Development of a Subcutaneous Formulation for Trastuzumab – Nonclinical and Clinical Bridging Approach to the Approved Intravenous Dosing Regimen

Abstract

A subcutaneous (SC) formulation has been developed for the humanized monoclonal antibody (mAb) trastuzumab (Herceptin™). Trastuzumab is approved as an intravenous (IV) formulation to treat patients with early and metastatic breast cancer [1] and metastatic gastric and gastro-esophageal junction (GEJ) cancers [2] whose tumors over-express the human epidermal growth factor receptor 2 (HER2). IV infusion of trastuzumab is a well-established treatment modality. SC administration, however, may overcome a number of disadvantages related to the currently approved route of administration [3,4]. Being less invasive and with an injection duration of approximately 5 min, SC dosing is expected to be more convenient for patients compared to IV infusion which takes about 30–90 min depending on the treatment cycle and individual tolerability. Especially, patients on single agent mAb therapy [5] and those receiving trastuzumab in combination with oral chemotherapy [6–8] are expected to benefit from this alternative route of administration. While IV infusion is typically administered in a hospital or physician’s office, SC administration may allow health care professional-assisted home- or patient self-administration in an outpatient setting [9]. This is dependent on a variety of factors including the dosing frequency and the duration of treatment. In this context, SC administration of trastuzumab could also reduce hospital resources and costs associated with IV administration, including pharmacy time for reconstitution, nursing time, as well as occupancy of day beds and infusion chairs [10,11]. This manuscript reviews the nonclinical pharmacology/pharmacokinetics and early clinical approach supporting the development of a novel SC formulation for trastuzumab. The main focus is on the bridging concept to the approved IV trastuzumab formulation and the selection of a fixed SC dose.

Introduction

This review describes the early development concept for a novel subcutaneous (SC) formulation for the humanized monoclonal antibody (mAb) trastuzumab (Herceptin™). Trastuzumab was approved for the humanized monoclonal antibody (mAb) trastuzumab as an alternative to established intravenous (IV) infusion. The ready-to-use liquid SC formulation is injected as a fixed dose in approximately 5 min, which is expected to increase patient’s convenience, reduce pharmacy preparation time, and administration costs overall. The trastuzumab dose as well as the dose of recombinant human hyaluronidase (rHuPH20), an enzyme that enables SC administration of volumes larger than 2 mL, was selected based on nonclinical xenograft, pharmacology, and pharmacokinetics mouse and minipig studies. The basic assumption for bridging from the IV to the SC regimen was that comparable trastuzumab serum trough concentrations would result in comparable efficacy. This hypothesis is confirmed by the results from the Phase 3 study in the neo-adjuvant/adjuvant setting. The safety profiles of the trastuzumab SC and IV formulations are comparable and consistent with the known safety profile of trastuzumab.

Hyaluronidase

SC injection of fluids is limited by the volume that can be painlessly injected into the interstitial space [12–14], and should typically not exceed 1–2 mL [15]. To achieve a dosing volume low enough to enable SC bolus injection, the IV trastuzumab formulation was concentrated from approximately 21 to 120 mg/mL. This highly con-
Table 1  Estimated steady-state serum trough concentrations by regimen.

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<tr>
<td></td>
<td>Median</td>
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<td>C_{trough} (mg/L)</td>
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<td>4mg/kg loading dose</td>
<td>64.9</td>
<td>44.2–107</td>
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<td>followed by 2mg/kg</td>
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<td>maintenance dose</td>
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<td>8mg/kg loading dose</td>
<td>24 (13.1–35.2)</td>
<td>28 (16.2–40.4)</td>
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<td>followed by 4mg/kg</td>
<td>n = 53</td>
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<td>maintenance dose</td>
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<tr>
<td>Median overall survival</td>
<td>22.9 (16.0–37.1)</td>
<td>25.8 (13.3–34.7)</td>
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<td>(95 % CI) (months)</td>
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a Data derived from studies M77004 (q1w in MBC) and BO15899 (q1w in non-small cell lung cancer)

b Data derived from studies W016229 (q3w in MBC) and BO15935 (q3w in MBC)

Table 2  Tumor response rates, time to disease progression and overall survival with the once weekly dosing schedule using different doses of trastuzumab [35].

