analysis of protein aggregation is an increasing challenge. Aggregation is potentially encountered during various steps of the manufacturing process, which include fermentation, purification, formulation and during storage. Different factors, such as temperature, protein concentration, agitation stress, freezing and thawing, solvent and surfactant effects, and chemical modifications, might influence the aggregation behavior of a therapeutic protein. During development of a highly concentrated antibody formulation the aggregation tendency of the protein has to be monitored and controlled by the addition of various excipients and surfactants [Kiese S. et al., J. Pharm. Sci., 2008; 97(10); 4347-4366]. The challenge to prepare suitable highly concentrated, stable pharmaceutical formulation of the pharmaceutically active anti-HER2 antibody in accordance with the present invention is increased by the fact that two different proteins have to be formulated in one liquid formulation in such a way that the formulation remains stable over several weeks and the pharmaceutically active ingredients remain active during proper storage.

T~~In a first aspect t~~he present ~~invention~~disclosure provides a highly concentrated, stable pharmaceutical formulation of a pharmaceutically active anti-HER2 antibody or a mixture of such antibody molecules for subcutaneous injection, which are ready for use.

More particularly the highly concentrated, stable pharmaceutical formulation of a pharmaceutically active anti-HER2 antibody formulation of the present ~~invention~~disclosure comprises:

- about 50 to 350 mg/ml anti-HER2 antibody;
- about 1 to 100 mM of a buffering agent providing a pH of 5.5 ± 2.0;
- about 1 to 500 mM of a stabilizer or a mixture of two or more stabilizers, whereby optionally methionine is present as a secondary stabilizer e.g. in a concentration of 5 to 25 mM;
- 0.01 to 0.1 % of a nonionic surfactant; and
- an effective amount of at least one hyaluronidase enzyme.

In a first aspect, the present disclosure provides a highly concentrated, stable pharmaceutical liquid formulation of a pharmaceutically active anti-HER2 antibody for subcutaneous injection essentially consisting of:

a. 120 ± 18 mg/ml anti-HER2 antibody;

b. 1 to 50 mM of a histidine buffer providing a pH of 5.5 ± 0.6;

c. 15 to 250 mM of α, α-trehalose dihydrate or sucrose and 5 to 25 mM of methionine; and

d. 0.01 to 0.08 % of a nonionic surfactant, wherein said anti-HER2 antibody is Trastuzumab.

T~~In a further aspect t~~he present ~~invention~~disclosure provides for use of a formulation for the preparation of a medicament useful for treating a disease or disorder amenable to treatment with an anti-HER2 antibody such as e.g. cancer or a non-malignant disease in a subject comprising administering the formulation described herein to a subject in an amount effective to treat the said disease or disorder. In another aspect, the present disclosure provides the use of a highly concentrated, stable pharmaceutical anti-HER2 antibody formulation for subcutaneous injection as defined herein for the preparation of a medicament useful for treating a disease or disorder amenable to treatment with an anti-HER2 antibody, optionally cancer or a non-malignant disease in a subject. The anti-HER2 antibody can be co-administered concomitantly or sequentially with a chemotherapeutic agent.

TIn another aspect the present inventiondisclosure provides there are provided methods of treating a disease or disorder which is amenable to treatment with an anti-HER2 antibody (e.g. cancer or a non-malignant disease) in a subject comprising administering the formulation described herein to a subject in an amount effective to treat the said disease or disorder. The cancer or a non-malignant disease will generally involve HER2-expressing cells, such that the HER2 antibody in the therapeutic pharmaceutical SC formulation in accordance with the present inventiondisclosure is able to bind to the affected cells.

The present inventiondisclosure also provides pharmaceutical compositions consisting of a highly concentrated, stable pharmaceutical formulation of a pharmaceutically active anti-HER2 antibody or a mixture of such antibody and a suitable amount of at least one hyaluronidase enzyme in the form of a kit comprising both injection components and suitable instructions for their subcutaneous administration.

In another aspect, the present disclosure provides a pharmaceutical formulation comprising the highly concentrated, stable pharmaceutical anti-HER2 antibody formulation for subcutaneous injection as defined herein for reconstitution with 150 to 16'000 U/ml, 2'000 U/ml or 12'000 U/ml of a hyaluronidase enzyme.

In another aspect, the present disclosure provides a pharmaceutical formulation comprising the highly concentrated, stable pharmaceutical anti-HER2 antibody formulation for subcutaneous injection as defined herein for reconstitution with 150 to about 16'000 U/ml, 2000 U/ml or 12000 U/ml of a rHuPH20.

TA further aspect of the present inventiondisclosure relates to injection devices comprising a highly concentrated, stable pharmaceutical formulation in accordance with the present inventiondisclosure. In another aspect, the present disclosure provides an injection device comprising a highly concentrated, stable pharmaceutical anti-HER2 antibody formulation as defined herein. Such formulation may consist of a pharmaceutically active anti-HER2 antibody or a mixture of such antibody molecules and suitable excipients as outlined below and may additionally comprise a soluble hyaluronidase glycoprotein either as a combined formulation or as a separate formulation for co-administration.

The highly concentrated, stable pharmaceutical formulation of a pharmaceutically active anti-HER2 antibody formulation of the present inventiondisclosure may be provided in liquid form or may be provided in lyophilized form. In accordance with the teachings in WO 97/04801 the antibody concentration in the reconstituted formulation can be increased by reconstitution of a lyophilized formulation to provide a protein concentration in the reconstituted formulation which is about 2-40 times greater than the protein concentration in the mixture before the lyophilization step.

The anti-HER2 antibody concentration is 100 to 150 mg/ml, e.g. 120 ± 18 mg/ml, about 110 mg/ml, about 120 mg/ml or about 130 mg/ml.

The concentration of the buffering agent providing a pH of 5.5 ± 2.0 is 1 to 50 mM, e.g. 10 to 30 mM or about 20 mM. Various buffering agents are known to the person skilled in the art as outlined further below. The buffering agent can be a histidine buffer, e.g. L-histidine/HCl. In a particular embodiment the pH of the L-histidine/HCl buffer is about 5.5 or about 6.0.

In another aspect, the present disclosure provides a kit for subcutaneous injection of a highly concentrated, stable anti-HER2 antibody formulation comprising:

A) A vial containing:

a. 120 +/- 18 mg/ml anti-HER2 antibody;

b. 1 to 50 mM of a histidine buffer providing a pH of 5.5 ± 0.4;

c. 15 to 250 mM of α, α-trehalose dihydrate or sucrose and 5 to 25 mM of methionine;

d. 0.01 to 0.08 % of a nonionic surfactant, wherein said anti-HER2 antibody is Trastuzumab; and

B) A vial containing 150 to 16'000 U/ml, 2000 U/ml or 12'000 U/ml of a hyaluronidase enzyme.

The stabilizer (used synonymously with the term "stabilizing agent" in the present patent description) is e.g. a carbohydrate or saccharide or a sugar admitted by the authorities as a suitable additive or excipient in pharmaceutical formulations, e.g. α,α-trehalose dihydrate or sucrose. The concentration of the stabilizer is 15 to 250 mM, or 150 to 250 mM, or about 210