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ORIGINAL RESEARCH ARTICLE

Comparative Nonclinical Assessments of the Proposed Biosimilar PF-05280014 and Trastuzumab (Herceptin®)

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Abstract

Background and Objectives Trastuzumab (Herceptin®) is a humanized monoclonal antibody (mAb) that binds to the HER2 protein. PF-05280014 is being developed as a potential biosimilar to trastuzumab products marketed in the United States (trastuzumab-US) and European Union (trastuzumab-EU). Nonclinical studies were designed to evaluate the similarity of PF-05280014 to trastuzumab-US and trastuzumab-EU using in vitro structural and functional analyses, and in vivo pharmacokinetic and immunogenicity assessments.

Methods Peptide mapping was utilized to determine structural similarity. Functional similarity was assessed via an in vitro tumor cell growth inhibition assay. CD-1 male mice were administered a single-dose (0, 1, 10, or 100 mg/kg) of PF-05280014, trastuzumab-US, or trastuzumab-EU. Mice were monitored for clinical signs and body weight changes over a 4-month period. At approximately 720, 1,080, 1,440, 2,160, and 2,880 h post-dose, terminal blood samples were collected and assayed for PF-05280014, trastuzumab-US, or trastuzumab-EU concentrations and anti-drug antibodies (ADA). Values for C_{max}, area under the concentration time curve (AUC), clearance (CL), volume of distribution (V_{ss}), half-life ($t_{½}$), and the presence of ADA were determined.

Results In this report, peptide mapping of PF-05280014, trastuzumab-US, and trastuzumab-EU showed similar chromatographic profiles in a side-by-side analysis. The tumor cell growth inhibition of PF-05280014 was similar to trastuzumab-US and trastuzumab-EU. C_{max} and $AUC_{0-\infty}$ values in mice were similar and dose-dependent across the mAbs at all doses, and CL and V_{ss} values were similar and dose-independent. The CL values across doses ranged from 0.193 to 0.350 mL/h/kg (PF-05280014), from 0.200 to 0.346 mL/h/kg (trastuzumab-US), and from 0.193 to 0.335 mL/h/kg (trastuzumab-EU). V_{ss} values across doses ranged from 84.9 to 120 mL/kg (PF-05280014), 86.7 to 130 mL/kg (trastuzumab-US), and 85.4 to 116 mL/kg (trastuzumab-EU). The incidence of ADA was low (~10%) and also similar across all dose levels and the three mAbs. The lower exposure generally observed in ADA-positive animals did not impact the overall PK interpretation. All animals survived to their scheduled terminal blood collection with no mAb-related differences in body weight gain or clinical signs.

Conclusions PF-05280014, trastuzumab-US, and trastuzumab-EU were well tolerated during the 4-month observation period following a single dose of up to

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100 mg/kg. PF-05280014, trastuzumab-US, and trastuzumab-EU showed similar structural properties, tumor cell growth inhibition properties, and PK profiles. The incidence of ADA was low and similar across the three mAbs. The results of these studies support the development of PF-05280014 as a proposed biosimilar to Herceptin.

Key Points

PF-05280014, trastuzumab-US, and trastuzumab-EU were all well tolerated following a single dose of up to 100 mg/kg.

PF-05280014, trastuzumab-US, and trastuzumab-EU showed similar structural properties, tumor cell growth inhibition properties, and PK profiles.

The results of the evaluated studies support the development of PF-05280014 as a proposed biosimilar to the marketed trastuzumab products (Herceptin).

1 Introduction

Trastuzumab (Herceptin®) is a humanized recombinant IgG1 monoclonal antibody (mAb) that selectively inhibits signaling of the human epidermal growth factor receptor 2 (HER2) [1]. Herceptin® is licensed in the United States (US) [2] and approved in the European Union (EU) [3] for the treatment of HER-2-overexpressing breast and gastric cancers. The marketed products obtained from these two regions are referred to herein as trastuzumab-US and trastuzumab-EU, respectively. There is evidence that treatment with Herceptin blocks intracellular signaling, causes cytostatic G1 arrest, and induces growth inhibition in target cells [1, 4]. Like many other licensed or approved biotherapeutics products, trastuzumab is nearing the expiration of at least one of its patents, allowing development of biosimilar drug therapies.

