

Subcutaneous trastuzumab: development of a new formulation for treatment of HER2-positive early breast cancer

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Abstract: Trastuzumab is a monoclonal antibody directed against the human epidermal growth factor receptor 2 (HER2). HER2 is amplified or overexpressed in about 15% of breast cancers and is associated with aggressive disease. Clinical benefits of trastuzumab have been established in the treatment of both early and metastatic HER2-positive breast cancer. Patients with HER2-positive early breast cancer have to be treated with trastuzumab for one year in combination with and sequentially after chemotherapy. This requires that trastuzumab is intravenously infused over 30–90 minutes every 3 weeks for one year which is time-consuming for both the patient and the health care provider. Consequently, a subcutaneous formulation of trastuzumab using a recombinant human hyaluronidase has been developed. Recombinant human hyaluronidase transiently increases absorption and dispersion in the subcutaneous space of large therapeutic proteins, such as monoclonal antibodies, allowing subcutaneous administration of trastuzumab in about 5 minutes. Thus, subcutaneous trastuzumab could represent a new treatment option that could have benefit to both the patient and the health care system. This review focuses on the development of the subcutaneous trastuzumab formulation and analyzes clinical trials assessing the pharmacokinetics, efficacy, and safety of this new formulation.

Keywords: trastuzumab, hyaluronidase, human epidermal growth factor receptor 2, breast cancer

Introduction

Trastuzumab is a recombinant humanized monoclonal antibody that targets the external domain of the human epidermal growth factor receptor 2 (HER2), a tyrosine kinase transmembrane receptor. HER2 is amplified and/or overexpressed in approximately 15% of breast cancers.^{1,2} This amplification is associated with aggressive disease and confers a poor prognosis.¹ In the metastatic setting, significant clinical benefits of trastuzumab have been demonstrated when this agent is used in combination with chemotherapeutic agents.^{3,4} In the adjuvant setting, use of trastuzumab is recommended by US and European guidelines on the basis of data from four large trials.^{5–9} Those trials have shown a significant reduction in the relative risk of recurrence of 33%–52% and a significant improvement in overall survival of about 35% in patients with early breast cancers treated for one year with trastuzumab.^{5–9}

Trastuzumab therapy in patients with HER2-positive early breast cancer consists of a loading dose of 8 mg/kg infused intravenously over 90 minutes followed by 6 mg/kg infused over 30–90 minutes every 3 weeks. Even if trastuzumab has been shown to be cost-effective in the adjuvant setting,¹⁰ this therapy remains time-consuming both for the patient and the health care provider. Therefore, a new formulation

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enabling subcutaneous administration of trastuzumab using recombinant human hyaluronidase has been developed. The administration time via the subcutaneous route is about 5 minutes, which may lead to an improvement in quality of life for patients. This review describes the development of the subcutaneous trastuzumab formulation, as well as its efficacy and safety.

Subcutaneous route: alternative to intravenous administration

The subcutaneous route could constitute a convenient and easy to use alternative to intravenous administration. The benefit of subcutaneous administration compared with the intravenous route has been examined in the treatment of antibody deficiencies.¹¹ Several studies have demonstrated that in patients previously treated with intravenous immunoglobulins, subcutaneous immunoglobulin administration is more convenient and improves quality of life as well as satisfaction with treatment.^{12,13} This improvement is consistently associated with patients' treatment preference for subcutaneous administration. Furthermore, reducing time in hospital could bring benefit to both the patient and the health care system.¹⁴

In addition to the advantages of subcutaneous administration, potential limitations exist. The structure of the subcutaneous space limits the volume of drug that can be administered and precludes the delivery of drugs into the vascular compartment. Indeed, the subcutaneous space comprises the extracellular matrix composed of the glycosaminoglycan, hyaluronan, that forms a viscous barrier to fluid diffusion in the extracellular matrix. In contrast with the other components of the extracellular matrix, such as collagen, hyaluronan has a rapid turnover, with a half-life of about 12 hours in the skin.¹⁵ Because of these characteristics, hyaluronan constitutes a potential and unique target for modifying the extracellular matrix. Consequently, degrading hyaluronan may be a method to increase the permeability of the matrix temporarily and thereby enhance the delivery of drugs and fluids through the extracellular matrix and into the circulation.