Scientific Basis for Dose Selection Approach

The aim of the dose finding studies was to select a SC dose that achieves trastuzumab serum trough concentrations (C_{trough}) at least as high as those with the approved IV formulation while maintaining the clinical dosing frequency. The selected dose was subsequently to be confirmed by a formal pharmacokinetic non-inferiority test as co-primary endpoint in a Phase 3 study (Section 6.4). The key assumption supporting this dose selection approach is that clinical response is driven by the concentration of a given mAb and that optimal efficacy is obtained when all accessible target sites are saturated [24–27].

Selection of IV trastuzumab doses

Trastuzumab is approved as a once-weekly (q1w) or 3-weekly (q3w) dosing regimen, with the q1w regimen developed initially [28, 29]. The dose levels for these regimens were selected from xenograft models to exceed the target concentration that showed a superior outcome against tumor growth (data on file F. Hoffmann-La Roche Ltd) [30]. These studies supported the selection of 20μg/mL as a targeted minimum trastuzumab C_{trough}. A loading dose was used for the IV formulation in order to obtain trastuzumab concentrations in the target range more rapidly. The q1w loading (4mg/kg) and maintenance (2mg/kg) doses achieve this trough concentration in the majority of patients for the entire duration of treatment (Table 1, data on file F. Hoffmann-La Roche Ltd).

Subsequently, a q3w IV dosing regimen was developed on the basis of mimicking the average exposure obtained with the q1w regimen [31]. This less frequent dosing regimen consists of an 8mg/kg loading dose followed by q3w 6mg/kg maintenance doses. Based on a population pharmacokinetic analysis with data primarily from the MBC setting, the predicted median C_{trough} (over a period of 3 weeks at steady-state) for the q1w and q3w regimens are 64.9 and 47.3mg/L, respectively (Table 1, data on file F. Hoffmann-La Roche AG). With these steady-state median concentrations, more than 90% of the patients exceed the target C_{trough} of 20μg/mL at the approved IV dose levels.

Concept of target site saturation to support selection of trastuzumab SC dose

Trastuzumab specifically binds to HER2 expressed on mammalian cells. A population pharmacokinetics model for trastuzumab showed that it exhibits linear pharmacokinetics in the range of clinical serum concentrations [32, 33], indicating saturation of accessible target sites. This hypothesis is supported by data published by Mager et al. [34], showing that the specific and saturable interaction of antibodies with the target receptor influences their disposition. Once accessible target sites are saturated, linear pharmacokinetics are observed.

The conclusion that the approved IV trastuzumab regimens lead to saturation of accessible target sites (HER2 receptors) and, therefore, to optimal clinical efficacy is supported by a plateau effect in the dose-efﬁcacy relationship that has been described previously [35]. No statistically signiﬁcant difference in response rate was seen in MBC patients between the approved q1w IV regimen (4mg/kg loading dose; 2mg/kg maintenance dose) and a second unapproved q1w IV regimen using a double dose (8mg/kg loading dose; 4mg/kg maintenance dose) (Table 2).

Based on the above considerations, the dose for the q3w SC trastuzumab formulation was selected with the aim of achieving trastuzumab serum trough concentrations at least as high as those obtained with the q3w IV trastuzumab formulation. It is therefore expected that trastuzumab serum concentrations upon SC administration that are non-inferior to those after IV administration would result in a comparable clinical beneﬁt.

The Phase 3 HannaH trial (BO22227) comparing efﬁcacy, pharmacokinetics and safety of the IV and SC formulations in patients
with HER2-positive breast cancer (neo-adjuvant followed by adjuvant setting) has been conducted to confirm this hypothesis (Section 6.4).

**Nonclinical Pharmacology and Pharmacokinetics Studies Supporting Development of the SC Formulation for Trastuzumab**

The nonclinical pharmacology and pharmacokinetics studies were conducted to support (i) the bridging from the approved IV administration of trastuzumab to the SC administration route and (ii) the selection of the rHuPH20 concentration in the trastuzumab SC formulation.