Regulatory agencies in the EU and the US have developed final or draft specific guidance documents describing their criteria for a biologic to be considered a biosimilar [5–9]. Biosimilars are biologic drugs designed to be highly similar to the licensed or approved biologic product ("the reference product") in regards to key analytic (e.g., structural identity, potency, and in vitro functions), nonclinical (e.g., pharmacokinetic, immunogenicity, safety and tolerability profiles), and clinical (e.g., pharmacokinetics, pharmacodynamics, immunogenicity, efficacy, safety and tolerability) profiles [4–8]. Unlike small molecule generic drugs that are identical to the licensed or approved reference drug, biosimilars are highly similar, but not identical, to the reference drug. Manufacturing processes for biologics typically are proprietary, and without access to this information other pharmaceutical companies have to produce biosimilars using existing technology to reverse engineer the biosimilar to be highly similar to the reference product profile [5–9]. These regulatory and scientific guidances have indicated that the in-depth physicochemical and functional characterization of a proposed biosimilar should include appropriately robust and orthogonal analytical methods that enable a comprehensive molecular, functional, and structural biosimilarity assessment [10–12].

PF-05280014 is a humanized recombinant IgG1 mAb directed against the extracellular domain of the HER2 receptor being developed as a potential biosimilar of the reference products marketed as Herceptin® in the US and EU (trastuzumab-US and trastuzumab-EU, respectively). In nonclinical studies, these reference products have been shown to induce apoptosis in human tumor cell lines overexpressing HER2 and shrink tumor xenograft implants in athymic mice [13–15]. Furthermore, since trastuzumab does not recognize the mouse counterpart of human HER2 [16], non-target-mediated and FcRn-dependent clearance of PF-05280014, trastuzumab-US, and trastuzumab-EU can be compared in mice [17]. During the development of PF-05280014, the proposed biosimilar was subjected to extensive side-by-side physicochemical and functional characterization using state of the art robust and orthogonal methodologies with reference product Herceptin sourced from the US and the EU (Ng et al. manuscript in preparation). The focus of the current manuscript is to report the nonclinical comparisons including in vivo pharmacokinetics (PK), anti-drug antibody (ADA) responses, and tolerability of PF-05280014, trastuzumab-US, and trastuzumab-EU. In addition, we report the comparative in vitro peptide maps and tumor cell growth inhibition properties.

2 Methods

2.1 Physicochemical and Functional Similarity

We determined the level of physicochemical similarity between PF-05280014, trastuzumab-US, and trastuzumab-EU by generating individual tryptic peptide maps for the three mAbs. The trypsin-digested peptides for each mAb were separated on a reversed-phase high performance liquid chromatography using a 2.1 × 150 mm Waters BioSuite C18 column (Waters Corp., Milford, MA) monitored at 214 nm. Each peptide was identified by liquid chromatography mass spectrometry using ultrahigh resolution quadrupole time-of-flight mass spectrophotometer.

To determine the level of in vitro functional similarity, PF-05280014, trastuzumab-US, and trastuzumab-EU were

analyzed in an in vitro tumor cell proliferation inhibition assay using the HER2-overexpression metastatic breast carcinoma cell line, SKBR-3. Cellular viability was detected using CellTiter Glo (Promega, Inc., Madison, WI), a commercially available bioluminescent detection kit designed to measure mammalian cell viability through the presence of ATP present in live cells [18]. Briefly, SKBR-3 cells derived from a metastatic breast adenocarcinoma that over-expresses the HER2 receptor were seeded into a 96-well tissue culture treated plates and cultured for 24 h. The media from the cell plate was removed and serially diluted PF-05280014, trastuzumab-US, or trastuzumab-EU were transferred into the appropriate wells. Inhibition of cell growth was inversely proportional to the amount of ATP present in each well, as determined by measuring luminescence using the CellTiter Glo detection kit. The resulting dose-response curves for the three mAbs allowed determination of their relative potency with respect to each other.

2.2 Animals

Male CD-1 mice (Charles River Laboratories, Portage, MI) were used in the in vivo PK/tolerability study; the strain was selected to match data available for the reference products. There were ten groups (one control group, and three groups for each mAb at doses of 1, 10, or 100 mg/kg), each containing 55 mice. At the initiation of dosing, animals were 6–7 weeks old and weighed between 25.5 and 43.4 g. This in vivo study was designed to provide data to support the subsequent clinical development of PF-05280014 as a potential biosimilar.

