT, USA) receptor binding assay was used to quantify the binding of human IgG Fc domain and Fc neonatal receptor (FcRn). The assay measured the dose-dependent signal decrease observed when an Fc-containing sample is added to a

reaction containing FcRn-His (Amgen, Inc. Thousand Oaks, CA, USA) and Fc-biotin (Amgen, Inc. Thousand Oaks, CA, USA). When ABP 980 or trastuzumab is present at sufficient concentrations to inhibit the binding of FcRn to the biotinylated human Fc, a dose-dependent decrease in emission is measured using a plate reader. Activity was determined by comparing the sample response to the response obtained for the reference standard. The sample binding relative to the reference standard was determined using a four-parameter logistic model fit (SoftMax[®] Pro Software, Molecular Devices, Sunnyvale, CA, USA). Each sample was tested in three independent assays, and the final valid result for a given sample was reported as the mean of the three measurements. Results were reported as percentage relative binding values.

2.13 Fc gamma Receptor Type 2a (FcγRIIa) and FcγRIIIa Binding

Relative binding to Fc gamma receptor type 2a (FcγRIIa) (131H), FcγRIIIa (158 V), and FcγRIIIa (158F) was quantified using AlphaLISA[®] assays (Perkin Elmer, Shelton, CT, USA). Recombinant, purified human FcγRIIa (131H), FcγRIIIa (158 V) and FcγRIIIa (158F) glutathione S-transferase (GST)-fusion proteins were generated at Amgen Inc. Thousand Oaks, CA, USA through either a CHO or 293 cell expression system. The AlphaLISA[®] assay is an Amplified Luminescent Proximity Homogenous Assay (Alpha) designed to measure the level of Fc gamma receptor (FcγR) binding to the Fc portion of IgG1 mAbs. The assay contained two bead types, an acceptor bead and a donor bead. The acceptor bead is coated with glutathione and binds recombinant human FcγR-GST. The donor beads are coated with streptavidin, which binds to biotinylated human IgG1 (Amgen Inc. Thousand Oaks, CA, USA). When trastuzumab or ABP 980 is present at sufficient concentrations to inhibit the binding of FcγR-GST to the biotinylated human IgG1, a dose-dependent decrease in emission is measured using a plate reader. The sample binding relative to the reference standard was determined using a four-parameter logistic model fit (SoftMax[®] Pro Software, Molecular Devices, Sunnyvale, CA, USA). Each sample was tested in three independent assays, and the final valid result for a given sample was reported as the mean of the three measurements. Results were reported as percentage relative binding values for FcγRIIa (131H), FcγRIIIa (158 V) or FcγRIIIa (158F).

2.14 Human Epidermal Growth Factor Receptor 2 (HER2) Binding Enzyme-Linked Immunosorbent Assay (ELISA)

A solid-phase enzyme-linked immunosorbent assay (ELISA) was used to determine the binding of human HER2 to ABP

980 and trastuzumab. Recombinant human HER2 (rHER2) (AcroBiosystems, Newark, DE, USA) was coated onto the wells of a microtiter ELISA plate. After washing the wells to remove unbound protein, the remaining uncoated portions of the plate were blocked using a blocking buffer. Plates were then washed and a serial dilution of reference standard, control and sample(s) was added to the appropriate wells and incubated. Wells were again washed and goat anti-human IgG (Fc fragment) conjugated to horseradish peroxidase (HRP) (ThermoFisher, Waltham, MA, USA) was added. After a final wash, a peroxidase substrate/chromogen solution was added to the wells. The substrate changes color in the presence of HRP, in proportion to the amount of ABP 980 or trastuzumab bound to HER2 by the samples and standard. The reaction was stopped with 1.0 M phosphoric acid, and absorbance was measured with a microplate reader. The test-sample binding relative to the reference standard was determined using a four-parameter logistic model fit (SoftMax[®] Pro Software, Molecular Devices, Sunnyvale, CA, USA). Each sample was tested in three independent assays, and the final valid result for a given sample was reported as the mean of the three measurements. Results were reported as percentage relative binding values.

2.15 Inhibition of Proliferation in BT-474 Cells

BT-474 cells were incubated with varying concentrations of reference standard, assay control, and samples. After a timed incubation, Cell-Titer Glo[®] Luminescent Cell Viability Assay Reagent (Promega, Madison, WI, USA) system was added to the assay plates. Addition of Cell-Titer Glo[®] reagent results in cell lysis and generation of luminescence signal, which is proportional to the amount of adenosine triphosphate (ATP) present. The amount of ATP present was directly proportional to the number of viable cells in the culture and inversely proportional to the concentration of ABP 980 or trastuzumab. The test-sample bioactivity relative to the reference standard was determined using a four-parameter logistic model fit. Each sample was tested in three independent assays, and the final valid result for a given sample was reported as the mean of the three measurements. Results were reported as percentage relative potency.

2.16 NK92 Antibody-Dependent Cellular Cytotoxicity (ADCC) Assay

To assess ADCC, HCC2218 cells that overexpress HER2 receptors were used as target cells, and NK92-M1 cells, stably transfected with human FcγRIIIa (158 V), were used as effector cells. Target cells were loaded with calcein-AM (Sigma-Aldrich, St. Louis, MO, USA). ADCC activity was assessed by adding effector cells to the opsonized target cells at an effector-to-target ratio of 25:1 and incubated for

approximately 1 h. Calcein released from lysed target cells was determined by measuring the fluorescence of the supernatant. Each sample was tested in three independent assays, and the final valid result for a given sample was reported as the mean of the three measurements. The test-sample bioactivity relative to the reference standard was determined using a four-parameter logistic model fit (SoftMax[®] Pro Software, Molecular Devices, Sunnyvale, CA, USA). Results were reported as percentage relative NK92 ADCC.

2.17 Peripheral Blood Mononuclear Cell (PBMC) ADCC Assay

Peripheral blood mononuclear cells (PBMC) isolated from FcγRIIIa-158 V/F heterozygous donors were used as effector cells. SKBR3 HER2-expressing breast cancer cells were used as target cells in the assay. Target and effector cells were incubated with test samples for 18–24 h. After incubation, CytoTox Glo[®] Cytotoxicity Assay Reagent was added to the assay plates. CytoTox Glo[®] measures the presence of intracellular protease activity (dead-cell protease) that is released from membrane-compromised cells. The quantity of dead-cell protease in the medium, and thus luminescence, is directly proportional to the number of dead cells in the culture. Each sample was tested in three independent assays, and the final valid result for a given sample was reported as the mean of the three measurements. The test-sample bioactivity relative to the reference standard was determined using a four-parameter logistic model fit (PLA 2 Software, Steggmann Systems GmbH, Rodgau, Germany). Results were reported as percentage relative PBMC ADCC.

3 Results

The similarity testing plan and analytical methods used for the physicochemical and functional characterization of ABP 980 and trastuzumab are summarized in Table 1. Results of key structural and function characterization tests are presented here.

3.1 Primary Structure

The primary structures of ABP 980 and trastuzumab were investigated by several complementary characterization methods, including intact mass, reduced and deglycosylated HC and light chain (LC) mass analysis, peptide mapping, and glycan map. Results of the intact mass and reduced and deglycosylated HC and LC mass analyses are summarized in Table 2. The results showed ABP 980 and trastuzumab have the same molecular mass, which is also consistent with the theoretical mass. Reduced peptide map overlays are displayed in Fig. 1a. The chromatograms for the three

products are visually similar; no new or missing peaks were identified. In addition, the same N-linked glycosylation site at N³⁰⁰ was detected in the same glycopeptide in ABP 980 and trastuzumab. These results demonstrate that ABP 980 and trastuzumab have the same amino acid sequences.

The glycan profile of mAbs, specifically levels of afucosylated, galactosylated, and high mannose species, can influence binding to the FcγRIIIa receptor, which mediates ADCC activity. The percentage values of individual glycan species and glycan groups and glycan map profiles for representative lots of ABP 980, trastuzumab (US), and trastuzumab (EU) are shown in Table 2 and Fig. 1b. Although the abundance of certain individual glycan species, such as A1G0F, differ, similar levels of the key glycan groups—afucosylated, galactosylated, sialylated, and high mannose species—were present in ABP 980 compared to trastuzumab. However, the ranges for afucosylated and galactosylated species for trastuzumab (US) and trastuzumab (EU) are fairly wide. These combinations of glycans species, particularly the afucosylated and galactosylated species, influence binding to FcγRIIIa and ADCC activity, as shown in Fig. 2. This figure shows predicted NK92 ADCC, a formula for which was derived using JMP statistical software by determining which product quality attributes had a statistically significant impact on the measured NK92 ADCC. Then, the leverage of each statistically significant factor was determined to compute the predicted NK92 ADCC for any given glycan combination. High mannose, up to a level of 5%, was not found to have a significant impact on binding to FcγRIIIa and ADCC activity (Fig. 2d) (p value = 0.5505). All three products, ABP 980, trastuzumab (US), and trastuzumab (EU), follow the same glycan–ADCC relationship, as illustrated by the combined data set shown in Fig. 2. No new peaks were observed in the ABP 980 glycan map compared with trastuzumab.