Using hyaluronidase to improve subcutaneous administration

Background

Hyaluronidase belongs to a family of enzymes that rapidly degrade hyaluronan.¹⁶ Hyaluronidase catalyzes the cleavage of the hyaluronan long chain polymer and this breakdown

of hyaluronan by hyaluronidase in the subcutaneous space enhances the permeation of coadministered agents.^{17,18} Animal-derived hyaluronidases have been used clinically for more than 60 years in several clinical applications, including ophthalmic surgery¹⁹ and rehydration.²⁰ However, if purified animal-derived hyaluronidase is used in the clinical setting, the animal extracts remain contaminated with other proteins that may induce an allergic reaction.^{21,22} To overcome these concerns, a soluble purified recombinant hyaluronidase has been developed,²³ and is approved by the US Food and Drug Administration for administration as an adjuvant to increase the absorption and dispersion of other injected drugs.

Development of recombinant human hyaluronidase

Six hyaluronidase genes have been identified in the human genome.²⁴ Of these, only the PH20 gene product is a neutral pH-active hyaluronidase,²⁵ and hydrolyzes hyaluronan under physiologic conditions. The human PH20 enzyme is a glycoprotein anchored to the plasma membrane of the sperm through a glycosyl-phosphatidylinositol moiety. Identification of this human hyaluronidase promoted the molecular engineering of a recombinant human PH20 enzyme (rHuPH20) lacking the glycosyl-phosphatidylinositol moiety and rendering rHuPH20 soluble.²³

The efficacy of rHuPH20 in enhancing the delivery of therapeutics via the subcutaneous route was first established in *in vivo* studies.²³ These studies showed that rHuPH20 creates channels approximately 200 nm in size by degrading hyaluronan in the extracellular matrix, indicating that large therapeutic proteins like monoclonal antibodies can diffuse through these channels. These effects of hyaluronidase on the extracellular matrix are temporary and are reversed completely within 24 hours of injection.

The clinical safety of rHuPH20 was first demonstrated in a double-blind, placebo-controlled, single-dose study in which no allergic reactions were reported in 100 healthy volunteers receiving a single subcutaneous injection.²⁶ Several clinical studies confirmed these findings and further provided evidence of the efficacy of rHuPH20 in enhancing subcutaneous fluid or drug administration in healthy adults,²⁷ in patients with serious illness,²⁸ and in children.²⁹ The therapeutics coadministered with rHuPH20 have been primarily insulin,³⁰ antibiotics,³¹ and narcotics.²⁷ The pharmacokinetic profile and safety of subcutaneous morphine coadministered with rHuPH20 was compared with that of subcutaneous morphine given alone and with intravenous morphine in a Phase IV double-blind, randomized, crossover study in healthy adults.²⁷

Compared with subcutaneous coadministration of morphine with saline, coadministration with rHuPH20 enhanced the pharmacokinetic profile of subcutaneous morphine, with an improvement of 42% in absorption of morphine and an increase of 29% in maximum plasma morphine concentration (C_{max}). Furthermore, compared with intravenous morphine, subcutaneous morphine with or without rHuPH20 resulted in similar total systemic exposure, and appeared to be well tolerated and safe, with fewer systemic adverse events.

Similarly, comparison of three administration routes of ceftriaxone (subcutaneous ceftriaxone with or without rHuPH20 and intravenous ceftriaxone) in a Phase I study³¹ showed that the peak plasma concentration occurred earlier (one hour) and was higher (+12%) with subcutaneous ceftriaxone plus rHuPH20 than following subcutaneous ceftriaxone plus placebo. Ceftriaxone exposure was comparable among the three treatments and well tolerated.

Taken together, the results of these preclinical and clinical studies support the development of a new formulation of trastuzumab using rHuPH20.