2.3 Dose Preparation, Dose Analysis, and Dose Administration

To achieve the dose levels of 1, 10, and 100 mg/kg with a dose volume of 5 mL/kg, PF-05280014 stock solution (22 mg/mL) was diluted in histidine/sucrose buffer. Both trastuzumab-US and trastuzumab-EU, as lyophilized commercial products, were first reconstituted in sterile water for injection according to the product label to achieve the label concentration of 21 mg/mL, and then further diluted in histidine/trehalose buffer. The control group was administered PF-05280014 diluent and each of the other groups received one of the three mAbs at 1, 10, or 100 mg/kg by bolus intravenous (IV) injection into the tail vein.

The mAb dose concentrations were verified by ultraviolet spectrophotometry by measuring absorbance at 280 nm [19]. Stability of the formulations under these conditions was confirmed. All dose preparations were within ±10 % of the target concentrations (mean values ranging from 102.3 to 105.0 % of target), with the exception of the 2.0 mg/mL PF-05280014 formulation (mean 114.3 % of target). Because this deviation was small (14 %, compared to the ±10 % acceptable range) and dose-normalized PK data demonstrated that the interpretation of the exposure data was not impacted by this change, the nominal dose (10 mg/kg) was used for this group. The doses (1, 10, and 100 mg/kg) were selected to match PK data available for the reference products.

2.4 Pharmacokinetics

Because blood from each mouse was collected via terminal cardiac puncture, only one sample per mouse was obtained. Thus, composite profiles for each mAb were generated for PK evaluation. Samples were collected at approximately 0.5, 6, 24, 96, 168, 336, 720, 1,080, 1,440, 2,160, and 2,880 h post-dose in each group. Five animals/group/time point were anesthetized with carbon dioxide inhalation and at least 0.7 mL of blood was collected via cardiac puncture from each animal; necropsy was not performed. Blood samples were allowed to clot at room temperature, and the serum was harvested following centrifugation and stored at −60 to −80 °C until analyzed.

Serum concentrations of trastuzumab were determined via a single validated enzyme-linked immunosorbent assay (ELISA) that was cross-validated for PF-05280014, trastuzumab-US, and trastuzumab-EU. PF-05280014, trastuzumab US, or trastuzumab EU was captured using recombinant HER2 extracellular domain adsorbed on a microtiter plate. Bound trastuzumab was detected using a goat anti-human immunoglobulin (IgG) antibody conjugated with horseradish peroxidase. The substrate, 3,3′,5,5′ tetramethylbenzidine, was used for the colorimetric readout. Sample concentrations were determined by interpolation from a calibration curve that was fit using a five parameter logistic model ($1/y^2$ weighting). The lower limit of quantitation was 20.0 ng/mL. Concentration values below the lower limit of quantitation were considered to equal zero for descriptive statistics and PK analyses.

Noncompartmental PK analysis was performed on mean serum concentration data using WinNonlin Professional Edition (Pharsight Corporation, Version 5.2, Mountain View, CA) with nominal doses and sampling times to generate the composite PK profile for each mAb. Parameters included maximum serum concentration of the administered mAb (C_{max}), area under the concentration-time curve (AUC), elimination half-life ($t_{1/2}$), systemic clearance (CL), and volume of distribution at steady-state (V_{ss}).

The serum AUC to a specified time point ($AUC_{0–2,880}$) and total exposure ($AUC_{0–\infty}$) were estimated using the

linear trapezoidal rule [20]. The percent extrapolation in the $AUC_{0-\infty}$ calculations was determined as follows: $[(AUC_{0-\infty}) - AUC_{0-2,880})/AUC_{0-\infty}] \times 100$. This percent extrapolation calculation was used to determine if the $AUC_{0-2,880}$ values were representative of the overall predicted concentration-time curves.

2.5 Anti-drug Antibody Detection

Samples for ADA testing consisting of approximately 0.12 mL of whole blood were collected from each animal in the PF-05280014, trastuzumab-US, and trastuzumab-EU groups prior to dosing and at one time point/animal after dosing. Pre-dose samples were collected via submandibular puncture and post-dose samples were collected via cardiac puncture at approximately 720, 1,080, 1,440, 2,160, or 2,880 h post-dose. All samples were allowed to clot at room temperature. The serum was harvested following centrifugation and was stored at −60 to −80 °C until analyzed. Pre-dose (baseline) and terminal time point serum samples were analyzed for the presence of ADA using validated electrochemiluminescence assays (Meso Scale Discovery, Rockville, MD) specific for PF-05280014, trastuzumab-US, or trastuzumab-EU. These samples were used to generate composite profiles for each administered mAb.

