PHARMACEUTICAL COMPOSITION OF PEMBROLIZUMAB AND USE THEREOF

Field of the invention

The present invention relates to the field of pharmacy and medicine, specifically to pharmaceutical compositions of anti-PD-1 antibody pembrolizumab, which may be used for treating malignant neoplasms or infectious diseases.

Background of the invention

Programmed cell death protein 1 (PD-1) is an inhibitory member of the CD28 receptor family and is located on the cell surface of T-lymphocytes, B-cells, monocytes, NK cells and dendritic cells (Jin H.T., Ahmed R., Okazaki T. Role of PD-1 in regulating T-cell immunity. Curr Top Microbiol Immunol. 2011; 350: 17-37). PD-1 is a transmembrane receptor from the immunoglobulin family and consists of 288 amino acids. The protein structure includes an extracellular IgV domain, a spacer arm, a transmembrane domain and a cytoplasmic domain. The latter includes 2 tyrosine-containing sequences (ITIM and ITSM) involved in signaling in a cell (Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev. 2010;236:219-242. doi:10.1111/j.1600-065X.2010.00923.x).

PD-1 has 2 inhibitory ligands, PD-L1 and PD-L2, which are also transmembrane receptors and play an important role in immune homeostasis. PD-L1 is expressed on T- and B-cells, dendritic cells, macrophages, endothelial, hematopoietic and epithelial cells. In addition, PD-L1 expression has been detected on cells of many types of malignant tumors, such as melanoma, renal cell carcinoma, non-small cell lung cancer, head and neck tumors, gastrointestinal tract tumors, ovarian cancer, lymphomas, leukemias (Han Y., Liu D., Li L. PD-1/PD-L1 pathway: current researches in cancer. Am J Cancer Res. 2020; 10(3): 727-742). PD-L2 has limited expression on activated macrophages and dendritic cells and binds mainly to the PD-1 receptor. The main factor increasing the expression of PD-L1 and PD-L2 is the anti-inflammatory cytokine IFNy.

The PD-1 receptor and the PD-L1 ligand thereof play a significant role in the survival and progression of malignant neoplasms. As noted above, PD-L1 receptor expression is increased on the surface of many types of malignant cells. The PD-L1/PD-1 interaction stimulates the development of immunosuppression in the tumor microenvironment, and thus protects tumor cells from the activity of cytotoxic CD8+ T cells. The PD-1/PD-L1 system is a promising therapeutic target (Wu Y., Chen W., Xu Z.P., Gu W. PD-L1 Distribution and Perspective for Cancer Immunotherapy-Blockade, Knockdown, or Inhibition. Front Immunol. 2019; 10: 2022, Ju X., Zhang H., Zhou Z., Wang Q. Regulation of PD-L1 expression in cancer and clinical implications in immunotherapy. Am J Cancer Res. 2020; 10(1): 1-11).

Known is anti-PD-1 antibody pembrolizumab, which is a humanized monoclonal G4 (IgG4) antibody to human PD-1 receptor (PCT/US2008/007463). It was produced by combining variable

sequences of a murine high-affinity antibody to PD-1 receptor and a human IgG4 kappa framework containing a stabilizing S228P mutation in the Fc fragment. It selectively blocks the binding of the PD-1 receptor to ligands thereof, reactivating antitumor immunity. Activation of the immune response stimulates the elimination of tumor cells.

Pembrolizumab has shown high efficacy in treatment of various types of malignant tumors: melanoma, non-small cell lung cancer, small cell lung cancer, head and neck cancer, classical Hodgkin lymphoma, urothelial carcinoma, stomach cancer, high microsatellite instability malignant neoplasms, hepatocellular carcinoma, esophageal cancer, cervical cancer, Merkel cell carcinoma, renal cell cancer, endometrial cancer, etc. Pembrolizumab is also known to be used for treatment of infectious diseases. Currently, there is an ongoing study of pembrolizumab in the therapy of other diseases or disorders in which inhibition of PD-1 activity may be desirable.

The prior art provides Keytruda, a therapeutic product which includes pembrolizumab, sucrose, polysorbate 80 and a histidine buffer (PCT/US2012/031063). Despite this, there is still a need for novel improved stable pharmaceutical compositions of pembrolizumab.

Brief description of drawings

Figure 1 is a graph of aggregate content determined by SE HPLC (%) versus storage time at a temperature of 25 ± 2 °C for the monoclonal antibody pembrolizumab in the test formulations.

Figure 2 is a graph of monomer content determined by SE HPLC (%) versus storage time at a temperature of 25 ± 2 °C for the monoclonal antibody pembrolizumab in the test formulations.

Figure 3 is a graph of basic fraction content determined by IE HPLC (%) versus storage time at a temperature of 25 ± 2 °C for the monoclonal antibody pembrolizumab in the test formulations.