**Preclinical pharmacology studies**
**IV/SC bridging study with trastuzumab in a mouse xenograft model**

A pharmacology study in a mouse xenograft model was designed to bridge between the IV and SC dosing regimens and to demonstrate similar pharmacodynamic activity of trastuzumab at the same minimum serum concentration after IV and SC administration, i.e., irrespective of the administration route. BALB/c nu/nu mice were transplanted subcutaneously with the non-small cell lung cancer cell line Calu-3, which has high expression of HER2. This xenograft model was successfully used in our laboratory for pharmacology studies with the anti-HER2 antibodies pertuzumab and trastuzumab [36, 37]. Approximately 4 weeks after cell transplantation, mice (n=10 per dose group) were treated with 0, 1, 3 or 10 mg/kg trastuzumab by either SC or IV administration once weekly for a period of 6 weeks. SC administration was done on the flank contralateral to the tumor xenograft. The trastuzumab SC formulation contained 4600 U/ml rHUPH20 to aid in dispersion and uptake of trastuzumab from the SC space. The trastuzumab dose levels for SC and IV dosing were selected based on a previous single dose IV and SC pharmacokinetics study in mice (data not shown). Simulated multiple-dose pharmacokinetics profiles indicated that SC and IV dosing at the same dose level yield about 50% lower maximum trastuzumab serum concentrations following SC administration, while trastuzumab trough levels were similar for both dosing routes. Thus, dose levels of 1, 3, and 10 mg/kg were chosen for both SC and IV administration. Tumor size was assessed throughout the treatment period and was correlated with route, dose and terminal serum trough levels of trastuzumab.

Tumor growth inhibition tended to be dose dependent (Fig. 1). Following administration of trastuzumab at a dose of 1 mg/kg by SC or IV administration, there was no apparent tumor growth inhibition (average ± SD trough serum concentrations were 0.62 ± 0.36 and 0.73 ± 0.42 μg/mL after IV and SC administration, respectively). Following IV or SC administration of 3 mg/kg trastuzumab, tumor growth inhibition was evident with no difference between dose routes (median tumor growth inhibition 38% and 39% after IV and SC dosing determined on day 71 after tumor cell inoculation). Average trastuzumab C_{trough} (± SD) prior to the last treatment was similar for both routes of administration (9.4 ± 5.4 and 10.4 ± 11.9 μg/mL after IV and SC dosing, respectively). Also at 10 mg/kg IV or SC trastuzumab, tumor growth inhibition was not significantly different between dosing routes. A trend towards higher tumor growth inhibition after IV administration (median tumor growth inhibition 66% and 48% after IV and SC dosing determined on day 71 after tumor cell inoculation) was consistent with a trend towards higher serum trough concentrations after IV dosing (95.7 ± 26.5 and 61.3 ± 33.8 μg/mL after IV and SC dosing, respectively). The trend to lower average exposure and tumor growth inhibition after SC administration is probably a chance result due to very low exposure in a few mice of the 10 mg/kg SC dose group. Overall, the results of the study suggest that the SC vs. IV dosing routes do not influence trastuzumab efficacy in this xenograft model given that similar trastuzumab trough concentrations are reached.

**Fig. 1** Effect of trastuzumab administered either IV or SC on Calu-3 NSCLC xenografts in female Balb/c nude mice. Treatment was performed on indicated days after tumor cell inoculation. Tumor volume (mm^3) was determined twice weekly and is shown as median and interquartile range (n=9–10 animals per group).
Dye dispersion study in mice

Hyaluronidases (both animal-derived and rHuPH20) can be used in 2 different administration modes to facilitate SC administration: (i) sequential administration of hyaluronidase followed by administration of the drug, or (ii) concomitant administration of hyaluronidase and drug in a co-formulation. In the approved setting, rHuPH20 (Hylenex™) is pre-injected to allow cleavage of hyaluronan prior to subsequent infusion of larger volumes of SC formulations, while the SC trastuzumab formulation contains rHuPH20 as an excipient. We hypothesized that the concomitant administration in a co-formulation as in the trastuzumab SC formulation would require higher hyaluronidase doses as compared to sequential administration to achieve the same permeation enhancing effect. Dye dispersion studies are an established approach to study the permeation enhancing effects of hyaluronidases [19,38].