The positive control (affinity purified rabbit anti-trastuzumab-EU IgG), negative control (pooled normal CD-1 mouse serum), and study serum samples were co-incubated with biotinylated PF-05280014 and ruthenium-labeled PF-05280014, or biotinylated trastuzumab-US and ruthenium-labeled trastuzumab-US, or biotinylated trastuzumab-EU and ruthenium-labeled trastuzumab-EU (to match the mAb administered to each individual animal). Anti-drug antibodies were captured via the biotinylated-PF–05280014, biotinylated-trastuzumab-US, or biotinylated-trastuzumab-EU using streptavidin-coated Meso Scale Discovery Multi-Array® plates. Final detection was achieved using ruthenylated-trastuzumab and tripropylamine to produce an electrochemiluminescent signal that was measured using the Meso Scale Discovery Sector™ Imager 6000. Assay responses were quantified in relative light units (RLU). To ensure that ADA induction was being measured, the pre-dose (baseline) sample served as the control for each animal. Samples that were negative post-dose were considered negative for ADA whether or not the pre-dose sample was also negative. Samples that were negative pre-dose and positive post-dose were considered positive for ADA induction. Samples that were positive both pre- and post-dose were considered positive if the post-dose titer was at least one dilution factor higher (0.3 or log 2, where 2 was the serial dilution factor) compared with the titer value for corresponding pre-dose sample. Trastuzumab was not detected in any of the control samples; thus, samples from the control group were not evaluated for ADA.

2.6 In-Life Observations

Animals were checked twice daily for mortality, signs of pain or distress, and any other abnormalities. Cage-side observations were performed 2 and 4 h after dosing (including an examination of the injection site), and once daily throughout the 4-month (2,880 h) observation period. Body weights were recorded prior to dosing and weekly throughout the study. Overall assessment of tolerability was based on mortality, clinical signs, body weight, and body weight change.

3 Results

3.1 Biochemical and In vitro Functional Properties

The tryptic peptide map for PF-05280014 was similar to those obtained for trastuzumab-US and trastuzumab-EU (Fig. 1). The detected peptides constitute at least 98 % of the amino acid sequence of trastuzumab. The small portion (<2%) of the antibody sequence that was not detected corresponds to small peptides that are not retained on the analytical chromatographic column. The complete amino acid sequence, including those small peptides not observed in the tryptic peptide map, was confirmed in a separate LC/MS analysis at the subunit level (Ng et al. manuscript detailing extensive structural and functional biosimilarity characterization in preparation) for each mAb. These results demonstrate that the primary sequences of PF-05280014, trastuzumab-US, and trastuzumab-EU were identical in peptide sequence to each another.

In the tumor cell growth inhibition assay using SKBR-3 cells, the dose-response curves for PF-05280014, trastuzumab-US, and trastuzumab-EU were superimposable (Fig. 2). These data indicate that PF-05280014 is similar to trastuzumab-US and trastuzumab-EU in both its amino acid sequence and expected in vitro antiproliferative properties.

3.2 In-Life Observations

All animals survived until their scheduled terminal PK time point. There were no mAb-related clinical signs or effects on mean body weight or body weight change in any of the dose groups.

3.3 Pharmacokinetics

PF-05280014, trastuzumab-US, and trastuzumab-EU demonstrated similar serum concentration profiles over the