Trastuzumab: mechanisms of action

Trastuzumab targets the extracellular domain of HER2³² and exerts its antitumor effect by inhibiting the HER2 downstream signaling transduction pathway.³³ Suppression of HER2 signaling activity by trastuzumab induces cell cycle arrest and apoptosis, as well as inhibition of angiogenesis. Other mechanisms of action include antibody-dependent cell-mediated cytotoxicity³⁴ and inhibition of HER2 shedding.³⁵ Through these mechanisms, trastuzumab improves survival in HER2-positive metastatic breast cancer^{3,4} and early breast cancer.⁵⁻⁹

Pharmacokinetics, efficacy, and safety of subcutaneous trastuzumab

Pharmacokinetic parameters

The similarities between the pharmacokinetic parameters of subcutaneous trastuzumab and those of intravenous trastuzumab have been demonstrated in an open-label, two-part, Phase I/Ib study in 24 healthy male volunteers and 42 patients with HER2-positive early breast cancer (Table 1).³⁶ The first part of this study aimed at defining the optimal dose of subcutaneous trastuzumab that could result in a pharmacokinetic profile for trastuzumab similar to that associated with intravenous trastuzumab administration. Subcutaneous doses of trastuzumab tested in healthy volunteers ranged from 6 mg/kg to 10 mg/kg, and were compared with an approved intravenous trastuzumab

dose of 6 mg/kg. For a trastuzumab dose of 8 mg/kg, a subcutaneous injection of about 5 mL of the liquid formulation of trastuzumab, containing rHuPH20 2000 U/mL, was used in a 70 kg subject. A skin test with rHuPH20 was performed prior to subcutaneous administration. Pharmacokinetic analysis showed that a subcutaneous dose level of 8 mg/kg trastuzumab achieved a trastuzumab exposure comparable with that obtained using an intravenous dose of 6 mg/kg trastuzumab (Table 1). These results were further validated in the second part of this study in patients with HER2-positive early breast cancer receiving 8 mg/kg or 12 mg/kg of subcutaneous trastuzumab.

The safety of the subcutaneous formulation was also evaluated in this study (Table 1). The number of adverse events ($n = 217$) reported in 55 of the 58 subjects in the subcutaneous group was lower than that reported in the intravenous group (50 adverse events in 11 of 12 subjects in the intravenous group). However, the proportion of subjects experiencing at least one adverse event in the subcutaneous and intravenous groups was similar (55/58 versus 11/12; $P = 1.000$).

Irrespective of the method of administration and dose of trastuzumab, the majority of adverse events were of mild intensity. Eighteen injection site reactions were reported in 58 patients receiving subcutaneous trastuzumab, most of which were considered to be mild in intensity. Further, the incidence of infusion-related reactions was lower in those treated with subcutaneous trastuzumab than in those treated with intravenous trastuzumab.

The results of this Phase I study showed that administering trastuzumab via the subcutaneous route does not impact the trastuzumab exposure, and also defined the optimal dose for subcutaneous trastuzumab. These results have been further used to select a fixed 600 mg subcutaneous dose of trastuzumab. The pharmacokinetic, efficacy, and safety profiles of this fixed subcutaneous dose were evaluated in a noninferiority randomized, open-label, multicenter Phase III HannaH (enHANced treatment with NeoAdjuvant Herceptin) study.³⁷

Efficacy and safety

The HannaH study compared the pharmacokinetics, efficacy, and safety of subcutaneous trastuzumab versus intravenous trastuzumab in patients with locally advanced, inflammatory, or early HER2-positive breast cancer treated in the neoadjuvant setting (Table 1). A total of 596 patients were randomly assigned to receive either a fixed 600 mg dose of subcutaneous trastuzumab plus chemotherapy for eight cycles