3.2 Higher-Order Structure

ABP 980 and trastuzumab were characterized by FTIR for secondary structure, by near UV CD for tertiary structure, and by DSC for thermal stability. The results are shown in Table 2 and Fig. 3. The FTIR profiles of ABP 980 and trastuzumab are visually similar, with peaks at around 1639 cm⁻¹ and 1689 cm⁻¹, indicating the presence of a predominantly antiparallel beta-sheet structure typical of antibodies. The spectral similarity analysis demonstrates that the FTIR spectra are ≥ 95% similar. The near UV CD profiles of ABP 980 and trastuzumab are visually similar with peaks corresponding to optical activities of tryptophan, tyrosine, and phenylalanine, superimposed on the broad disulfide signal from 250 to 320 nm. The intensity of these features reflects the native structure and demonstrates that the disulfide bonds and aromatic amino acids are in the expected environment due to the proper folding of the proteins. The spectral

Table 1 Attributes and analytical techniques for characterization of ABP 980 and trastuzumab reference product

Category	Analytical techniques and attributes
Primary structure	Whole mass by mass spectrometry: intact molecular mass and mass spectrum profile Reduced and deglycosylated molecular masses of HC and LC: molecular masses of HC and LC and mass spectrum profile Reduced peptide map: amino acid sequence, peptide map profile, post-translational modifications Non-reduced peptide map: disulfide structure and peptide map profile Glycan map by HILIC HPLC: afucosylation, high mannose, galactosylation, afucosylated galactosylation, sialylation, glycan map profile cIEF: isoelectric point, profile Amino acid analysis: extinction coefficient Identity by ELISA
Higher-order structure	FTIR: spectral similarity, spectral profile Near UV CD: spectral similarity, spectral profile DSC: T_{m1} , T_{m2} , profile
Particles and aggregates	HIAC: $\geq 2 \mu\text{m}$, $\geq 5 \mu\text{m}$, $\geq 10 \mu\text{m}$, $\geq 25 \mu\text{m}$ particles MFI: $\geq 5 \mu\text{m}$ particles and $\geq 5 \mu\text{m}$ non-spherical particles SV-AUC: HMW, profile SE-HPLC-LS: Molar mass, profile
Product-related substances and impurities	SE-UHPLC: HMW, main peak, LMW, profile rCE-SDS: HC + LC, LMW + MMW, NGHC, profile nrCE-SDS: pre-peaks, main peak, profile
Thermal stability and degradation	SE-HPLC: HMW, main peak degradation rCE-SDS: HC + LC, LMW + MMW, NGHC degradation nrCE-SDS: pre-peak degradation Proliferation inhibition bioassay: potency degradation
General properties	Protein content: protein content, reconstituted protein concentration Reconstitution time
Process-related impurities	HCP ELISA 2D-DIGE LC/MS Protein A ELISA qPCR
Fab-mediated biological activity	Potency: proliferation inhibition (BT-474 cells) HER2 binding: ELISA, SPR Inhibition of AKT phosphorylation Proliferation inhibition (NCI-N87 cells) Proliferation inhibition synergy with chemotherapeutic (NCI-N87 cells) Lack of proliferation inhibition (non-amplified HER2 MCF7 cells)
Fc-mediated biological activity	FcRn binding FcγRIIIa (158 V) binding FcγRIIIa (158F) binding FcγRIa binding FcγRIIa (131H) binding FcγRIIb binding FcγR binding on primary macrophages FcγRIIIb binding C1q binding
Fab and Fc-mediated biological activity	NK92 ADCC activity PBMC ADCC activity Lack of ADCC (HER2-negative cells) ADCP activity Lack of CDC activity

2D-DIGE two-dimensional differential in-gel electrophoresis, *ADCC* antibody-dependent cellular cytotoxicity, *ADCP* antibody-dependent cellular phagocytosis, *CDC* complement-dependent cytotoxicity, *cIEF* capillary isoelectric focusing, *DSC* differential scanning calorimetry, *ELISA* enzyme-linked immunosorbent assay, *FcγR* Fc gamma receptor, *FcγRIa* Fc gamma receptor type 1a, *FcγRIIa* Fc gamma receptor type 2a, *FcγRIIb* Fc gamma receptor type 2b, *FcγRIIIa* Fc gamma receptor type 3a, *FcRn* Fc neonatal receptor, *FTIR* Fourier-transformed infrared, *HC* heavy chain, *HER2* human epidermal growth factor receptor 2, *HILIC* hydrophilic interaction liquid chromatography, *HMW* high molecular weight, *HPLC* high-performance liquid chromatography, *LC* light chain, *LC/MS* liquid chromatography with mass spectrometry, *LMW* low molecular weight, *MFI* micro-flow imaging, *NGHC* non-glycosylated heavy chain, *nrCE-SDS* non-reduced capillary electrophoresis with sodium dodecyl sulfate, *PBMC* peripheral blood mononuclear cells, *qPCR* quantitative polymerase chain, *reactionnrCE-SDS* reduced capillary electrophoresis with sodium dodecyl sulfate, *SDS* sodium dodecyl sulfate, *SE-HPLC-LS* size-exclusion high-pressure liquid chromatography–light scattering, *SE-UHPLC* size-exclusion ultra high-performance liquid chromatography, *SPR* surface plasmon resonance, *SV-AUC* sedimentation velocity analytical ultracentrifugation, *UV CD* ultraviolet circular dichroism

Table 2 Summary of structural attributes for ABP 980, trastuzumab (US), and trastuzumab (EU)

Attribute	ABP 980 Range (n)	Trastuzumab (US) Range (n)	Trastuzumab (EU) Range (n)
Intact molecular weight, difference between observed and theoretical (ppm)			
Glycosylation: A2G0F:A2G0F	37–42 (4)	28–43 (3)	28–33 (3)
Glycosylation: A2G0F:A2G1F	22–28 (4)	19–23 (3)	19–20 (3)
Glycosylation: A2G1F:A2G1F, A2G0F:A2G2F	22–25 (4)	18–19 (3)	15–20 (3)
Glycosylation: A2G1F:A2G2F	25–31 (4)	17–21 (3)	19–25 (3)
Reduced and deglycosylated light chain, difference between observed and theoretical (ppm)	35–37 (4)	36–37 (3)	36–36 (3)
Reduced and deglycosylated heavy chain, difference between observed and theoretical (ppm)	20–22 (4)	21–22 (3)	21–22 (3)
Glycan profile (%)			
Galactosylation	42.7–57.0 (22)	21.8–53.4 (23)	22.0–51.4 (33)
High mannose	1.5–4.2 (22)	2.1–6.5 (23)	1.8–4.8 (33)
Afucosylation	7.6–9.1 (22)	5.1–8.6 (23)	5.1–9.2 (33)
Sialylation	0.8–1.7 (22)	1.2–2.4 (23)	1.1–2.6 (33)
Glycosylation at Asn300 (%) (n)	99.3–99.8 (13)	98.6–99.1 (21)	98.5–99.2 (33)
FTIR: spectral similarity, relative to trastuzumab (US), (%)	99.5–100.0 (7)	99.4–100.0 (6)	99.3–100.0 (6)
Near UV CD: spectral similarity, relative to trastuzumab (US) (%)	98.1–99.2 (7)	98.5–100 (6)	97.1–99.4 (6)
DSC: T_{m1} (°C)	70.6–70.9 (11)	70.7–71.0 (9)	70.7–71.0 (9)
DSC: T_{m2} (°C)	82.5–82.7 (11)	82.5–82.6 (9)	82.5–82.6 (9)
LO: ≥ 2 - μ m particles/mL	8–1466 (6)	64–361 (30)	102–534 (6)
LO: ≥ 5 - μ m particles/mL	1–237 (30)	10–45 (6)	6–28 (6)
LO: ≥ 10 - μ m particles/mL	0–40 (30)	1–9 (6)	1–4 (6)
LO: ≥ 25 - μ m particles/mL	0–8 (30)	0–2 (6)	0–1 (6)
MFI: ≥ 5 - μ m particles/mL	0–100 (28)	72–414 (6)	50–144 (6)
MFI: ≥ 5 - μ m non-spherical particles/mL	0–52 (28)	64–330 (6)	26–108 (6)
SV-AUC: HMW	< LOQ	< LOQ	< LOQ
SE-HPLC–LS: molar mass of monomer (kDa)	144–148 (7)	144–148 (6)	143–148 (6)
SE-HPLC–LS: molar mass of pre-peak (kDa)	281–318 (7)	283–313 (6)	281–314 (6)
Protein content (mg/vial)			
ABP 980 (440-mg presentation) vs trastuzumab (US) (n)	422–441 (7)	424–447 (21)	–
ABP 980 (150-mg presentation) vs trastuzumab (EU) (n)	148–157 (22)	–	150–159 (30)
Protein concentration in mg/mL (n)	20.0–21.4 (29)	20.6–21.6 (20)	20.4–21.3 (30)

DSC differential scanning calorimetry, FTIR Fourier-transformed infrared, HMW high molecular weight, LO light obscuration, LOQ limits of quantitation, MFI micro-flow imaging, n number of lots analyzed, SE-HPLC–LS size-exclusion high-pressure liquid chromatography–light scattering, SV-AUC sedimentation velocity analytical ultracentrifugation, trastuzumab (EU) European Union-authorized trastuzumab, trastuzumab (US) United States Food and Drug Administration-licensed trastuzumab, UV CD ultraviolet circular dichroism

similarity analysis demonstrates that the near UV CD spectra are $\geq 95\%$ similar. The DSC results show that the thermal transition temperatures are similar between ABP 980 and trastuzumab. Taken together, these results indicate that ABP 980 has similar higher-order structures to trastuzumab.