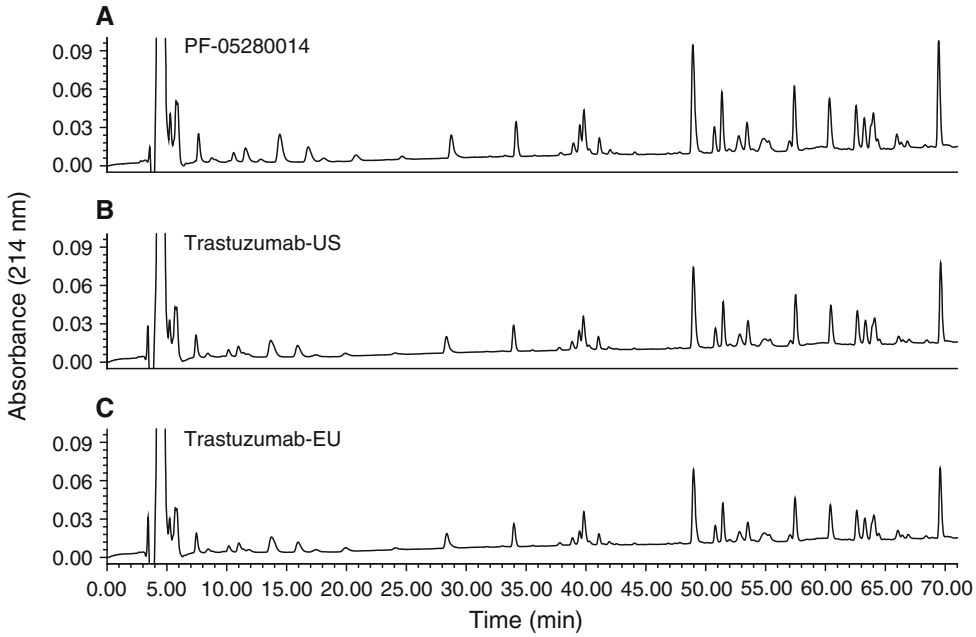

Fig. 1 Comparison of tryptic peptide maps of PF-05280014, trastuzumab-US, and trastuzumab-EU

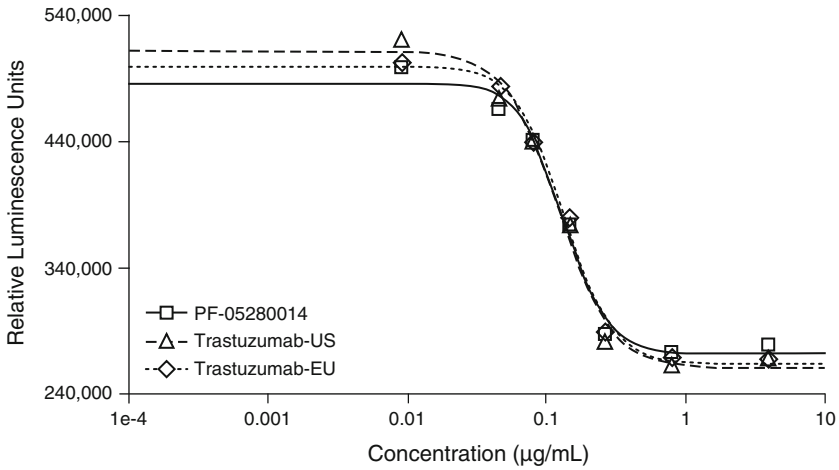

Fig. 2 Comparison of in vitro functional activity of PF-05280014 with trastuzumab-US and trastuzumab-EU measured in tumor cell growth inhibition assay using SKBR-3 cells

first 24 h at all three doses evaluated (Fig. 3) and through 2,880 h (Fig. 4). PK parameters C_{max}, $AUC_{0–2,880}$, $AUC_{0–\infty}$, $t_{1/2}$, CL, and V_{ss} were also similar across the three mAbs (Table 1). The percent extrapolation in the $AUC_{0–\infty}$ values based on the $AUC_{0–2,880}$ values ranged from 0.081 % to 1.28 %, indicating minimal extrapolation of these data. Both CL and V_{ss} appeared to be independent of the administered dose for all three mAbs. The $t_{1/2}$ for PF-05280014 was similar to, and generally overlapped with, the $t_{1/2}$ of trastuzumab-US and trastuzumab-EU. When data from mice that tested positive for ADA were excluded from the mean calculations, there was no impact on the overall PK interpretation (Table 2). Comparisons of the C_{max} and AUC ratios indicate that PF-05280014 had similar exposures to trastuzumab-US and trastuzumab-EU at all three doses regardless of whether data from all mice were used for the comparisons or when data from samples from those mice testing positive for ADA were excluded (Table 3).