Table 1 Clinical trials of subcutaneous trastuzumab in patients with HER2+ early stage breast cancer

| Trial name (clinicaltrials.gov identifier) | Study design | Treatment | n | Primary outcome | Other outcomes |
|--|---|--|---|--|---|
| NCT00800436 | Phase I Open-label, two-part, dose-finding and dose confirmation study | Part 1: dose-finding IV: 6 mg/kg SC: 6 mg/kg; 10 mg/kg; 8 mg/kg Part 2: dose confirmation SC: 8 mg/kg; 12 mg/kg | 66 including 24 healthy male volunteers | 8 mg/kg SC resulted in exposure comparable with that obtained with 6 mg/kg IV | Subjects with at least 1 AE IV: 11/12 (92%) SC: 55/58 (95%) No SAE |
| HannaH (NCT00950300) | Phase III Noninferiority, randomized, open-label, study | 600 mg SC + chemotherapy 6 mg/kg IV + chemotherapy | 596 | SC was noninferior to IV in terms of C_{trough} and pCR | Patients with at least 1 AE IV: 280/298 (94%) SC: 289/297 (97%) Patients with at least 1 SAE IV: 37/298 (12%) SC: 62/297 (21%) |
| PrefHer (NCT01401166) Ongoing trial | Phase II Randomized, crossover study with two cohorts | Cohort 1 with crossover 600 mg SC using a vial 6 mg/kg IV Cohort 2 with crossover 600 mg SC using a ready to use injection device 6 mg/kg IV | 400 | Patients' preference | Health care professional satisfaction with SC |
| SafeHer (NCT01566721) Ongoing trial | Phase III Two-cohort open label study | SC by assisted administration SC by self-administered by single-use injection device | 2500 | Incidence of adverse events | DFS Patient satisfaction with SC Single use injection device |

Abbreviations: SC, subcutaneous trastuzumab; IV, intravenous trastuzumab; C_{trough} , trastuzumab serum trough concentration; pCR, pathologic complete response; AE, adverse event; SAE, serious adverse event; DFS, disease-free survival.

before surgery and subcutaneous trastuzumab alone for 10 cycles after surgery (subcutaneous arm), or an initial 8 mg/kg body weight intravenous loading dose of trastuzumab followed by a 6 mg/kg maintenance dose, both in combination with chemotherapy for a total of eight cycles before surgery, as per the standard intravenous regimen, and intravenous trastuzumab alone for 10 cycles after surgery (intravenous arm). In both arms, the neoadjuvant chemotherapy consisted of four cycles of docetaxel followed by four cycles of fluorouracil, epirubicin, and cyclophosphamide. The total treatment duration was one year in both arms. Coprimary endpoints were the serum trough trastuzumab concentration (C_{trough}) and pathologic complete response. The trastuzumab serum C_{trough} is the lowest serum concentration measured before surgery (predose cycle 8, by which time a steady-state drug concentration should be reached).

Noninferiority of the subcutaneous formulation compared with the intravenous formulation was demonstrated for both pharmacokinetic and efficacy endpoints. Indeed, the mean observed C_{trough} was 69.0 $\mu\text{g/mL}$ in the subcutaneous arm and 51.8 $\mu\text{g/mL}$ in the intravenous arm with a ratio

(mean subcutaneous C_{trough} /mean intravenous C_{trough}) of 1.33 (90% confidence interval [CI] 1.24–1.44). The lower bound of the 90% CI was superior to the prespecified noninferiority margin, indicating that the two methods of administration lead to comparable trastuzumab serum trough concentrations. The pathologic complete response rate in patients who received the subcutaneous formulation was 45.4%, while the rate was 40.7% in patients receiving the intravenous formulation. The difference in pathologic complete response rate between the two arms was 4.7% (95% CI –4–13.4), with a lower bound of the 95% CI that was superior to the prespecified noninferiority margin. These results indicate that subcutaneous trastuzumab was noninferior to intravenous trastuzumab with regard to pharmacokinetic profile and pathologic complete response rate. Similar results were found in the two arms for overall response rate (87.2% and 88.8%, respectively) and for median time to response (6 weeks in each arm). Estrogen receptor status was the only factor that showed a major impact on pathologic complete response in both arms, with estrogen receptor-negative patients experiencing better outcomes. Predose body weight and

trough levels at cycle 8 did not affect pathologic complete response rates.