Figure 4 is a graph of monomer content determined by CE under non-reducing conditions (%) versus storage time at a temperature of 25 ± 2 °C for the monoclonal antibody pembrolizumab in the test formulations.

Figure 5 is a graph of the content of the sum of heavy and light chains determined by CE under reducing conditions (%) versus storage time at a temperature of 25 ± 2 °C for the monoclonal antibody pembrolizumab in the test formulations.

Figure 6 is a graph of relative specific activity (%) versus storage time at a temperature of 25 ± 2 °C for the monoclonal antibody pembrolizumab in the test formulations.

Detailed description of the invention

Definitions

Unless defined otherwise herein, all technical and scientific terms used in connection with the present invention will have the same meaning as is commonly understood by those skilled in the art.

Furthermore, unless otherwise required by context, singular terms shall include plural terms, and the plural terms shall include the singular terms.

As used in the present description and claims that follow, unless otherwise dictated by the context, the words "have", "include," and "comprise" or variations thereof such as "has", "having," "includes", "including", "comprises," or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

The term "pharmaceutical composition" refers to a composition and/or formulation comprising pembrolizumab in a therapeutically effective amount and excipients or auxilliary substances (carriers, diluents, fillers, solvents, etc.), the choice and proportions of which depend on the type and route of administration and dosage.

The term "aqueous composition" as used herein refers to a water-based composition, the water in the composition may be: water, water for injections, physiologic saline (0.9%-1.0% aqueous solution of sodium chloride).

The term "freeze-dried" used herein refers to a formulation that has been subjected to a process known in the art as freeze-drying, which includes freezing the formulation followed by removal of ice from the frozen contents.

The pharmaceutical composition is "stable" if the active agent retains physical stability and/or chemical stability and/or biological activity thereof during the specified shelf life at storage temperature, for example, of 2-8 °C. Further, the active agent may retain both physical and chemical stability, as well as biological activity. Storage period is adjusted based on the results of stability test in accelerated or natural aging conditions.

The term “long-term storage” or “long term stability” is understood to mean that a pharmaceutical composition may be stored for three months or more, for six months or more, for one year or more, and the composition may have a minimum stable shelf life of at least two years as well. Generally speaking, the terms "long term storage" and "long term stability" further include stable storage durations that are at least comparable to or better than the stable shelf life typically required for currently available commercial formulations of the anti-PD-1 antibody pembrolizumab, without losses in stability that would render the formulation unsuitable for its intended pharmaceutical application.

The term "buffering agent" refers to an acid or base component (typically a weak acid or weak base) of the buffer or buffer solution. A buffering agent helps to maintain the pH value of a given solution at or near to a pre-determined value, and the buffering agents are generally chosen to complement the pre-determined value. A buffering agent may be a single compound which gives rise to a desired buffering effect, especially when said buffering agent is mixed with (and suitably capable of proton

exchange with) an appropriate amount (depending on the pre-determined value desired) of corresponding "acid/base conjugate" thereof.

The term “buffer” or “buffer solution” refers to an aqueous solution comprising a mixture of an acid (typically a weak acid, such as e.g. acetic acid, citric acid) and a conjugated base thereof (such as e.g. an acetate or citrate salt, e.g. sodium acetate, sodium citrate, as well as hydrates of said salts, e.g. sodium acetate trihydrate) or alternatively a mixture of a base (typically a weak base, e.g. histidine) and a conjugated acid thereof (e.g. histidine hydrochloride or histidine hydrochloride monohydrate or L-histidine hydrochloride (h/c) monohydrate (m/h) or L-histidine h/c m/h or histidine h/c m/h). The pH value of a “buffer solution” changes only slightly upon addition thereto of a small quantity of strong base or strong acid, as well as upon dilution or concentration due to the “buffering effect” imparted by a “buffering agent”.

In the present application, a "buffer system" comprises one or more buffering agent(s) and/or an acid/base conjugate(s) thereof, and more suitably comprises one or more buffering agent(s) and an acid/base conjugate(s) thereof, and most suitably comprises one buffering agent and an acid/base conjugate thereof. Unless specified otherwise, any concentrations referred herein to a "buffer system" (a buffer concentration) may suitably refer to the combined concentration of buffering agent(s) and/or acid/base conjugate(s) thereof. In other words, concentrations referred herein to a "buffer system" may refer to the combined concentration of all the relevant buffering species (i.e. the species in dynamic equilibrium with one another, e.g. citrate/citric acid). The overall pH of the composition comprising the relevant buffer system is a reflection of the equilibrium concentration of each of the relevant buffering species (i.e. the balance of buffering agent(s) to acid/base conjugate(s) thereof).

Buffer solutions may be, for example, acetate, phosphate, citrate, histidine, succinate and other buffer solutions. Generally, the pharmaceutical composition preferably has a pH in the range from 4.0 to 8.0.