3.4 Anti-Drug Antibodies

Induction of ADA in animals was determined by comparing the pre-dose (baseline) and post-dose serum ADA results in the same animal. Following administration of PF-05280014, trastuzumab-US, or trastuzumab-EU, the overall incidence of ADA in mice was low. Specifically, 8/74 (10.8 %), 6/75 (8 %), and 8/75 (10.7 %) of the animals tested positive for the induction of antibodies against PF-05280014, trastuzumab-US, and trastuzumab-EU, respectively (Table 4). Most animals that tested positive for the induction of anti-PF-05280014, anti-trastuzumab-US, or anti-trastuzumab-EU antibodies at 720, 1,080, 1,440, 2,160 or 2,880 h post-dose had lower trastuzumab exposure compared with the animals

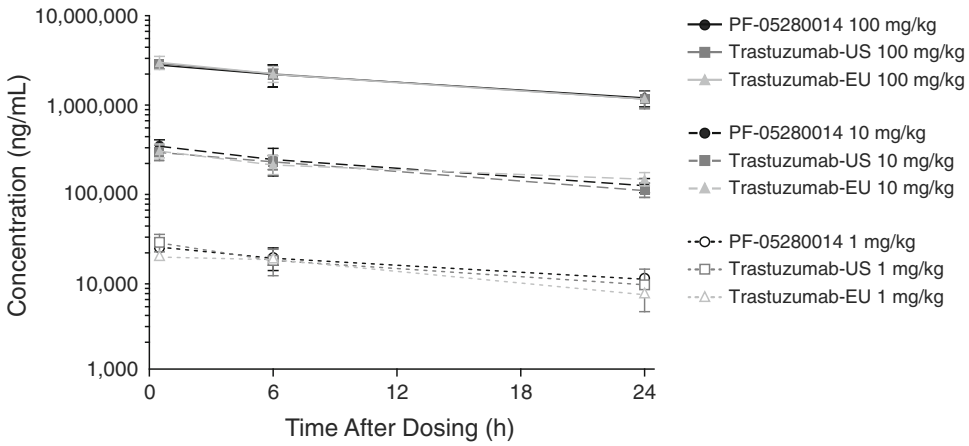

Fig. 3 Mean (±SD) concentrations of PF-05280014, trastuzumab-US, or trastuzumab-EU over 24 h after a single bolus intravenous injection of 1, 10, and 100 mg/kg in male CD-1 mice

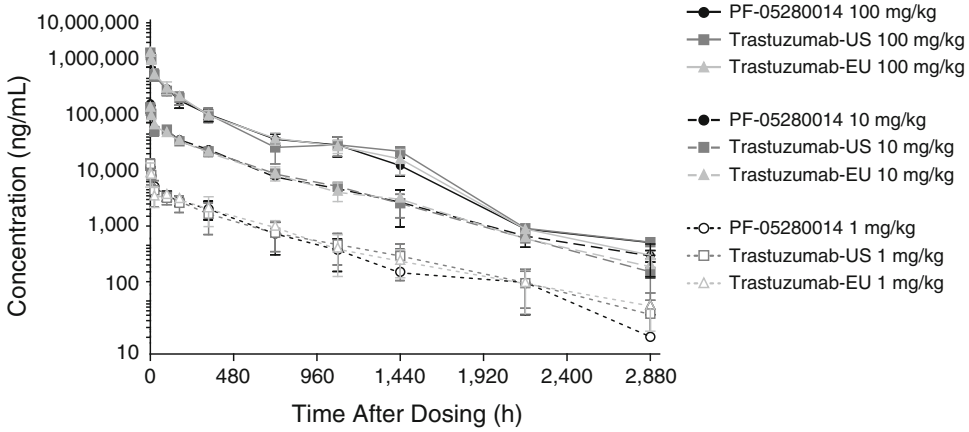

Fig. 4 Mean (±SD) concentrations of PF-05280014, trastuzumab-US, and trastuzumab-EU over time for the whole study duration up to 2,880 h after a single bolus intravenous injection of 1, 10, and 100 mg/kg in male CD-1 mice

Table 1 PK parameters (all mice)

Biologic	Dose level (mg/kg)	C_{max} ± SD (μg/mL)	$AUC_{0-2,880}$ ± SEM (μg·h/mL)	$AUC_{0-\infty}$ [a] (μg·h/mL)	$t_{1/2}$ (h)	CL (mL/h/kg)	V_{ss} (mL/kg)
PF-05280014	1	22.8 ± 1.90	4,200 ± 287	4,220	380	0.237	104
	10[b]	318 ± 49.4	51,400 ± 1,560	51,800	440	0.193	84.9
	100	2,520 ± 219	285,000 ± 11,000	286,000	309	0.350	120
Trastuzumab-US	1	26.3 ± 5.83	4,050 ± 334	4,080	416	0.245	129
	10	269 ± 52.7	49,800 ± 1,430	50,000	352	0.200	86.7
	100	2,620 ± 332	289,000 ± 12,500	289,000	320	0.346	130
Trastuzumab-EU	1	18.6 ± 8.55	4,590 ± 337	4,650	536	0.215	113
	10	281 ± 49.9	51,500 ± 1,370	51,800	392	0.193	85.4
	100	2,700 ± 450	298,000 ± 10,300	298,000	280	0.335	116

[a] The percent extrapolation in the $AUC_{0-\infty}$ values ranged from 0.081 to 1.28 %; [b] nominal dose

that tested negative, although this correlation could not be made for every animal that tested positive.