A dye dispersion study in nude mice was performed to compare permeation enhancing effects of rHuPH20 either following sequential administration of rHuPH20 and a tracer dye or following administration of a co-mixture of tracer dye and rHuPH20. This dye dispersion study was conducted using the intradermal (ID) route. This route was selected to facilitate visualization of dye dispersion and to accommodate anatomical differences between human and non-human subcutis. The subcutis in furred animals like rodents gives little resistance to bulk fluid flow [19]. By contrast, the ID space in rodents provides resistance to bulk fluid flow and, thus, is a closer approximation to the anatomical resistance to bulk fluid flow in the SC space of humans. The dye dispersion experiment in NCR nu/nu mice (Taconic Laboratories, USA) was conducted as described elsewhere [17].

rHuPH20 (100 U/mL, total dose 2 U) was injected ID at 1, 5, and 15 min prior to an ID injection of Trypan Blue dye (n=6 mice/cohort). The dye dispersion area was then measured at 1, 2.5, 5, and 20 min post ID injection of dye. Dye dispersion areas from sequentially delivered rHuPH20 and Trypan Blue dye were compared to dye dispersion areas following a co-mixture ID injection of Trypan Blue with increasing doses of rHuPH20 (100, 500, and 5000 U/mL, total doses 2, 10 and 100 U, respectively). A statistically significant, dose-dependent, increase in dye dispersion area was observed with the co-mixture cohorts of 2, 10 and 100 U (100, 500, and 5000 U/mL) of rHuPH20 (Fig. 2). In comparison, dye dispersion areas of animals injected with 2 U (100 U/mL; 0.88 μg/mL) of rHuPH20 followed by a subsequent sequential injection of Trypan Blue dye at 1, 5, and 15 min were not statistically different from each other, suggesting that by 1 min, the majority of the local dispersion reaction had reached completion. Sequential injections yielded faster dye spreading as compared to co-mixture administration of the same enzyme dose.

These results demonstrate that 5–50-fold more rHuPH20 is required in a co-mixture solution to approach maximal spreading effects as observed with sequential administration, in the time frame relevant for SC injections, e.g., 5 min or less. Thus, administration of co-mixture solutions containing 500–5000 U/mL rHuPH20 achieved a dispersion profile similar to sequential administration of a solution with 100 U/mL rHuPH20 followed by Trypan Blue dye injection.

Pharmacokinetic study in minipigs

A pharmacokinetic study with trastuzumab SC in minipigs explored the effect of various rHuPH20 concentrations on the SC absorption of trastuzumab in order to guide rHuPH20 concentration selection for the formulation used in the first clinical study with trastuzumab SC. The minipig was chosen because its skin and the texture of the SC tissue are considered to be similar to those of humans with a fibrous tissue network connecting dermis and deep fascia/muscle [39]. In addition, the minipig has
been demonstrated to be a predictive model for human pharmacokinetics of mAbs after both IV and SC administration [40]. Female Göttingen minipigs (Ellegaard Götingen Minipigs A/S, Dalmose, Denmark) (n = 5/dose group) received a single SC dose of trastuzumab at 108 mg/animal (about 13–14 mg/kg) containing either 0, 2000 or 6000 U/mL rHuPH20. Another group received a SC dose of trastuzumab at 216 mg/animal (about 26.7 mg/kg) containing 2000 U/mL rHUPH20 to explore dose-linearity of SC absorption. Serum concentrations of trastuzumab were analyzed with a specific ELISA.

Fig. 3 shows the average serum concentration-time profiles for the SC dose groups dosed at 108 mg/animal. After SC administration, trastuzumab absorption in minipigs was more rapid with rHuPH20-containing formulations. Median time to maximum serum levels (Tmax) was shortened from 72 h without rHuPH20 to 24 h for all rHuPH20-containing formulations. Average maximum trastuzumab serum levels (± SD) for the rHuPH20-containing formulations trended towards an increase relative to the levels obtained for the formulation without rHuPH20 (101 ± 21.7, 126 ± 13.2 and 129 ± 6.78 μg/mL at 0, 2000, and 6000 U/mL rHuPH20, respectively, at 108 mg trastuzumab).