"Stabilizer" refers to an excipient or a mixture of two or more excipients that provide the physical and/or chemical stability of the active agent. Stabilizers may be amino acids, for example, but not limited to, arginine, histidine, glycine, lysine, glutamine, proline; surfactants, for example, but not limited to, polysorbate 20 (trade name: Tween 20), polysorbate 80 (trade name: Tween 80), polyethylene-polypropylene glycol and copolymers thereof (trade names: Poloxamer, Pluronic, sodium dodecyl sulfate (SDS); antioxidants, for example, but not limited to, methionine, acetylcysteine, ascorbic acid, monothioglycerol, sulfurous acid salts, etc.; chelating agents, for example, but not limited to, ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), sodium citrate, etc.

The terms "osmotic agent" or "tonicity-regulating agent", as well as "osmolyte", as used herein, refer to an excipient that can provide the required osmotic pressure of a liquid antibody solution. In some embodiments, the tonicity-regulating agent may increase the osmotic pressure of a liquid antibody liquid antibody formulation to isotonic pressure such that said liquid antibody formulation is physiologically compatible with the cells of the tissue of a subject's organism. In another embodiment, the tonicity-regulating agent may contribute to increased stability of antibodies. "Isotonic" formulation is a formulation that has an osmotic pressure equivalent to that of human blood. Isotonic formulations typically have an osmotic pressure from about 239 to 376 mOsm/kg. A tonicity agent may be in an enantiomeric (e.g. L- or D-enantiomer) or racemic form; in the form of isomers such as alpha or beta, including alpha, alpha; or beta, beta; or alpha, beta; or beta, alpha; in the form of a free acid or free base; in the form of a salt; in a hydrated form (e.g. monohydrate or dihydrate), or in an anhydrous form. Exemplary osmotic agents are, but not limited to, sugars (trehalose, trehalose dihydrate, sucrose, glucose), polyols (mannitol, sorbitol), amino acids (proline or L-proline, arginine, glycine), or salts (sodium chloride, potassium chloride, magnesium chloride).

As used herein, the term "solubilizer" refers to a pharmaceutically acceptable non-ionic surfactant. Both one solubilizer and combinations of solubilizers may be used. Exemplary solubilizers are, without limitation, polysorbate 20 or polysorbate 80, poloxamer 184 or poloxamer 188, or PLURONIC®.

Typically, amino acids are L-amino acids. For example, if histidine and histidine hydrochloride monohydrate are used, it is typically L-histidine and L-histidine hydrochloride monohydrate. For example, if proline is used, it is typically L-proline. Amino acid equivalents, for example, pharmaceutically acceptable proline salts (for example, proline hydrochloride) may also be used.

The term "medicament" or "formulation" is a substance (or a mixture of substances as a pharmaceutical composition) in the form of tablets, capsules, solutions, ointments and other ready forms intended for restoration, improvement or modification of physiological functions in humans and animals, and for treatment and prophylaxis of diseases, for diagnostics, anesthesia, contraception, cosmetology and others.

The term “use” applies to the possibility to use the pharmaceutical composition of pembrolizumab according to the present invention to treat, relief the course of diseases, expedite the remission, reduce the recurrence rate for diseases or disorders.

The term “method of treatment” applies to the possibility to use the pharmaceutical composition of pembrolizumab according to the present invention to treat, relief the course of diseases, expedite the remission, reduce the recurrence rate for the diseases or disorders. "Treat" or "treatment", "prophylaxis" of a disease, disorder or condition may comprise the prevention or delay of the onset of clinical

symptoms of a disease, disorder or condition developing in human, the inhibition of a disease, disorder or condition, i.e. stop, reduction or delay of the development of a disease or a relapse thereof (in case of maintenance therapy) or at least one clinical or subclinical symptom thereof, or the alleviation or easement of a disease, i.e. the causing of regression of a disease, disorder or condition.

The term "parenteral administration" refers to administration regimens, typically performed by injection (infusion), and includes, in particular intravenous, intramuscular, intraarterial, intratracheal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, intraarticular, subcapsular, subarchnoid, intraspinal, epidural and intrasternal injection or infusion.