4 Discussion

In vitro evaluation of PF-05280014, trastuzumab-US, and trastuzumab-EU demonstrated that all three mAbs have the identical amino acid sequences based on a comparison of their tryptic peptide maps and LC/MS data. The in vitro tumor cell growth inhibition curves for PF-05280014, trastuzumab-US, and trastuzumab-EU were also superimposable. Taken together, these data demonstrate that PF-05280014 is identical to trastuzumab-US and trastuzumab-EU in sequence identity, and similar to them in its in vitro tumor cell growth

Table 2 PK parameters (excluding those testing positive for ADA)

Biologic	Dose level (mg/kg)	C_{max} ± SD (μg/mL)	$AUC_{0-2,880}$ ± SEM (μg·h/mL)	$AUC_{0-\infty}$ [a] (μg·h/mL)	$t_{1/2}$ (h)	CL (mL/h/kg)	V_{ss} (mL/kg)
PF-05280014	1	22.8 ± 1.90	4,650 ± 258	4,670	346	0.214	109
	10[b]	318 ± 49.4	52,000 ± 1,440	52,400	439	0.191	86.5
	100	2,520 ± 219	287,000 ± 11,000	288,000	307	0.347	122
Trastuzumab-US	1	26.3 ± 5.83	4,320 ± 294	4,380	529	0.228	134
	10	269 ± 52.7	49,800 ± 1,430	50,000	352	0.200	86.7
	100	2,620 ± 332	289,000 ± 12,500	289,000	320	0.346	130
Trastuzumab-EU	1	18.6 ± 8.55	4,730 ± 333	4,800	544	0.208	118
	10	281 ± 49.9	51,500 ± 1,380	51,700	392	0.193	85.3
	100	2,700 ± 450	298,000 ± 10,300	298,000	290	0.335	116

[a] The percent extrapolation in the $AUC_{0-\infty}$ values ranged from 0.104 to 1.54 %; [b] nominal dose

Table 3 Comparison of C_{max}, $AUC_{0-2,880}$, and $AUC_{0-\infty}$ values for PF-05280014, trastuzumab-US, and trastuzumab-EU after a single bolus intravenous injection

Dose level (mg/kg)	C_{max}			$AUC_{0-2,880}$			$AUC_{0-\infty}$		
	PF:US	PF:EU	US:EU	PF:US	PF:EU	US:EU	PF:US	PF:EU	US:EU
All animals									
1	0.87	1.23	1.41	1.04	0.915	0.88	1.03	0.91	0.88
10[a]	1.18	1.13	0.96	1.03	1.00	0.97	1.04	1.00	0.97
100	0.96	0.93	0.97	0.99	0.96	0.97	0.99	0.96	0.97
Data from mice testing positive for ADA excluded									
1	0.87	1.23	1.41	1.08	0.98	0.91	1.07	0.97	0.91
10[a]	1.18	1.13	0.96	1.04	1.01	0.97	1.05	1.01	0.97
100	0.96	0.93	0.97	0.99	0.96	0.97	1.00	0.97	0.97

ADA anti-drug antibodies; *EU* trastuzumab EU; *PF* PF-05280014; *US* trastuzumab-US

[a] Nominal dose

Table 4 Incidence of mice testing positive for ADA

Biologic	Dose level (mg/kg)	Number of ADA-positive animals/Number of animals tested						% Total incidence
		720 h	1,080 h	1,440 h	2,160 h	2,880 h	Total	
PF-05280014	1	1/5	1/5	3/5	0/5	0/5	5/25	20.0
	10[b]	1/5	0/5	1/5	0/5	0/5	2/25	8.0
	100	0/5	0/5	1/4[a]	0/5	0/5	1/24[a]	4.2
Trastuzumab-US	1	1/5	1/5	0/5	1/5	2/5	5/25	20.0
	10[b]	0/5	0/5	0/5	0/5	0/5	0/25	0
	100	0/5	0/5	0/5	1/5	0/5	1/25	4.0
Trastuzumab-EU	1	0/5	2/5	1/5	1/5	1/5	5/25	20.0
	10[b]	0/5	0/5	1/5	0/5	0/5	1/25	4.0
	100	0/5	0/5	0/5	0/5	2/5	2/25	8.0