Compartmental pharmacokinetic modeling was done using a non-linear mixed-effects model. The structural model was composed of 2 compartments with both a linear clearance and saturable clearance from the first compartment (“serum compartment”) and linear inter-compartment exchange as described elsewhere [40]. Additionally, first-order absorption from an absorption site compartment was assumed in the case of SC administration. For model development, additional data after IV administration of trastuzumab to minipigs were used (data not shown). Compartmental pharmacokinetic modeling revealed approximately 2-fold higher estimates for the absorption rate constants associated with the rHuPH20-containing formulations relative to the rate constant associated with the control formulation without rHuPH20 (0.828, 1.66, and 1.71 day⁻¹ at 0, 2000 and 6000 U/mL rHuPH20). Thus, there was no further increase in absorption rate when increasing the rHuPH20 concentration from 2000 to 6000 U/mL. The similar absorption rate constants at these rHuPH20 concentrations are consistent with the super-imposable trastuzumab serum concentration-time curves from these formulations during the absorption phase (Fig. 3). Compartmental modeling using a population PK approach (non-linear mixed effects model) revealed no relevant difference in the absorbed fraction of trastuzumab across formulations. The estimate for the absorbed fraction of trastuzumab was 85.4 ± 2.6% when utilizing data from all SC dose group (mean ± standard error of the population mean parameters estimated from compartmental PK analysis). An increase in the absorbed fraction from rHuPH20 containing formulations was not expected considering the high absorbed fraction of trastuzumab without rHuPH20. Non-compartmental PK analysis suggested a higher interindividual variability in the fraction absorbed/bioavailability as compared to the compartmental PK analysis. Thus, for the trastuzumab formulation containing 2000 U/mL rHuPH20 SC bioavailability estimates ranged from 67 to 94%, while individual fraction absorbed estimates from compartmental PK analysis ranged from 73 to 85%. The higher variability in the estimates from non-compartmental analysis may be due to the fact that due to the parallel group design of the study the SC bioavailability estimates from non-compartmental analysis reflect interindividual differences in both absorption and disposition kinetics. Doubling of the trastuzumab dose had no obvious impact on the SC absorption rate (or process). At the 216 mg trastuzumab SC dose, exposure was roughly twice as high as that for the 108 mg trastuzumab SC dose when using the same formulation containing 2000 U/mL rHuPH20 (average AUC₀–672 h ± SD: 29 600 ± 3 360 and 73 600 ± 15 200 μg · h/mL at 108 and 216 mg trastuzumab).

Overall, these data from minipigs demonstrate an approximately 2-fold more rapid absorption of SC administered trastuzumab from rHuPH20-containing formulations compared to the formulation without rHuPH20. This effect on the absorption rate was similar with both of the tested rHuPH20 concentrations, i.e., the increase in the rHuPH20 concentration from 2000 to 6000 U/mL did not lead to a further absorption enhancement. These data support the use of 2000 U/mL rHuPH20 in the clinical trastuzumab SC formulation.

Clinical

The clinical studies conducted to bridge from the approved IV to the SC administration route were designed to define the SC dose required to achieve trastuzumab serum trough concentrations at least as high as with IV administration, to subsequently demonstrate bio-non-inferiority (non-inferior Ctrough) and to provide supportive efficacy data. In addition to systemic side effects, special focus was on injection reactions related to SC administration of 5 mL. The formation of anti-drug antibodies (ADAs) and their potential impact on the exposure to trastuzumab and correlation with adverse events has been monitored throughout the clinical program, as the route of absorption might impact the formation of ADAs [41].
The Phase 1 dose finding study that formed the basis for the clinical bridging approach from the approved q3w IV to the q3w SC formulation is described in the following section.

Clinical dose finding study
The aims of the clinical dose finding study were to support selection of the SC dose of trastuzumab expected to result in trastuzumab serum trough concentrations at least as high as those achieved with the IV formulation and to assess and compare the safety and tolerability of IV and SC dosing [42], (Table 3). The open-label study was conducted in both healthy male volunteers and female patients with HER2-positive early breast cancer (EBC) in the adjuvant setting. To avoid under-dosing patients during dose selection, in part 1 of the trial pre-defined SC doses were initially studied in healthy male subjects. It was expected that the pharmacokinetics profile in female patients in the adjuvant setting was comparable to that in male subjects, as tumor tissue that may have affected trastuzumab elimination was removed. To be able to account for potential differences in the pharmacokinetics profile in the 2 populations, 2 cohorts, 1 consisting of 6 female patients (Cohort 1) and the other of 6 healthy male subjects (Cohort 2) received a single 6 mg/kg dose of the approved IV formulation. 3 different SC dose levels were subsequently studied in an adaptive fashion in 3 cohorts of 6 healthy male subjects each. The SC trastuzumab dose levels as well as the HuPH20 concentration of 2000U/mL were guided by preclinical animal pharmacology and pharmacokinetics studies (Section 4). As the SC bioavailability in the minipig was approximately 85% with a comparably large inter-subject variability (range 67 – 94% for the trastuzumab formulation containing 2000U/mL rHuPH20), SC doses were pre-selected to cover the observed range of bioavailability. The initially selected trastuzumab SC doses of 6 and 10 mg/kg (cohorts 3 and 4) assume a SC bioavailability of 100% and of 60%, respectively. Following interim non-compartmental pharmacokinetics analysis from cohorts 1, 2, 3, and 4, cohort 5 was opened, in which healthy male subjects received an adjusted dose of 8 mg/kg that was expected to result in comparable trastuzumab serum trough concentrations to the 6 mg/kg IV dose.