3.3 Particles and Aggregates

Particles and aggregates of ABP 980 and trastuzumab were assessed using a combination of test methods capable of detecting the presence and/or quantifying the amounts of particles and aggregates of various sizes and shapes

(spherical or non-spherical) with a focus on proteinaceous particles. Subvisible particles were evaluated quantitatively by LO and characterized by MFI for morphology, specifically the presence of non-spherical particles, which could be proteinaceous, as opposed to spherical particles often corresponding to silicone oil droplets. Particle concentrations for ≥ 2 -, ≥ 5 -, ≥ 10 -, and ≥ 25 - μ m sizes are shown in Table 2. The subvisible particles, as characterized by LO and MFI, show ABP 980 and trastuzumab products have low and similar amounts of subvisible particles in the ≥ 2 -, ≥ 5 -, ≥ 10 -, and ≥ 25 - μ m sizes, and similar amounts of non-spherical particles in the ≥ 5 - μ m size range.

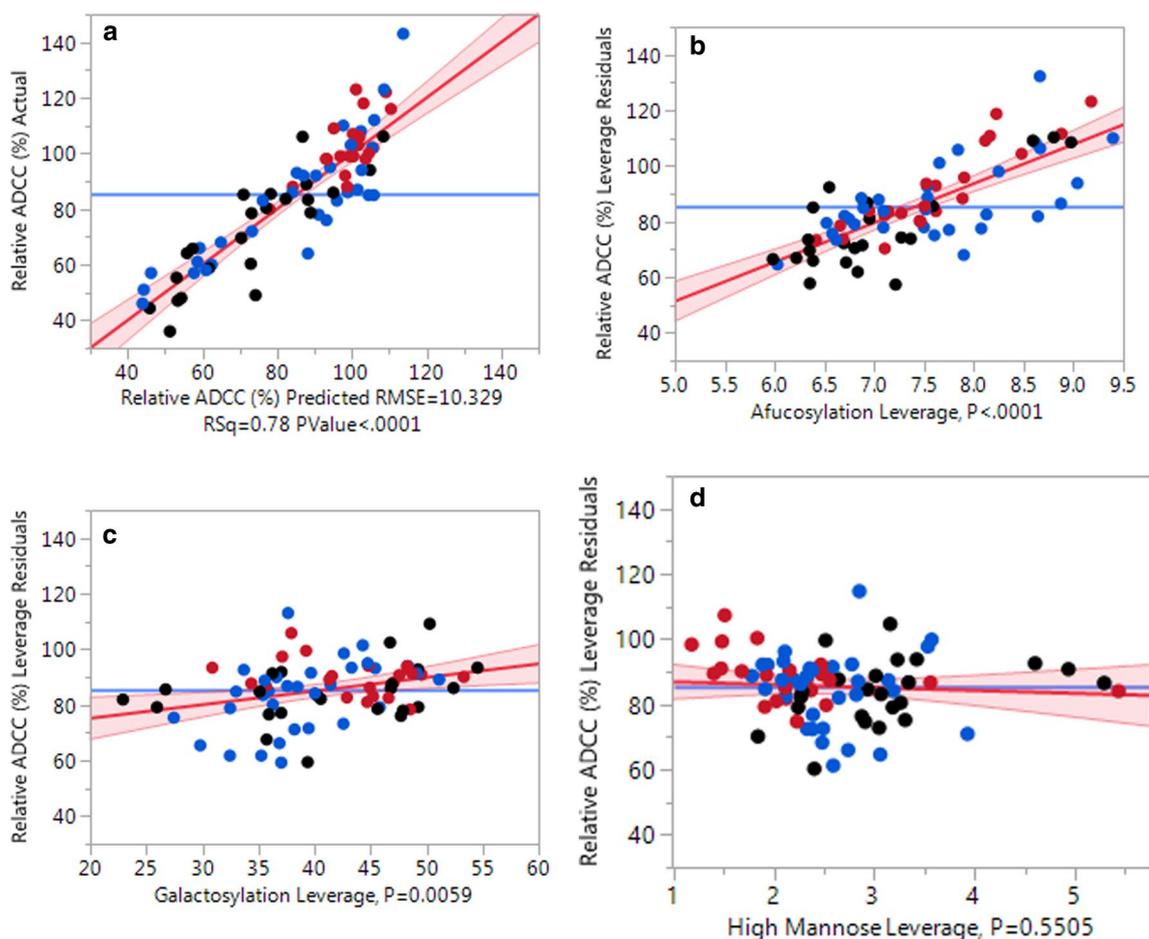


Fig. 2 Correlation of NK92 ADCC with glycan composition: **a** overall structure–function model; **b** relative contribution of afucosylation; **c** relative contribution of galactosylation; and **d** relative con-

tribution of high mannose. Red=ABP, blue=trastuzumab EU, and black=trastuzumab US. ADCC antibody-dependent cellular cytotoxicity

trastuzumab and ABP 980 block downstream signaling and ligand-independent proliferation of HER2-overexpressing tumor cells. Binding to HER2 is mediated by the fragment antigen binding (Fab) region of trastuzumab and ABP 980.

An ELISA was performed to compare the relative binding of ABP 980, trastuzumab (US), and trastuzumab (EU) to HER2. Results are shown in Table 3 and demonstrate that the products have similar HER2 target binding.

3.7 Potency: Inhibition of Proliferation in BT-474 cells

Potency was compared between ABP 980 and trastuzumab by measuring their ability to inhibit proliferation of BT-474, a HER2-overexpressing breast carcinoma cell line. Relative potency ranges are shown in Table 3. These results support the conclusion that ABP 980 has similar potency as compared to trastuzumab (US) and trastuzumab (EU).

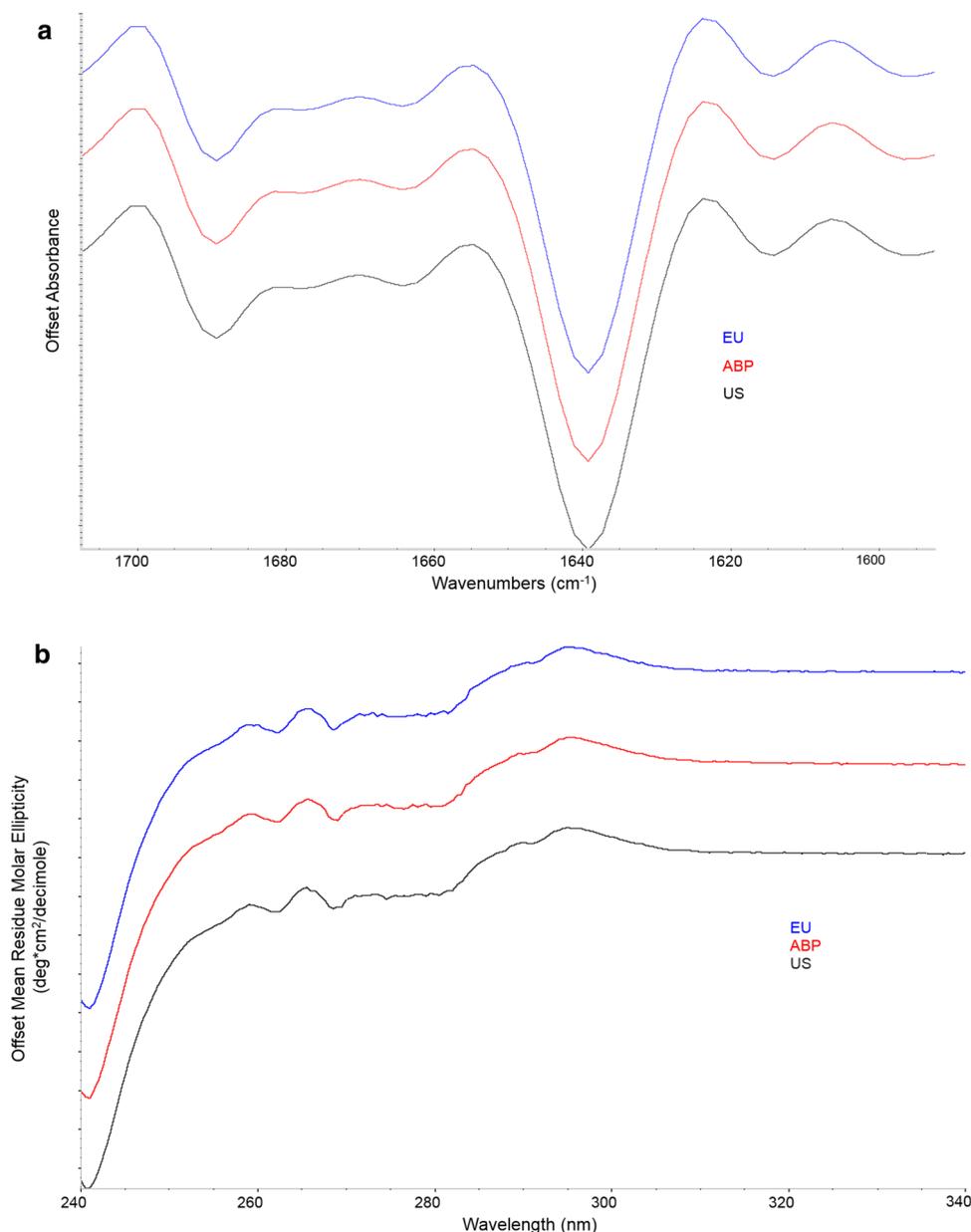
3.8 Relative FcRn, FcγRIIa and FcγRIIIa Binding