Abbreviations

IC – incoming control,

FT – freeze-thaw,

k_D – diffusion interaction parameter,

Rh – hydrodynamic radius,

SH – shake,

T – temperature,

T ons – melting onset temperature,

T1 – first melting temperature,

T2 – second melting temperature,

T agr – aggregation temperature,

TS – thermal stress,

TS50 96H – quality parameter following thermal stress at 50°C for 96 hours,

Δ TS50 96H – change in the quality parameter following stressing (thermal stress at 50°C for 96 hours),

TS50 – quality parameter following thermal stress at 50°C for 10 days,

Δ TS50 – change in the quality parameter following stressing (thermal stress at 50°C for 10 days),

Δ abs – absolute change in the charged variant profile determined by method 21,

SH800 96H – quality parameter following stirring (shake) for 96 hours,

Δ SH800 96H – change in the quality parameter following stressing (stirring (shake) for 96 hours),

SH800 – quality parameter following stirring (shake) for 14 days,

Δ SH800 – change in the quality parameter following stressing (stirring (shake) for 14 days),

FT(-20)*5 – quality parameter following 5 freezing cycles,

FT(-20)*3 – quality parameter following 3 freezing cycles,

Δ FT(-20)*5 – change in the quality parameter following stressing (5 freezing cycles),

Δ FT(-20)*3 – change in the quality parameter following stressing (3 freezing cycles),

Acid 3.5 1H – quality parameter following acid hydrolysis to pH 3.5 and aging for 1 hour,

Acid 4.0 – quality parameter following acid hydrolysis to pH 4.0 and aging for 10 days,

Δ Acid 3.5 1H – change in the quality parameter following stressing (acid hydrolysis to pH 3.5 and aging for 1 hour),

Δ Acid 4.0 – change in the quality parameter following stressing (acid hydrolysis to pH 4.0 and aging for 10 days),

Basic 8.5 1H – quality parameter following basic hydrolysis to pH 8.5 and aging for 1 hour,

Basic 8.0 – quality parameter following basic hydrolysis to pH 8.0 and aging for 10 days,

Δ Basic 8.5 1H – change in the quality parameter following stressing (basic hydrolysis to pH 8.5 and aging for 1 hour),

Δ Basic 8.0 – change in the quality parameter following stressing (basic hydrolysis to pH 8.0 and aging for 10 days),

Ox 0.1% – quality parameter following oxidation with hydrogen peroxide for 4 hours,

Δ Ox 0.1% – change in the quality parameter following stressing (oxidation with hydrogen peroxide for 4 hours),

UV – quality parameter following photo stress, dose ICH×1,

Δ UV – change in the quality parameter following stressing (photo stress, dose ICH×1),

n/a – not determined,

UV – UV exposure,

IE HPLC – ion exchange high-performance liquid chromatography,

CE – capillary electrophoresis,

Non-red. – non-reducing conditions,

SW – software,

Red. – reducing conditions

SA – specific activity (potency),

SE HPLC – size-exclusive high-performance liquid chromatography.

The present invention discloses pharmaceutical compositions of anti-PD-1 antibody pembrolizumab, which may be used as a medicinal product for treating malignant neoplasms or infectious diseases.

During formulation selection, we took into account the purpose, route of administration and tolerability of the drug product (for example, reduction of discomfort during administration), as well as the stability and preservation of activity of protein molecule within the formulation.

In one aspect, the present invention relates to a pharmaceutical composition comprising:

(i) pembrolizumab;

(ii) histidine

(iii) histidine hydrochloride monohydrate;

(iv) glycine;

(v) trehalose and poloxamer 188, or

proline; and

(vi) water for injection.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) pembrolizumab;

(ii) histidine

(iii) histidine hydrochloride monohydrate;

(iv) glycine;

(v) trehalose and poloxamer 188; and

(vi) water for injection.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) pembrolizumab;

(ii) histidine

(iii) histidine hydrochloride monohydrate;

(iv) glycine;

(v) proline; and

(vi) water for injection.

The concentration of pembrolizumab contained in the pharmaceutical compositions of the present invention may vary depending on the desired properties of the compositions, as well as on the particular conditions, methods and purposes of use of the pharmaceutical compositions.

In some embodiments of the invention, pembrolizumab is present at a concentration of 5-50 mg/ml.

In some embodiments of the invention, histidine is present at a concentration of 0.087-0.432 mg/ml.

In some embodiments of the invention, histidine hydrochloride monohydrate is present at a concentration of 0.464-0.931 mg/ml.

In some embodiments of the invention, glycine is present at a concentration of 1-2 mg/ml.

In some embodiments of the invention, trehalose is present at a concentration of 70-130 mg/ml.

In some embodiments of the invention, poloxamer 188 is present at a concentration of 0.8-1.2 mg/ml.

In some embodiments of the invention, proline is present at a concentration of 20-34 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) 5-50 mg/ml pembrolizumab;

(ii) 0.087-0.432 mg/ml histidine;

(iii) 0.464-0.931 mg/ml histidine hydrochloride monohydrate;

(iv) 1-2 mg/ml glycine;

(v) 70-130 mg/ml trehalose and 0.8-1.2 mg/ml poloxamer 188, or

20-34 mg/ml of proline; and

(vi) water for injection to 1 ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) 5-50 mg/ml pembrolizumab;

(ii) 0.087-0.432 mg/ml histidine;

(iii) 0.464-0.931 mg/ml histidine hydrochloride monohydrate;

(iv) 1-2 mg/ml glycine;

(v) 70-130 mg/ml trehalose and 0.8-1.2 mg/ml poloxamer 188; and

(vi) water for injection to 1 ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) 5-50 mg/ml pembrolizumab;

(ii) 0.087-0.432 mg/ml histidine;

(iii) 0.464-0.931 mg/ml histidine hydrochloride monohydrate;

(iv) 1-2 mg/ml glycine;

(v) 20-34 mg/ml of proline; and

(vi) water for injection to 1 ml.