In part 2 of the study, 40 patients with HER2-positive EBC in the adjuvant setting were administered either the SC dose of 8 mg/kg (n = 20) or a higher dose of 12 mg/kg (n = 20) to cover a greater range of exposure, which was considered relevant in terms of assessing systemic and local adverse events in the target patient population prior to entering into a larger Phase 3 trial.

Pharmacokinetics, safety and tolerability results: SC dose finding in humans
Detailed study results from the dose finding study have been reported previously [42]. SC administration of trastuzumab was generally well tolerated. Most AEs (72%) were mild in severity. There was no apparent dose-related increase in AEs receiving SC trastuzumab. In patients receiving SC trastuzumab, 18 administration site AEs were reported, the majority of which were of mild intensity (erythema [n = 7], discoloration [n = 5], injection site swelling [n = 2], injection site discomfort [n = 1], injection site reaction [n = 1]). There were 2 instances of moderate injection site pain. There were no clinically significant changes from time-matched baseline values in ECG parameters following doses of the study drug. There were no deaths, serious AEs, treatment withdrawals due to AEs or laboratory abnormalities, or dose modifications in this study.

Table 4 shows the trastuzumab serum pharmacokinetics following IV and SC administration. Exposure (AUC0–inf) in patients receiving 8 mg/kg SC trastuzumab was comparable to that in patients receiving 6 mg/kg IV trastuzumab (1800 day*μg/mL vs. 2090 day*μg/mL), indicating a SC bioavailability of approximately 80%, comparable to the results from the study in minipigs. At the same dose level, trastuzumab serum concentrations measured at 22 days post-dose with 37.8μg/mL and 27.5μg/mL were slightly higher with the SC compared to the IV dose, respectively. The corresponding coefficients of variation (CV%) were with 27.5% and 27.1% in the same range, indicating a comparable variability in trastuzumab serum concentrations following both routes of administration. As expected, the Cmax followed the 8 mg/kg SC dose was 88.4 μg/mL which was markedly lower compared to the IV dose of 6 mg/kg (185 μg/mL). Cmax and AUC did not appear to deviate from dose proportionality over the range of SC trastuzumab doses administered (6–12 mg/kg) in both Part 1 and Part 2 [42].

A total of 8 out of 58 (14%) participants who had been administered subcutaneous trastuzumab had a positive test result for anti-drug antibodies (ADAs). The binding characteristics of these ADAs have been described in more detail previously [46].
anti-trastuzumab antibodies following a confirmatory binding assay. 2 of these participants were tested positive at 5 months post-administration. The AE and pharmacokinetics profiles in participants with a positive test result for anti-trastuzumab antibodies were in the range of the study population. Due to a lack of an available assay at the time of conducting the study, no neutralizing anti-trastuzumab assay was performed. All participants’ samples were negative for neutralizing anti-rHuPH20 antibodies. As the formation of anti-trastuzumab antibodies may depend on the dosing regimen, the frequency of anti-trastuzumab antibodies and the potential implications are investigated further in the Phase 3 HannaH study.