Trastuzumab and ABP 980 are IgG1 antibodies that bind to multiple FcγRs and the FcRn through their Fc domains. Binding to FcRn has the potential to influence the PK profile of an antibody given its role in serum recycling of IgG, and is thus of interest to evaluate as part of the similarity assessment.

Relative FcRn binding ranges for ABP 980, trastuzumab (US), and trastuzumab (EU) are shown in Table 3. The relative binding ranges of ABP 980, trastuzumab (US), and trastuzumab (EU) demonstrate similar FcRn binding for the three products, supporting the PK equivalence demonstrated in the previously reported PK clinical study, which compared ABP 980 exposure to that of trastuzumab sourced from the US and EU regions [13].

FcγRIIa has the most widespread expression pattern of the FcγRs, expressed on macrophages, dendritic cells, neutrophils, basophils, eosinophils, and platelets. It is a

Fig. 3 Higher order structure comparisons of trastuzumab (EU) (top, blue line), ABP 980 (middle, red line), and trastuzumab (US) (bottom, black line): **a** Fourier-transformed infrared spectroscopy (FTIR) and **b** near ultraviolet-circular dichroism (UV CD)



low-affinity receptor, interacting primarily with IgG in complexed or aggregated form and plays a role in pathogen clearance through ADCP. Trastuzumab has been reported to exhibit ADCP activity, although the clinical relevance of this effector function has not been determined [6, 16, 17]. Fc γ RIIa has two common allelic variations at position 131, histidine (131H) and arginine (131R). The binding affinity for these variants for IgG1 antibodies has been reported to be within 1.5-fold [18]; thus binding to either variant is considered representative. Relative binding ranges to Fc γ RIIa (131H) are shown in Table 3 and demonstrate similar binding for the three products.

Fc γ RIIIa is a pro-inflammatory Fc γ R expressed on effector cells, including natural killer (NK) cells. Fc γ RIIIa

mediates ADCC activity, and binding to this receptor is influenced by the glycan profile, specifically the levels of afucosylated, galactosylated, and high mannose species [19–22]. Fc γ RIIIa has two allelic variants that have been characterized as the high-affinity variant (158 V) and low-affinity variant (158F) [18]. Relative Fc γ RIIIa (158 V) and Fc γ RIIIa (158F) binding ranges for ABP 980, trastuzumab (US), and trastuzumab (EU) are shown in Table 3.

Similar to the observations reported in Kim et al. [23], Amgen also observed high variability in Fc γ RIIIa binding for a subset of trastuzumab lots that correlated with changes in specific glycans. Overall, ABP 980 exhibits similar binding to both variants of Fc γ RIIIa, albeit within a narrower range, as compared to trastuzumab (US) and trastuzumab

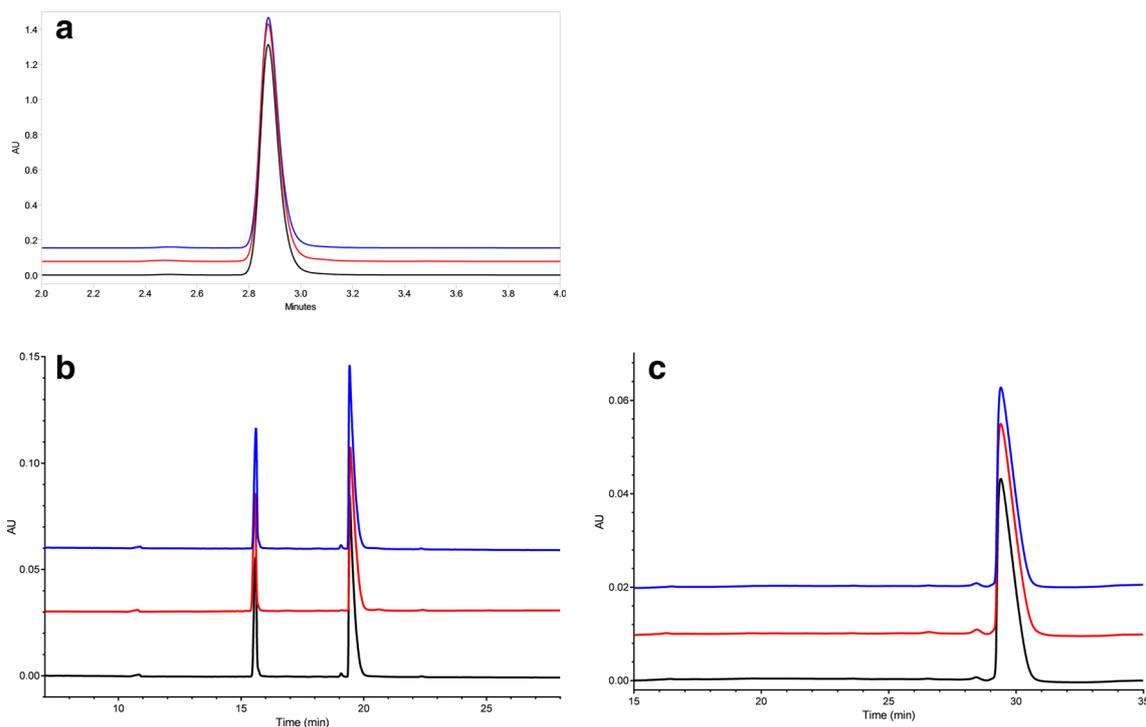


Fig. 4 Purity comparison of trastuzumab (EU) (top, blue line), ABP 980 (middle, red line), and trastuzumab (US) (bottom, black line): **a** size variant profiles assessed by size-exclusion high-performance

liquid chromatography; **b** size variant profiles assessed by reduced capillary electrophoresis; and **c** size variant profiles assessed by non-reduced capillary electrophoresis

Table 3 Summary of key functional activities for ABP 980, trastuzumab (US), and trastuzumab (EU)

Key functional activity	ABP 980 Range (n)	Trastuzumab (US) Range (n)	Trastuzumab (EU) Range (n)
Relative inhibition of proliferation in BT-474 (potency) range (%)	96–122 (22)	88–121 (23)	88–124 (33)
HER2 relative binding range (%)	86–109 (22)	90–117 (23)	85–119 (33)
Relative FcRn binding range (%)	93–116 (22)	78–108 (22)	86–106 (33)
Relative FcγRIIa binding range (%)	90–103 (16)	81–106 (13)	85–108 (13)
Relative FcγRIIIa (158 V) binding range (%)	86–112 (22)	48–120 (23)	48–104 (33)
Relative FcγRIIIa (158F) binding range (%)	97–110 (12)	51–105 (14)	51–115 (11)
Relative PBMC ADCC activity range (%)	82–124 (22)	51–115 (23)	35–119 (33)
Relative NK92 ADCC activity range (%)	79–117 (22)	36–106 (23)	46–143 (33)

ADCC antibody-dependent cellular cytotoxicity, *FcγRIIa* Fc gamma receptor type 2a, *FcγRIIIa* Fc gamma receptor type 3a, *FcRn* Fc neonatal receptor, *HER2* human epidermal growth factor receptor 2, *PBMC* peripheral blood mononuclear cells

(EU). Additionally, the FcγRIIIa binding results are consistent with the range of glycan and ADCC activity observed.