ADA anti-drug antibodies

[a] Results from one animal were inconclusive (the animal was positive in screening, negative with titer; however, limited sample volume precluded confirmation testing or additional analysis); [b] nominal dose

inhibition profile. Additional physicochemical and functional side-by-side characterization of PF-5280014 with trastuzumab-US and trastuzumab-EU using state of the art robust and orthogonal analytical methodologies will be published in a separate paper (Ng et al. manuscript in preparation).

Because trastuzumab is a mAb engineered to recognize the extracellular domain of the human HER2 receptor, it does not recognize the mouse homolog, *neu* [16]. Therefore, PF-05280014, trastuzumab-US, and trastuzumab-EU would not be expected to interact with the endogenous mouse receptor, *neu*, thereby enabling comparative PK evaluation of non-target- and FcRn-mediated disposition of these three mAbs [17].

PF-05280014, trastuzumab-US, and trastuzumab-EU were well tolerated following a single IV dose up to 100 mg/kg. All animals survived to their scheduled terminal blood collection time point, and there were no mAb-related clinical signs or changes in body weight or body weight gain over the course of the 4-month study. The study protocol specified possible clinical pathology, necropsy, and/or tissue collection as additional safety-related endpoints if warranted by unscheduled death or euthanasia; because these did not occur in this study, these additional safety-related endpoints were not evaluated.

The plasma concentration-time profile of PF-05280014 was similar to those of trastuzumab-US and trastuzumab-EU at all three doses within the first 24 h and through the 4-month post-dose observation period. In addition, the C_{max} and $AUC_{0-\infty}$ values for PF-05280014, trastuzumab-US, and trastuzumab-EU were similar at all doses in this study. Moreover, the C_{max} data reported here are consistent with the data on the reference products previously submitted to regulatory agencies [16]. The percent extrapolation in the $AUC_{0-\infty}$ values based on the $AUC_{0-2,880}$ values ranged from 0.081 to 1.28 %, indicating minimal extrapolation. These data demonstrate that the observed values for $AUC_{0-2,880}$ accounted for almost all the mAb administered to the mice, thereby validating the results. The CL and V_{ss} were independent of the dose and were comparable across the three mAbs. The $t_{1/2}$ for PF-05280014 was similar to those values for trastuzumab-US and trastuzumab-EU and was consistent with the data reported for the reference products after a single IV dose in mice [21]. Additionally, the C_{max} values observed in this study were similar to those previously reported by trastuzumab [16]. There were also no mAb- or vehicle control-related clinical signs observed in this study.

The incidence of ADA induction was low ($\sim$10%) and similar across the three mAbs. In mice with ADA, the plasma concentrations of the administered mAb were lower in general compared with mice without ADA. Nevertheless, there was no impact on the overall PK profile evaluation because of the overall low incidence of ADA within each dose group. These data indicate that PF-05280014, trastuzumab-US, and trastuzumab-EU are not highly immunogenic in mice. Because immunogenicity in animals has limited predictive value for immunogenicity in humans [22, 23], these parameters will need to be evaluated in clinical studies.

5 Conclusion

As the patents of licensed biotherapeutics begin to lapse, there is an increasing interest in developing biosimilars with the same target, mechanism of action, safety, and efficacy as the patented molecules to potentially increase patient access to valuable therapies at lower cost. Based on the comparative peptide mapping, in vitro cell growth inhibition profiles and in vivo PK and ADA data reported in this paper, PF-05280014 appears to be similar to trastuzumab-US and trastuzumab-EU. These nonclinical data support the clinical development of PF-05280014 as a potential biosimilar. Subsequent to the nonclinical study reported here, a phase I study was conducted in healthy volunteers to determine the biosimilarity between PF-05280014, trastuzumab-US, and trastuzumab-EU (REFLECTIONS B327-01; NCT01603264). A phase III study is currently being conducted in metastatic breast cancer patients (REFLECTIONS B327-02; NCT01989676).

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