**Fixed SC dose**

While intravenously-administered trastuzumab is dosed on a weight-adjusted basis (8 mg/kg loading dose followed by q3w maintenance doses of 6 mg/kg), in the Phase 3 study, the SC formulation was given at a fixed dose for all patients. This fixed dose was selected using the pharmacokinetics data from the Phase 1 dose-finding study applying a modeling and simulation approach (data on file F. Hoffmann-La Roche). The analysis indicated that a dose of 600 mg q3w would result in non-inferior C_{trough}, at cycle 8 in comparison to the 8 mg/kg loading dose followed by q3w maintenance doses of 6 mg/kg. Predicted C_{trough} values at cycle 1 following SC dosing did exceed the trastuzumab serum target concentration of 20 μg/mL and were comparable to those achieved with the IV loading dose, omitting the need for a loading dose (see section 4.1). Historically, anticancer agents were usually dosed on a weight- or body-surface area-adjusted basis. This practice is a result of a scale-up from animal toxicology studies to the human situation [43, 44]. Attempts have been made to develop novel mAbs with a fixed dose. Application of a fixed dose would avoid potential dose calculation errors and generally reduce health care professional’s resources related to prescribing and administration of the drug, as well as manufacturing, storing and shipping costs of the formulation [45–47]. The use of a fixed dose of trastuzumab is supported by a recent simulation study by Wang et al. [48] that compared the performance of body size-based and fixed dosing in reducing pharmacokinetics and/or pharmacodynamics variability in adults for 12 intravenously administered monoclonal antibodies (mAbs) with published population pharmacokinetics and/or pharmacodynamics models, including IV trastuzumab. The median and the 95th percentile intervals of the simulated concentration-time profiles following the 2 dosing approaches were compared. The focus of the comparison was the 95th percentile intervals of the concentration-time profiles. Overall, the 95th percentile intervals did not differ markedly between the fixed and body weight/body surface area-based dosing approaches across all the 12 mAbs studied. While the comparison by Wang et al. was generated for the IV formulation of antibodies, it is anticipated that the same conclusion would apply when comparing SC administered antibodies with a fixed vs. a body weight-adjusted dose.

**Phase 3 HannaH trial**

HannaH is a Phase 3, open-label study involving 596 women with HER2-positive early breast cancer. The study was designed to compare trastuzumab serum concentrations (C_{trough}), efficacy (pathological complete response, pCR) and safety of trastuzumab SC to that of trastuzumab IV (Fig. 4) [49]. Secondary endpoints included event-free survival and overall survival. The results from this recently analysed study showed that non-inferior trastuzumab serum trough concentrations upon SC dosing result in comparable efficacy (measured as pCR) to the IV regimen. No new safety signals were observed and the SC safety profile is consistent with the known safety profile of trastuzumab IV. The HannaH study demonstrated comparable efficacy of the subcutaneous formulation of trastuzumab to the standard IV infusion of trastuzumab in women with HER2-positive early breast cancer. Data from the study have been presented at the 8th European Breast Cancer Conference in Vienna [49] and have been submitted in a marketing application to regulatory authorities in the European Union.

**SC Trastuzumab Formulation and Injection Device**

Herceptin™ (trastuzumab) is currently marketed in Europe in a single-dose vial containing 150 mg trastuzumab in the form of a lyophilized powder (containing the antibody, α,α-trehalose dihydrate, L-histidine and L-histidine hydrochloride and polysorbate 20) which should be reconstituted for infusions with water for injection to yield an injection dose of approximately 21 mg/mL. Another single-dose vial containing 60 mg trastuzumab is used in Japan and Australia. In the USA and many other countries a multiple dosage vial containing 440 mg trastuzumab is marketed.

The development of a ready-to-use SC formulation of trastuzumab became feasible with the recent advances in the development of highly concentrated mAb formulations [50, 51] and the availability of rHuPH20 that enables rapid SC administration of trastuzumab. 

**Fig. 4** Phase 3 HannaH study design in HER2-positive nep-adjuvant/adjuvant patients.
Different to IV trastuzumab, which is dosed on a weight-adjusted basis, the SC formulation is administered at a fixed dose in all treatment cycles without the need for a loading dose and independent of a patient’s body weight. The trastuzumab SC formulation offers an alternative to the more invasive and lengthier IV administration. Importantly, for patients who have completed chemotherapy, the SC route might allow spending less time in clinics. The fixed dose and ready-to-use liquid formulation would also reduce pharmacy preparation time, reduce administration costs overall, and should be welcome in institutions with an infusion capacity bottleneck.

**Conflict of Interest**

Drs B. Bittner, W. Richter, F. Hourcade-Potelleret, C. McIntyre, F. Herting and J. Schmidt are employees of F. Hoffmann-La Roche Ltd., Dr M. Zepeda is an employee of Halozyme Therapeutics (San Diego).

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