3.9 Antibody-Dependent Cellular Cytotoxicity

ADCC occurs when an antibody binds to target cells through its Fab domain while the Fc domain simultaneously engages Fc receptors (e.g., FcγRIIIa) on the surface of effector cells. This leads to activation of the effector cell, granule

exocytosis, and target cell death. Trastuzumab has previously been reported to induce ADCC of cells that overexpress HER2, and ADCC activity has been implicated as an important activity in the clinical efficacy of trastuzumab [24].

Two ADCC assays were employed to compare the relative ADCC activity of ABP 980, trastuzumab (US), and trastuzumab (EU): an ADCC assay using PBMC as effector cells and SKBR3 cells as target cells and an ADCC

assay using engineered NK92 effector cells and HCC2218 target cells. There are multiple choices for effector cells used in ADCC methods including primary NK cells, engineered NK92 cells, reporter cell lines, and PBMC. All the choices show strengths and weaknesses [25]. Given that the time period for the functional similarity assessment of ABP 980 spanned close to 5 years, the use of engineered NK92 cells provided a consistent source of effector cells for comparison of results over the entire period of the assessment. PBMCs from healthy donors were also used to evaluate ADCC activity to provide additional information from a more physiologically relevant effector population to mimic the clinical population studied. Trastuzumab has been shown to mediate ADCC using PBMC in early breast cancer patients, metastatic breast cancer patients and healthy subjects [6] to a similar degree, confirming the clinical relevance of a PBMC ADCC assay.

Results are shown in Table 3 and demonstrate that ABP 980 exhibits similar ADCC activity in both assays, albeit within a narrower range, as compared to the variability observed in the trastuzumab reference product. The ADCC activity results are consistent with the wide ranges of glycan and FcγRIIIa binding observed in the reference product.

4 Conclusion

ABP 980 has been developed as a biosimilar to trastuzumab. In this report, sensitive and orthogonal methods were used to assess analytical and functional similarity between ABP 980 and both FDA-licensed trastuzumab and EU-authorized trastuzumab. These studies form part of the totality of evidence and follow the stepwise approach to evaluating biosimilars recommended by the FDA and EMA.

The results of these studies demonstrate that ABP 980 is analytically highly similar to trastuzumab with respect to identity, general properties, primary and higher-order structure, biological activity and carbohydrate structure. Where minor differences are noted, they have been evaluated and found unlikely to impact clinical performance. The totality of evidence, including the recently reported PK and clinical efficacy similarity of ABP 980 [13, 26], further supports that ABP 980 is highly similar to FDA-licensed trastuzumab and EU-authorized trastuzumab.

Acknowledgements Thanks to Gwen Maher, Nahira Miscalichi, Shannon Moser, Rupa Padaki, Drew Shami, Junyan Shu and Andrea Treptow for biological characterization support, Cynthia Li, Jie Wen, George Svitel and Nancy Jiao for higher order structure and aggregates and particles support, and Marsha Furtado and Andrew Dykstra for glycan mapping and peptide mapping methods support.

Compliance with Ethical Standards

Funding This study was funded by Amgen Inc.

Conflict of interest Katariina M. Hutterer, Alla Polozova, Scott Kuhns, Helen J. McBride, Xingxiang Cao, and Jennifer Liu are employees and stockholders of Amgen Inc.

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A randomized, single-blind, single-dose study evaluating the pharmacokinetic equivalence of proposed biosimilar ABP 980 and trastuzumab in healthy male subjects

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Received: 20 February 2017 / Accepted: 9 March 2017 / Published online: 24 March 2017
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Abstract

Purpose This study compared the pharmacokinetic (PK) profiles of the proposed biosimilar ABP 980 and trastuzumab in healthy males.

Methods In this single-blind study, 157 healthy males were randomized 1:1:1 to a single 6 mg/kg intravenous infusion of ABP 980, FDA-licensed trastuzumab [trastuzumab (US)], or EU-authorized trastuzumab [trastuzumab (EU)]. Primary endpoints were area under the serum concentration–time curve from time 0 to infinity (AUC_{inf}) and maximum observed serum concentration (C_{max}). To establish equivalence, the geometric mean ratio (GMR) and 90% confidence interval (CI) for C_{max} and AUC_{inf} had to be within the equivalence criteria of 0.80–1.25.

Results The GMRs and 90% CIs for C_{max} and AUC_{inf} , respectively, were: 1.04 (0.99–1.08) and 1.06 (1.00–1.12) for ABP 980 versus trastuzumab (US); 0.99 (0.95–1.03) and 1.00 (0.95–1.06) for ABP 980 versus trastuzumab (EU); and 0.96 (0.92–1.00) and 0.95 (0.90–1.01) for trastuzumab (US) versus trastuzumab (EU). All comparisons were within the equivalence criteria of 0.80–1.25. Treatment-emergent adverse events (TEAEs) were reported in 84.0, 75.0, and 78.2 of subjects in the ABP 980, trastuzumab (US), and trastuzumab (EU) groups, respectively. There were no deaths or TEAEs leading to study discontinuation and no binding or neutralizing anti-drug anti-bodies were detected.

Conclusions This study demonstrated the PK similarity of ABP 980 to both trastuzumab (US) and trastuzumab (EU),

and of trastuzumab (US) to trastuzumab (EU). No differences in safety and tolerability between treatments were noted; no subject tested positive for binding anti-bodies.

Keywords Biosimilar · Pharmacokinetics · Trastuzumab · HER2 · ABP 980 · Immunogenicity

Introduction

ABP 980 is being developed as a biosimilar to trastuzumab (Herceptin[®]). Herceptin[®] is approved for use in the United States (US), the European Union (EU), Japan, and much of the rest of the world for the treatment of metastatic breast cancer, early breast cancer, and metastatic gastric cancer [1, 2], and is the standard of care for subjects with human epidermal growth factor receptor 2 (HER2)-overexpressing breast cancer [3–5]. Trastuzumab and ABP 980 are produced by recombinant DNA technology in Chinese hamster ovary (CHO) cells and both monoclonal anti-bodies (mAbs) bind with high affinity and specificity to the extracellular domain of the HER2 [6]. Binding of trastuzumab to HER2 blocks receptor activation and subsequent proliferation of HER2-overexpressing cells [7]. In addition to blocking HER2-mediated proliferation, binding of trastuzumab to HER2 elicits anti-body-dependent cell-mediated cytotoxicity (ADCC) by binding to antigens expressed on target cells, while the anti-body Fc domain engages Fc receptors (e.g., FcγRIIIa) on the surface of immune effector cells. This binding sequence leads to the activation of the effector cell, granule exocytosis, and target cell death [7].

There is increasing interest in biosimilars as alternatives to their respective reference products, since they can provide additional safe and efficacious treatment options for patients. A biosimilar is a biologic product that is similar

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to a reference product with respect to quality, safety, and efficacy [8, 9]. Biosimilars are likely to have differences in product quality attributes owing to differences in cellular expression systems, processes, and product purification processes but should not have clinically meaningful differences with respect to safety, purity, and potency. Guidelines describing non-clinical and clinical considerations for the development of biosimilars, including mAbs, were issued by the European Medicines Agency (EMA) in 2012 and 2014 [10, 11], and by the United States Department of Health and Human Services and its Food and Drug Administration (FDA) in 2014 and 2015 [12, 13].

Both FDA and EMA guidelines are similar in that each recommends a stepwise approach beginning with comprehensive analytical characterization to establish structural and functional similarity of the biosimilar with the reference or innovator product, followed by a pharmacokinetic (PK) study and a pharmacodynamic (PD) study, if a suitable PD marker is available, to establish equivalence. Finally, a comparative clinical study is conducted in a sensitive population to confirm similar efficacy, safety, and immunogenicity of the proposed biosimilar to the reference product.

ABP 980 is being developed for the same indications, dosages, and route of administration as trastuzumab. Previous studies have shown that ABP 980 has the same amino acid sequence and is analytically and functionally similar to trastuzumab [14]. This Phase I, single-dose, PK study in healthy male subjects was conducted to determine the PK equivalence of ABP 980 to US FDA-licensed trastuzumab and EU-authorized trastuzumab, hereafter referred to as trastuzumab (US) and trastuzumab (EU), respectively. The secondary objectives were to compare the safety, tolerability, and immunogenicity of ABP 980, trastuzumab (US), and trastuzumab (EU). Finally, to establish a scientific bridge between reference products sourced from the US and the EU, the PK equivalence between trastuzumab (US) and trastuzumab (EU) was evaluated.

Materials and methods

This study was conducted in accordance with the provisions of the Declaration of Helsinki and in accordance with the ICH E6 Guidelines on Good Clinical Practice. All subjects signed an Institutional Review Board (IRB)/Independent Ethics Committee (IEC)-approved informed consent form before any study-specific procedures were performed.