Regarding the safety profile of the subcutaneous trastuzumab formulation, the proportion of patients who reported at least one adverse event was similar in the subcutaneous arm and the intravenous arm (97% and 94%, respectively, Table 1). The incidence of severe adverse events was also comparable between the two arms (52% [155 of 298] in the intravenous arm and 51.9% [154 of 297] in the subcutaneous arm). The most common severe adverse events were neutropenia (33.2% in the intravenous arm versus 29% in the subcutaneous arm), leucopenia (5.7% in the intravenous arm versus 4% in the subcutaneous arm) and febrile neutropenia (3.4% in the intravenous arm versus 5.7% in the subcutaneous arm). More patients had serious adverse events in the subcutaneous arm (21%) than in the intravenous arm (12%). This difference may be due to more infections at the injection site in the subcutaneous arm than in the intravenous arm (8.1% versus 4.4%, respectively).

Subcutaneous trastuzumab had a similar cardiac safety profile to that of intravenous trastuzumab. Indeed, 2.1% of patients in the intravenous arm and 2.4% of patients in the subcutaneous arm experienced a decrease in left ventricular ejection fraction. Altogether, the data show that the safety profiles of both formulations were comparable and consistent with the known safety profile of trastuzumab. Overall, the results of the HannaH study confirm that 600 mg of subcutaneous trastuzumab administered every 3 weeks is a valid treatment alternative to the 3-weekly intravenous regimen. This alternative treatment route may improve patient quality of life because the subcutaneous route is less invasive and reduces both administration time (5 minutes with the subcutaneous route versus 30–90 minutes with the intravenous route) and time spent in clinic or hospital.

Ongoing studies of subcutaneous trastuzumab

Two clinical trials, ie, the PrefHer and SafeHer (Table 1) are currently ongoing to evaluate further the benefit associated with a subcutaneous formulation of trastuzumab.

The PrefHer study [ClinicalTrials.gov, NCT01401166] is a randomized, open-label, crossover study evaluating patient preference and health care professional satisfaction with subcutaneous versus intravenous administration of trastuzumab in patients with HER2-positive early breast cancer. This study includes two cohorts (200 patients per cohort) assessing two methods of subcutaneous trastuzumab administration: in one cohort, subcutaneous trastuzumab administration is

performed using a vial, while in the other cohort subcutaneous administration is performed using an innovative ready-to-use injection device. In each of the cohorts, patients are randomized to receive either 600 mg of subcutaneous trastuzumab or 6 mg/kg of intravenous trastuzumab every 3 weeks for the first four cycles, and then cross over to the other treatment modality. The primary endpoint is the proportion of patients indicating an overall preference for either the subcutaneous or intravenous route of administration. Patient preference is assessed using pretreatment and post-treatment questionnaires.

The SafeHer study [ClinicalTrials.gov, NCT01566721] is a multicenter, two-cohort, nonrandomized, open-label study evaluating the safety and tolerability of two methods of administering subcutaneous trastuzumab as adjuvant therapy in patients with early HER2-positive breast cancer. Patients will receive 600 mg of subcutaneous trastuzumab every 3 weeks either by assisted administration using a conventional syringe and needle (vial formulation) or with assisted administration and self-administration using a single-use injection device (ready-to-use injection device).

Conclusion

Trastuzumab combined with rHuPH20 that opens up channels in the extracellular matrix of the skin enables trastuzumab to be administered via the subcutaneous route. The efficacy and safety of subcutaneous trastuzumab is comparable with that observed for intravenous trastuzumab, indicating that subcutaneous trastuzumab is a valid treatment alternative to intravenous administration. This alternative administration route may be considered as a more convenient and easier way to use trastuzumab, offering patients greater comfort and improving their quality of life.

Disclosure

The authors report no conflicts of interest in this work.

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