In some embodiments of the invention, pembrolizumab is present at a concentration of 15-35 mg/ml, or 20-30 mg/ml, or 25 mg/ml.

In some embodiments of the invention, histidine is present at a concentration of 0.200-0.319 mg/ml, or 0.200-0.250 mg/ml, or 0.210-0.240 mg/ml, or 0.210-230 mg/ml, or 0.215-0.230 mg/ml, or 0.215-0.225 mg/ml, or 0.220-0.225 mg/ml, or 0.221 mg/ml.

In some embodiments of the invention, histidine is L-histidine.

In some embodiments of the invention, histidine hydrochloride monohydrate is present at a concentration of 0.600-0.900 mg/ml or 0.650-0.850 mg/ml, or 0.700-0.800 mg/ml, or 0.730-0.770 mg/ml, or 0.750 mg/ml.

In some embodiments of the invention, histidine hydrochloride monohydrate is L-histidine hydrochloride monohydrate.

In some embodiments of the invention, glycine is present at a concentration of 1.3-1.7 mg/ml, 1.4-1.6 mg/ml, or 1.5 mg/ml.

In some embodiments of the invention, trehalose is present at a concentration of 70-100 mg/ml, or 70-90 mg/ml, or 70-85 mg/ml, or 75-85 mg/ml, or 80 mg/ml.

In some embodiments of the invention, trehalose is trehalose dihydrate.

In some embodiments of the invention, poloxamer 188 is present at a concentration of 0.9-1.1 mg/ml, or 0.95-1.05 mg/ml, or 1.0 mg/ml.

In some embodiments of the invention, proline is present at a concentration of 22-32 mg/ml, or 24-30 mg/ml, or 27 mg/ml.

In some embodiments of the invention, proline is L-proline.

In some embodiments of the invention, the composition has pH 5.1-6.1, 5.2-6.0, 5.3-5.9, 5.4-5.8 or 5.5-5.7.

In some embodiments of the invention, the composition has pH 5.6.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 80 mg/ml trehalose and 1.0 mg/ml poloxamer 188, or

27 mg/ml proline;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 80 mg/ml trehalose and 1.0 mg/ml poloxamer 188;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7.

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(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 80 mg/ml trehalose dihydrate and 1.0 mg/ml poloxamer 188;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 27 mg/ml proline;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7.

In some embodiments of the invention, the pharmaceutical composition has pH 5.6.

In one aspect, the present invention relates to a pharmaceutical composition of pembrolizumab, which is provided in dry (i.e. powder or granular) form for reconstitution in a suitable solvent (e.g. water) prior to administration. Such formulation may be prepared by, for example, lyophilisation, i.e. a process which is known in the art as freeze drying, and which involves freezing a product followed by removal of solvent from frozen material.

In one aspect, the present invention relates to a pharmaceutical composition of pembrolizumab produced by lyophilization of any of the above pharmaceutical compositions of pembrolizumab. Accordingly, the pharmaceutical compositions according to the present invention may be either aqueous pharmaceutical compositions or lyophilized pharmaceutical compositions (lyophilizates).

Lyophilizates are used to produce other dosage forms. For example, a lyophilizate for producing an injectable solution, a lyophilizate for producing a concentrate for producing an injectable solution. Lyophilizates are reconstituted by dissolving same in a suitable solvent, most typically in water for injection. Also, lyophilized compositions are first reconstituted in the required volume of solvent (most typically in water) and then further diluted in a suitable solvent (e.g. 5% glucose solution, 0.9% sodium chloride solution).

In some embodiments of the invention, the pharmaceutical composition of pembrolizumab is produced by lyophilization of the pharmaceutical composition of pembrolizumab according to the present invention.

In some embodiments of the invention, the pharmaceutical composition of pembrolizumab is produced by lyophilization of the pharmaceutical composition of pembrolizumab, comprising:

(i) 5-50 mg/ml pembrolizumab;

(ii) 0.087-0.432 mg/ml histidine;

(iii) 0.464-0.931 mg/ml histidine hydrochloride monohydrate;

(iv) 1-2 mg/ml glycine;

(v) 70-130 mg/ml trehalose and 0.8-1.2 mg/ml poloxamer 188, or

20-34 mg/ml of proline; and

(vi) water for injection to 1 ml.