Investigational product

ABP 980 was sourced from Amgen, Inc. (Thousand Oaks, CA, USA). Trastuzumab (US) was sourced from

Genentech, Inc. (South San Francisco, CA, USA), and trastuzumab (EU) was sourced from Roche Pharma AG (Grenzach-Wyhlen, Germany). ABP 980 and trastuzumab (US) were supplied in vials containing 440 mg of investigational product, and trastuzumab (EU) was supplied in vials containing 150 mg of investigational product. All investigational products were reconstituted to 21 mg/mL. The protein content of each of the drug lots obtained by the clinical sites was evaluated by Amgen, Inc. No adjustment to dosage was necessary as the differences in protein content between formulations were determined to be less than 5%.

Study design and subject population

This randomized, single-blind, single-dose, three-arm, parallel-group study in healthy adult males was conducted at a single center (Fig. 1). A total of 150 healthy male subjects were planned to be enrolled in the study.

Subjects were randomized in a ratio of 1:1:1, stratified by ethnicity (Japanese versus non-Japanese), to receive a single intravenous (IV) infusion of ABP 980 6 mg/kg, trastuzumab (US) 6 mg/kg, or trastuzumab (EU) 6 mg/kg over 90 min. Subjects, but not healthcare professionals administering the investigational product, were blinded to which treatment they were receiving.

Eligible participants included healthy adult men ≥ 18 – ≤ 45 years of age. Inclusion criteria comprised normal or clinically acceptable physical examination, clinical laboratory test values, vital signs, echocardiogram, and electrocardiogram (ECGs) at screening, body mass index (BMI) ≥ 18.0 and ≤ 30.0 kg/m² for non-Japanese subjects, and BMI ≥ 18.0 and ≤ 25.0 kg/m² for Japanese subjects. Physical examination, ECGs, and vital signs were repeated on Day-1.

Exclusion criteria included men of reproductive potential unwilling to practice a highly effective method of birth control, or refrain from donating sperm, for the duration of the study and for 5 months after treatment and men with pregnant partners. Additional exclusion criteria included a history or evidence of a clinically significant disorder, condition, or disease that could pose a risk to subject safety or interfere with the study; history or presence of conditions known to interfere with the distribution, metabolism, or excretion of drugs; history of surgery or major trauma within 12 weeks of screening, or surgery planned during the study; prior exposure to trastuzumab or related compounds; known or suspected sensitivity to products derived from mammalian cell lines; and subjects who were receiving or had received any investigational drug, device, or medication (prescription or over-the-counter) within 30 days or 5 half-lives (whichever was longer) of receiving study medication.

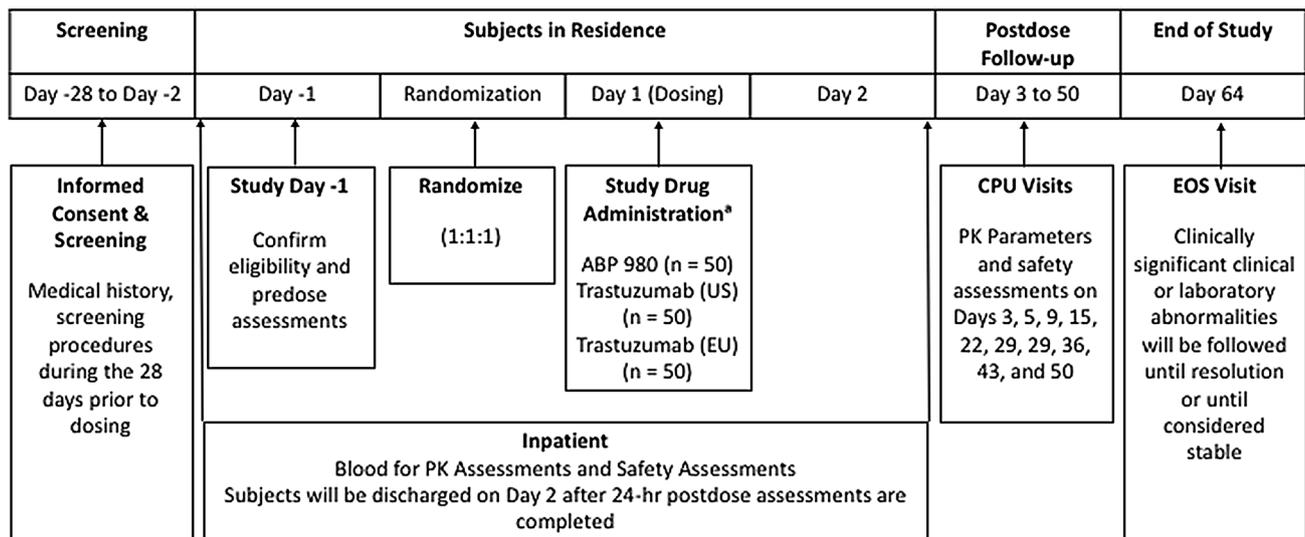


Fig. 1 Study design. CPU clinical pharmacology unit, EOS end of study, EU European Union, FDA Food and Drug Administration, IV intravenous, PK pharmacokinetic, US United States. ^aPlanned IV

dose: ABP 980 6 mg/kg, 440 mg vial; FDA-licensed trastuzumab 6 mg/kg, 440 mg vial; or EU-authorized trastuzumab 6 mg/kg, 150 mg vial

Subjects were screened and informed consent was obtained within 28 days of treatment administration. Day-1 assessment included medical history, physical examination, vital signs, and body weight. Body weight on Day-1 was used to calculate dose. Day 1 pre-dose procedures and assessments included vital signs and collection of samples for baseline assessment of hematology, chemistry, urinalysis, and ADAs.

Subjects remained in the study center or clinical pharmacology unit (CPU) for at least 24 h after dosing for safety and PK assessments. They were discharged on Day 2 after the 24-h assessments were completed. Subjects returned to the CPU on Days 3, 5, 9, 15, 22, 29, 36, 43, and 50, and Day 64 [end-of-study (EOS) visit] for safety evaluations and PK assessments. Vital signs and laboratory measurement were taken on Days 1 and 64 (EOS).

Sampling

Blood samples for serum ABP 980 or trastuzumab concentration determination were collected pre-dose, at 0.75 (mid-infusion), 1.5 (end of infusion), 2, 3, 4, 5, 6, 8, and 24 h after the start of dosing, and at each return visit to the CPU. Blood sampling for PK analysis during each return visit to the CPU, including the EOS visit, occurred per the scheduled timepoint.

A validated electrochemiluminescence (ECL) assay was used to quantify serum concentrations of ABP 980 and trastuzumab using a mouse anti-trastuzumab monoclonal anti-body (mAb) to capture the investigational product. After capturing ABP 980 or trastuzumab to the

immobilized anti-body, unbound materials were removed, followed by the addition of ruthenium labeled mouse anti-trastuzumab mAb to detect the captured ABP 980 or trastuzumab. A tripropylamine buffer was added to enhance the electrochemiluminescent signals. The ECL counts were directly proportional to the amount of ABP 980 or trastuzumab bound by the capture reagent. Conversion of ECL counts to concentrations was performed using the Gen5TM Secure Software v1.08.

Safety and tolerability were reviewed by the medical monitor on an ongoing basis. Adverse events (AEs) were monitored throughout the study. All AEs and serious AEs (SAEs) were reported. Adverse events were coded using MedDRA Version 17.0.

Anti-drug anti-bodies assay

Binding and neutralizing ADAs were detected with a two-tiered approach that included a screening assay and a confirmatory assay. Sampling for ADAs occurred on Day 1 pre-dose and at the EOS visit. A validated immunoassay was used to detect anti-bodies capable of binding ABP 980, trastuzumab (US), or trastuzumab (EU). All samples positive for binding ADAs were assessed for neutralizing anti-bodies capable of binding to ABP 980, trastuzumab (US), or trastuzumab (EU) using a target binding assay.

Study endpoints

The primary endpoints were AUC_{inf} and C_{max} for ABP 980, trastuzumab (US), and trastuzumab (EU). Secondary

endpoints included terminal elimination half-life ($t_{1/2}$); the time at which C_{\max} was observed (t_{\max}); AUC from time 0 to the last quantifiable concentration (AUC_{last}); and last measurable serum concentration (C_{last}) for ABP 980 and trastuzumab. Although AUC_{last} was not defined as a primary endpoint, to fully assess exposure to the investigational product, AUC_{last} was also statistically evaluated. Secondary safety and immunogenicity endpoints included the incidence of treatment-emergent AEs (TEAEs), SAEs, and ADAs.

Pharmacokinetic equivalence was established if the 90% confidence intervals (CIs) for the ratio of least square geometric means (GMs) of primary PK parameters comparing ABP 980 versus trastuzumab (US), ABP 980 versus trastuzumab (EU), and trastuzumab (US) versus trastuzumab (EU) fell within the standard equivalence criteria of 0.80 and 1.25.