In some embodiments of the invention, the pharmaceutical composition of pembrolizumab is produced by lyophilization of the pharmaceutical composition of pembrolizumab, comprising:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 80 mg/ml trehalose and 1.0 mg/ml poloxamer 188;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7.

In some embodiments of the invention, the pharmaceutical composition of pembrolizumab is produced by lyophilization of the pharmaceutical composition of pembrolizumab, comprising:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 80 mg/ml trehalose dihydrate and 1.0 mg/ml poloxamer 188;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7.

In some embodiments of the invention, the pharmaceutical composition of pembrolizumab is produced by lyophilization of the pharmaceutical composition of pembrolizumab, comprising:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 27 mg/ml proline;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7.

In some embodiments of the invention, the pharmaceutical composition of pembrolizumab, from which the lyophilized composition is produced, has pH 5.6.

In one aspect, the present invention relates to the use of the above pharmaceutical composition of pembrolizumab for treating a malignant neoplasm or an infectious disease.

In some embodiments, the invention relates to the use of the pharmaceutical composition of pembrolizumab comprising:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 80 mg/ml trehalose and 1.0 mg/ml poloxamer 188;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7,

for treating a malignant neoplasm or an infectious disease.

In some embodiments, the invention relates to the use of the pharmaceutical composition of pembrolizumab comprising:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 80 mg/ml trehalose dihydrate and 1.0 mg/ml poloxamer 188;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7,

for treating a malignant neoplasm or an infectious disease.

In some embodiments, the invention relates to the use of the pharmaceutical composition of pembrolizumab comprising:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 27 mg/ml proline;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7,

for treating a malignant neoplasm or an infectious disease.

In some embodiments of the invention, the pharmaceutical composition of pembrolizumab has pH 5.6.

In some embodiments, the invention relates to the use of the pharmaceutical composition of pembrolizumab produced by lyophilization of the above pharmaceutical composition of pembrolizumab for treating a malignant neoplasm or an infectious disease.

In some embodiments, the invention relates to the use of the pharmaceutical composition of pembrolizumab produced by lyophilization of the pharmaceutical composition of pembrolizumab comprising:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 80 mg/ml trehalose and 1.0 mg/ml poloxamer 188;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7,

for treating a malignant neoplasm or an infectious disease.

In some embodiments, the invention relates to the use of the pharmaceutical composition of pembrolizumab produced by lyophilization of the pharmaceutical composition of pembrolizumab comprising:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 80 mg/ml trehalose dihydrate and 1.0 mg/ml poloxamer 188;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7,

for treating a malignant neoplasm or an infectious disease.

In some embodiments, the invention relates to the use of the pharmaceutical composition of pembrolizumab produced by lyophilization of the pharmaceutical composition of pembrolizumab comprising:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 27 mg/ml proline;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7,

for treating a malignant neoplasm or an infectious disease.

In some embodiments of the invention, the pharmaceutical composition of pembrolizumab, from which the lyophilized composition is produced, has pH 5.6.

In one aspect, the present invention relates to the use of the above pharmaceutical composition of pembrolizumab for producing a medicinal product for treating a malignant neoplasm or an infectious disease.

In some embodiments of the invention, the malignant neoplasm is selected from the group: melanoma, non-small cell lung cancer, small cell lung cancer, head and neck cancer, primary mediastinal large B-cell lymphoma, urothelial cancer, stomach cancer, high microsatellite instability/DNA mismatch repair deficient (MMR) malignant neoplasms, hepatocellular cancer, cervical cancer, Merkel cell carcinoma, renal cell carcinoma, endometrial cancer, esophageal cancer, squamous cell skin cancer, basal cell carcinoma, breast cancer, colorectal cancer, prostate cancer, thyroid cancer, bladder cancer, non-Hodgkin lymphoma, Hodgkin lymphoma, pancreatic cancer, ovarian cancer, gallbladder cancer, malignant brain tumor, glioblastoma, tumor with high mutational burden.

The infectious disease may be caused by a viral, bacterial or fungal infection. In some embodiments of the invention, the infectious disease may be caused, for example, by human immunodeficiency virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, human papillomavirus, Epstein-Barr virus, human cytomegalovirus and herpes virus. Many of said diseases may be chronic diseases.

In some embodiments of the invention, said pharmaceutical composition of pembrolizumab of the present invention is intended for parenteral administration.

In some embodiments of the invention, said pharmaceutical composition of pembrolizumab of the present invention is intended for intramuscular, intravenous, or subcutaneous administration.

In some embodiments of the invention, said pharmaceutical composition of pembrolizumab of the present invention may be administered intravenously as an infusion.

The pharmaceutical composition of pembrolizumab according to the present invention may be used following dilution. To this end, the required volume of the composition is transfered from a vial to an infusion container comprising a sterile 0.9% sodium chloride solution or a sterile 5% dextrose solution. The resulting solution is stirred by gently turning the infusion container over.

The therapeutically effective amount of pharmaceutical composition of pembrolizumab according to the present invention depends on the condition of the subject, the severity of the condition, the previous therapy and the patient's history and response to the therapeutic agent. A suitable dose can be adjusted by the decision of the attending physician so that it can be administered to the patient once or through several injections.

In some embodiments of the invention, the subject of treatment, or patient, is a mammal, preferably a human subject. Said subject may be either male or female, of any age.

Pharmaceutical compositions according to the present invention may be stored in any suitable container. For example, a glass or plastic container, vial, ampoule, syringe, cartridge, or bottle of the desired volume. The containers may be provided with additional means for administration, such as droppers, auto-injectors.

A pharmaceutical composition according to the invention may be manufactured, packaged, or widely sold in the form of a single unit dose or a plurality of single unit doses in the form of a ready formulation. The term "single unit dose" as used herein refers to discrete quantity of a pharmaceutical composition containing a predetermined quantity of an active ingredient. The quantity of the active ingredient typically equals the dose of the active ingredient to be administered in a subject, or a convenient portion of such dose, for example, half or a third of such dose.

The pharmaceutical compositions may be administered as a single therapeutic agent or in combination with additional therapeutic agents as needed. Thus, in one embodiment, the present methods for treatment and/or prophylaxis are used in combination with administration of a therapeutically effective amount of another active agent. The other active agent may be administered before, during or following the administration of the pharmaceutical compositions according to the present invention. The other active agent may be administered as part of the present composition or, alternatively, as a separate formulation.

Examples

Methods

1. Preparation of pembrolizumab samples.

Antibody samples at a concentration of 5-50 mg/ml were prepared in Stirred Cell (Millipore) under pressure. To this end, the initial antibody formulation was placed in a cell, the protein was concentrated under a compressed air stream to a desired concentration under continuous stirring, following which at least 10-fold volume of an aqueous solution with the target formulation comprising buffering agents, osmotic agents and, if necessary, additional water soluble stabilizers was stepwise added to the cell. After antibody diafiltration, we continued concentrating to a concentration exceeding the target one, unloaded it from the cell, and the exact protein concentration was measured by UV spectroscopy. A concentrate of poloxamer 188 and an appropriate solution of excipients were then added to the sample to prepare a solution with protein at the target concentration.

Protein samples at a concentration of 20 mg/ml or greater were prepared in Pellicon cassettes (Millipore) in a tangential flow mode. To this end, the initial antibody formulation was placed in a diafiltration tank, the protein was concentrated to a desired concentration, at least 10-fold volume of the solution with the target formulation comprising buffering agents, and, if necessary, additional water soluble stabilizers was then supplied to the system. Following diafiltration, we continued concentrating to a concentration exceeding the target one, unloaded it from the system, and the exact protein concentration was measured. A concentrate of poloxamer 188 and an appropriate solution of excipients were then added to the sample to prepare a solution with protein at the target concentration.

When obtaining formulations comprising solubilizers, e.g. poloxamer 188, the surfactant concentrates were added to the antibody following diafiltering and concentrating, with the final dilution of the antibody to the target concentration with a solution of excipients.

During aseptic filling into the final container (for example, a sterile glass/plastic vessel, vial or syringe), the antibody solution was filtered using a 0.22 μm sterilizing membrane.

2. Determination of protein concentration in test samples

The protein concentration was measured by UV spectroscopy at a wavelength of 280 nm in UV transparent plates.

Each sample was diluted with the appropriate solution of excipients to a concentration of ~ 0.5 mg/ml. 150 μl of the diluted sample was placed into a well of UV spectrophotometry plate. Optical density of solutions in the plate wells was measured using a plate spectrophotometer at a wavelength of 280 nm. An appropriate solution of excipients was used as a reference solution.

Concentration (mg/ml) of protein (C) was calculated using the following formula:

$$C = \frac{A(280) \cdot b}{\varepsilon \cdot l}$$, where

A_{280} is a value of optical density at a wavelength of 280 nm;

ε is an extinction coefficient of test protein;

b is the total dilution factor for a sample;

l is layer thickness in a plate well; for 150 µl, l = 0.42 cm.

3. Determination of protein aggregation temperature by dynamic light scattering.

The point of aggregation of test proteins (at a concentration of 1 to 5 mg/ml) was determined using DynaPro Plate Reader II. To this end, 35 µl of the solution was placed into a well of a black polymer plate with an optically clear bottom, which was gradually heated in the instrument while constantly measuring scattered light intensity.

Measurement settings:

- Initial measurement temperature – 25 °C.
- Scattered light intensity at θ = 158°.
- Number of measurements per replicate – 3.
- Time per measurement – 5 s.
- Heating rate – 0.15 °C/min.
- Final temperature – 80 °C.