Statistical analysis

Serum trastuzumab and ABP 980 concentrations were listed and summarized descriptively by treatment and time-point using the PK concentration population including all subjects who were randomized and received any amount of investigational product and had at least one reported serum concentration of ABP 980 or trastuzumab. Mean trastuzumab and ABP 980 serum concentration–time data by treatment were presented graphically on semi-logarithmic and linear scales.

PK parameters were calculated using non-compartmental techniques (WinNonlin® Professional Network Edition, Version 6.3, Pharsight Corp, St. Louis, MO) for all subjects. Prior to statistical modeling, PK parameters were \log_e -transformed. Point estimates and 90% CIs for the mean difference in logarithmic PK parameters were estimated using an analysis of variance (ANOVA) model adjusted for treatment and ethnicity using the PK parameter population

including all subjects with an evaluable ABP 980 or trastuzumab serum concentration–time profile. The point estimates and 90% CIs for GM ratios (GMRs) were then calculated by transforming back to the original scale and PK similarity was established if the 90% CIs fell within the standard equivalence criteria of 0.80 and 1.25.

Safety analysis included descriptive summaries of AEs and the incidence of ADAs, using the safety population defined as all subjects who received any amount of investigational product.

Results

Subject disposition and characteristics

A total of 157 subjects were randomized to treatment with ABP 980 ($n=50$), trastuzumab (US) ($n=52$), and trastuzumab (EU) ($n=55$); 148 (94.3%) subjects completed the study; 9 (5.7%) subjects [3 subjects in the trastuzumab (US) group and 6 subjects in the trastuzumab (EU) group] discontinued from the study prematurely. One subject in the trastuzumab (EU) group prematurely discontinued infusion owing to an infusion reaction. This subject received a total volume infused of approximately 4.8 mg/kg and was excluded from the PK parameter and per protocol PK analysis. No subject had a medical or surgical history that prohibited them from participation in the study. Subject demographics are summarized in Table 1. Age, height, weight, and BMI were similar across treatment groups. The majority of subjects (62.4%) in the study were white.

Pharmacokinetic profiles

The mean serum concentration–time profiles were similar between treatments following a single 6 mg/kg IV infusion of ABP 980, trastuzumab (US), or trastuzumab (EU) over

Table 1 Demographic data and baseline characteristics

	ABP 980 ($n=50$)	Trastuzumab (US) ($n=52$)	Trastuzumab (EU) ($n=55$)
Mean age, years (SD)	25.8 (5.8)	25.4 (5.6)	25.6 (4.6)
Mean weight, kg (SD)	76.4 (11.1)	73.6 (10.8)	74.7 (11.8)
Mean height, cm (SD)	176.5 (6.25)	177.2 (7.37)	177.6 (8.01)
Mean BMI, kg/m ² (SD)	24.4 (2.8)	23.3 (2.5)	23.6 (3.4)
Race, n (%)			
Asian (1st generation Japanese)	10 (20.0)	10 (19.2)	11 (20.0)
Asian (other)	8 (16.0)	6 (11.5)	5 (9.1)
Black or African-American	3 (6.0)	0	0
White	28 (56.0)	34 (65.4)	36 (65.5)
Other	1 (2.0)	2 (3.8)	3 (5.5)

BMI body mass index, SD standard deviation

the entire course of the study (Fig. 2). Peak concentrations were observed approximately 1.5–5 h after the start of the infusion with similar C_{max} and t_{max} in all treatment groups.

Descriptive summary of PK parameters for ABP 980, trastuzumab (US), and trastuzumab (EU) is presented in Table 2. A total of 12 subjects [4 subjects in the trastuzumab (US) group and 8 subjects in the trastuzumab (EU)

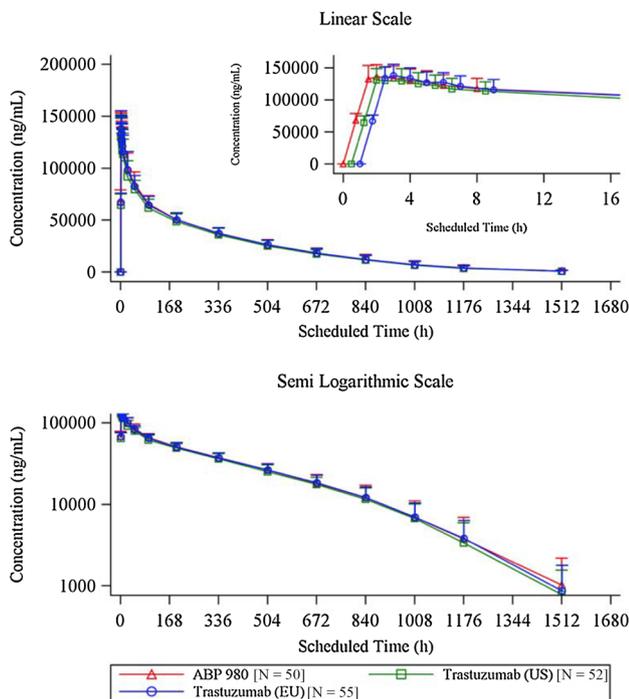


Fig. 2 Mean serum concentration–time profiles (PK concentration population) for ABP 980, trastuzumab (US), and trastuzumab (EU); linear scale (top) and expanded (0–16 h) scale (top inset); semi-logarithmic scale (bottom). Error bars SD

Table 2 Summary of pharmacokinetic parameters

Parameter	ABP 980	Trastuzumab (US)	Trastuzumab (EU)
C_{max} ($\mu\text{g/mL}$), GM (n) (GeoCV%)	139.9 (50) (13)	134.6 (52) (13)	140.5 (54) (12)
C_{last} ($\mu\text{g/mL}$), GM (n) (GeoCV%)	0.717 (50) (97)	0.574 (48) (91)	0.630 (46) (97)
AUC_{last} ($\mu\text{g h/mL}$), GM (n) (GeoCV%)	34,945 (50) (17)	33,160 (48) (17)	34,896 (46) (17)
AUC_{inf} ($\mu\text{g h/mL}$), GM (n) (GeoCV%)	35,224 (50) (18)	33,342 (48) (17)	35,123 (46) (18)
t_{max} (h), median (range) (n)	2.0 (1.52–5.00) (50)	2.0 (1.53–5.00) (52)	2.0 (1.55–24.00) (54)
$t_{1/2}$ (h), mean (SD) (n)	169.4 (40.82) (50)	154.0 (27.97) (48)	154.8 (39.78) (46)

GeoCV% geometric mean coefficient of variation, AUC area under the serum concentration–time curve, AUC_{inf} AUC from time 0 extrapolated to infinity, AUC_{last} AUC from time 0 to the last quantifiable concentration, CI confidence interval, C_{max} maximum serum concentration, LS least squares, t_{max} time at which the maximum serum concentration was observed, $t_{1/2}$ terminal elimination half-life, Max maximum, Min minimum, SD standard deviation, GM geometric mean, n number of non-missing observations

group] either terminated the study early or had multiple missing samples. These subjects were included in the PK Parameter Population, as C_{max} and t_{max} were considered to have been sufficiently characterized before the early termination or missing samples; however, AUC_{inf} was considered non-evaluable in these subjects and was, therefore, excluded from analysis. The GMs of PK parameters were similar across treatment groups following a single IV infusion of ABP 980, trastuzumab (US), and trastuzumab (EU) (Table 2). The GMs of C_{max} and AUC_{inf} were 139.9 $\mu\text{g/mL}$ and 35,224 $\mu\text{g h/mL}$ for ABP 980; 134.6 $\mu\text{g/mL}$ and 33,342 $\mu\text{g h/mL}$ for trastuzumab (US); and 140.5 $\mu\text{g/mL}$ and 35,123 $\mu\text{g h/mL}$ for trastuzumab (EU). The terminal $t_{1/2}$ was estimated to be 6–7 days. For all subjects in each treatment arm, AUC_{last} accounted for $\geq 90\%$ of the total AUC, confirming the adequacy of the duration of PK sampling across the three treatments.

The results of the statistical assessment of PK similarity for the overall population are shown in Table 3. The GMRs and 90% CIs for C_{max} and AUC_{inf} , respectively, were: 1.04 (0.99–1.08) and 1.06 (1.00–1.12) for ABP 980 versus trastuzumab (US); 0.99 (0.95–1.03) and 1.00 (0.95–1.06) for ABP 980 versus trastuzumab (EU); and 0.96 (0.92–1.00) and 0.95 (0.90–1.01) for trastuzumab (US) versus trastuzumab (EU). All comparisons were within the equivalence criteria of 0.80–1.25.

Safety results

Overall, the incidence of TEAEs was similar across treatment groups. Treatment-emergent AEs were reported in 42 (84.0%) subjects in the ABP 980 group, 39 (75.0%) subjects in the trastuzumab (US) group, and 43 (78.2%) subjects in the trastuzumab (EU) group. The majority of TEAEs were

Table 3 Statistical assessment of pharmacokinetic parameters

	PK parameters		
	C_{\max} ($\mu\text{g/mL}$) Adjusted LS GM (<i>n</i>)	AUC_{inf} ($\mu\text{g h/mL}$) Adjusted LS GM (<i>n</i>)	AUC_{last} ($\mu\text{g h/mL}$) Adjusted LS GM (<i>n</i>)
ABP 980	135.9 (50)	34061.4 (50)	33811.7 (50)
Trastuzumab (US)	131.2 (52)	32271.7 (48)	32113.6 (48)
Trastuzumab (EU)	136.8 (54)	33947.0 (46)	33748.2 (46)
Statistical analysis: ratio and 90% CI of adjusted least square geometric means			
ABP 980 versus trastuzumab (US)	1.04 (0.9948–1.0787)	1.06 (0.9974–1.1169)	1.05 (0.9967–1.1122)
ABP 980 versus trastuzumab (EU)	0.99 (0.9540–1.0338)	1.00 (0.9476–1.0624)	1.00 (0.9479–1.0589)
Trastuzumab (US) vs trastuzumab (EU)	0.96 (0.9213–0.9975)	0.95 (0.8973–1.0072)	0.95 (0.8998–1.0063)

Statistical model includes treatment and ethnicity as fixed effects

GM geometric means, LS least squares, *n* number of non-missing observations

mild to moderate in severity. Treatment-related AEs (i.e., TEAEs assessed as possibly or probably related to study drug) were reported in 33 (66.0%), 33 (63.5%), and 39 (70.9%) subjects in the ABP 980, trastuzumab (US), and trastuzumab (EU) groups, respectively.

There were no deaths or life-threatening TEAEs or TEAEs leading to study discontinuation. Two subjects reported TEAEs rated as severe; one subject in the trastuzumab (EU) group had an SAE of infusion-related reaction that was considered probably related to study drug; and one subject in the trastuzumab (US) group had multiple SAEs resulting from a motor bike accident, including pulmonary embolism secondary to deep vein thrombosis, not related to study medication. None of the SAEs resulted in premature study discontinuation.

The most frequently reported TEAEs (reported in >5% of subjects in any treatment group) were headache, upper respiratory tract infection, chills, pyrexia, myalgia,

nausea, epistaxis, arthralgia, and lethargy (Table 4). No new or unexpected safety signals were identified, and the safety profiles were consistent with what is known for trastuzumab.

Anti-drug anti-bodies

Immunogenicity was assessed at baseline just prior to dosing and at the EOS visit (Day 64). For all subjects, the concentrations of ABP 980 or trastuzumab at the end of the PK sampling period were well below the drug tolerance levels of 20 and 100 $\mu\text{g/mL}$ in the presence of 100 and 500 ng/mL ADA, respectively, indicating that ADA detection was not influenced by circulating drug levels. There were no pre-existing binding ADAs detected in baseline samples and no subjects had developed binding or neutralizing ADAs at the end of the study.

Table 4 Summary of treatment-emergent adverse events (safety population)

MedDRA preferred term	ABP 980 (<i>n</i> =50)		Trastuzumab (US) (<i>n</i> =52)		Trastuzumab (EU) (<i>n</i> =55)	
	<i>n</i> (%)	No. of events	<i>n</i> (%)	No. of events	<i>n</i> (%)	No. of events
Headache	16 (32.0)	21	19 (36.5)	26	24 (43.6)	30
Upper respiratory tract infection	21 (42.0)	25	18 (34.6)	20	20 (36.4)	22
Chills	3 (6.0)	3	6 (11.5)	7	7 (12.7)	8
Pyrexia	3 (6.0)	3	5 (9.6)	5	8 (14.5)	8
Myalgia	8 (16.0)	8	3 (5.8)	3	1 (1.8)	1
Nausea	2 (4.0)	2	5 (9.6)	5	4 (7.3)	4
Epistaxis	3 (6.0)	4	3 (5.8)	3	3 (5.5)	3
Arthralgia	5 (10.0)	5	1 (1.9)	1	1 (1.8)	1
Lethargy	3 (6.0)	3	2 (3.8)	2	0	0

Adverse events were coded using MedDRA Version 17.0

A TEAE is defined as an AE that was not present prior to treatment with investigational product, but appeared following treatment or was present at treatment initiation but worsened during treatment. Subjects with multiple events in the same category were counted only once in that category; subjects with events in more than 1 category were counted once in each of those categories

Discussion

This study was conducted to determine the PK similarity of the proposed biosimilar ABP 980 to trastuzumab. Study enrollment was restricted to healthy male subjects, because healthy subjects should provide the most homogeneous population for sensitive comparisons of the PK of ABP 980 and trastuzumab. The dose and sampling schedule chosen in this study were based on a previous study of trastuzumab [15]. A dose of 6 mg/kg provided sufficient exposure to study medication for an accurate evaluation of PK in healthy subjects within the dose range with linear kinetics and is, therefore, appropriate to detect potential PK differences between ABP 980, trastuzumab (US), and trastuzumab (EU). A dose of 6 mg/kg is also consistent with the prescribing instructions for Herceptin® [1, 2]. Based on $t_{1/2}$ of 6–6.8 days in healthy males in a prior pilot study for ABP 980 and trastuzumab (EU), 64 days of PK sampling post-infusion was considered sufficient to fully characterize the ABP 980 or trastuzumab PK profiles in healthy subjects.

This study was conducted to meet FDA and EMA guidelines for the development and approval of biosimilar agents [10–13]. Both agencies recommend a stepwise developmental approach designed to determine the similarity of the proposed biosimilar to the reference product with respect to physicochemical and functional characteristics, PK profile, and clinical efficacy, safety, and tolerability. The analytical and functional similarity of ABP 980 to trastuzumab sourced from the USA and EU has been extensively studied, and the results have been reported previously [14]. ABP 980 was shown to be similar to trastuzumab (US) and trastuzumab (EU) with respect to primary and higher order structure, HER2 binding affinity, inhibition of proliferation, and in vitro anti-body-dependent cell-mediated cytotoxicity. The results of this study further support that ABP 980 is similar to trastuzumab by demonstrating equivalence of ABP 980 to both trastuzumab (US) and trastuzumab (EU) with respect to PK profile.

A single 6 mg/kg IV infusion of ABP 980 in healthy male subjects resulted in a similar PK profile to both trastuzumab (US) and trastuzumab (EU) with respect to the primary PK parameters of AUC_{inf} and C_{max} . For both PK parameters, the 90% CIs were contained within the pre-specified standard equivalence margin of 0.8–1.25. The safety and tolerability of ABP 980, trastuzumab (US), and trastuzumab (EU) also were comparable and consistent with what is known for trastuzumab. No new or unexpected safety signals were noted.

The study design used here also allowed a direct comparison between trastuzumab (US) and trastuzumab (EU). Direct comparison between reference products sourced

from different regions also has important regulatory and clinical implications. Strong evidence for similarity between ABP 980 and trastuzumab, based on comprehensive analytical and functional assessment, is necessary to support clinical studies and ultimately extrapolation to all approved trastuzumab indications [16, 17]. The FDA and EMA have each developed guidelines that allow the use of foreign-sourced reference products in comparative clinical trials provided that there is sufficient scientific evidence demonstrating similarity between the foreign-sourced and locally-sourced reference products. In this three-arm study, the 90% CIs for AUC_{inf} and C_{max} were within the equivalence margin of 0.8 to 1.25 for trastuzumab (US) versus trastuzumab (EU), hence established PK bioequivalence between USA-sourced trastuzumab and EU-sourced trastuzumab, which is a required component to establish a scientific bridge between USA-sourced and EU-sourced trastuzumab, thus allowing use of one reference product in comparative Phase III clinical trials. Safety and tolerability also were comparable between trastuzumab (US) versus trastuzumab (EU).

As with all biologic agents, the risk for developing binding or neutralizing ADAs must be carefully assessed. In this study, immunogenicity was evaluated by assaying blood samples for the presence of binding or neutralizing ABAs on Day 1 before infusion and at the end-of-study visit. No subject in any treatment arm developed ADAs. This finding is consistent with previous studies demonstrating a low incidence of ADAs with trastuzumab [15, 18].

In conclusion, in this Phase I study, there were no differences between ABP 980, trastuzumab (US), and trastuzumab (EU) with respect to PK profile, safety, and tolerability after a single IV infusion. No subject tested positive for binding ADAs. In addition to the results of structural and functional characterization, these results provide further support that the proposed biosimilar ABP 980 is highly similar to FDA-licensed and EU-authorized trastuzumab reference products.

Acknowledgements Medical writing support was provided by Pasquale Iannuzzelli, Ph.D. (Innovation Communications Group) under the direction of Monica Ramchandani, Ph.D. (Amgen, Inc.).

Compliance with ethical standards

Conflict of interest Vladimir Hanes, Vincent Chow, Nan Zhang, and Richard Markus are employees and stockholders of Amgen Inc.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Funding This study was funded by Amgen Inc., Thousand Oaks, California.

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