The temperature trend and aggregation point were determined using the Dynamics V7 software.

4. Determination of protein melting point by differential scanning fluorimetry.

Sypro Orange fluorescent stain was added to the protein sample. The sample was analyzed in a CFX96 C1000 Touch Thermal Cycler amplifier in real time mode. Heating was from 25 to 85 °C, the detection channel was ROX. CFX Manager (Bio-Rad) software was used to process the results.

5. Determination of hydrodynamic radius of particles in solution by dynamic light scattering.

For analysis, 35 µl of sample at each concentration was placed into wells of a black polymer plate with an optically clear bottom. The analysis was performed using the DynaPro Plate Reader II instrument. Each well was analyzed 10 times. The resulting data was processed in the Dynamics V7 software.

6. Determination of diffusion interaction parameter (k_D) by dynamic light scattering.

A number of protein solutions from 30 mg/ml to 0.94 mg/ml were produced by stepwise dilution. Appropriate solutions of excipients were used as a solvent.

For analysis, 35 µl of sample at each concentration was placed into wells of a black polymer plate with an optically clear bottom. The analysis was performed using the DynaPro Plate Reader II instrument. Each well was analyzed 10 times. The resulting data was processed in the Dynamics V7 software, where dependence of the diffusion coefficient on protein concentration in solution was plotted and the inclination of line for the resulting dependence was determined.

7. Determination of thermal stability under 50°C thermal stress (TS50).

Test samples were divided into 2 aliquots of 150 μl each and placed into separate glass vials: 1 vial per composition was stored in a refrigerator at 5 ± 3 °C, the rest vials were placed in a thermostat and incubated at 50 °C for 96 hours. When selecting control points or following heating, the vials were removed from the thermostat, kept at room temperature for about 15 minutes, and transferred for analysis.

8. Determination of colloidal stability under shaking (SH800).

Test samples were divided into 2 aliquots of 150 μl each and placed into glass vials, 1 vial per formulation was stored in a refrigerator at 5 ± 3 °C, the rest vials were placed into a thermal shaker and shaken at 800 rpm at 5 ± 3 °C for 96 hours. During the selection of control points or following stress, the vials were removed from the thermal shaker and transferred for analysis.

9. Determination of colloidal stability under freeze-thaw (FT(-20)).

Test samples were divided into 2 aliquots and placed into plastic vials: 1 vial per formulation was stored in a refrigerator at 5 ± 3 °C, the rest vials were stored in a freezer at not higher than –18 °C until completely frozen. Thereafter, the vials were removed from the freezer, kept at room temperature until the contents were completely thawed; the solutions were mixed using a vortex and placed back into the freezer. This was repeated at least 3 times. Following stress, the vials were removed from the freezer, kept at room temperature until the contents were completely thawed; the solutions were mixed using a vortex and transferred for analysis.

10. Determination of stability under acid hydrolysis (Acid).

Test samples were divided into 2 aliquots and placed into polymer vials: 1 vial per formulation was stored in a refrigerator at 5 ± 3 °C (input control may be transferred for analysis once for all studies at the start of storage), pH for the rest vials was adjusted to 3.5 ± 0.1 with hydrochloric acid solution while stirring, thereafter, they were transferred to a refrigerator for storage at 5 ± 3 °C. After 1 hour, hydrolysis was quenched while stirring by adding sodium hydroxide solution to the initial pH value. The solutions were then transferred for analysis.

11. Determination of stability under basic hydrolysis (Basic).

Test samples were divided into 2 aliquots and placed into polymer vials: 1 vial per formulation was stored in a refrigerator at 5 ± 3 °C (input control may be transferred for analysis once for all studies at the start of storage), pH for the rest vials was adjusted to 8.5 ± 0.1 with sodium hydroxide solution while stirring, thereafter, they were transferred to a refrigerator for storage at 5 ± 3 °C. After 1 hour, hydrolysis was quenched while stirring by adding hydrochloric acid solution to the initial pH value. The solutions were then transferred for analysis.

12. Accelerated aging.

The test samples were divided into separate aliquots (one aliquot for the input control – it is allowed to transfer for analysis once for all studies at the start of storage) and placed in separate sterile glass vials: part of the vials for each formulation was placed in the refrigerator for storage at 5 ± 3 °C (input control), the rest vials were placed in a thermostat and incubated at 25 ± 2 °C for 6 months, periodically selecting control points according to the plan. When selecting control points and following storage, the vials were removed from the thermostat and transferred for analysis.

13. Determination of sample purity by size-exclusion high-performance liquid chromatography (SE HPLC).

Column: Tosoh TSK-GelG3000SWXL 7.8 mm ID × 30 cm, 5 μm.

Pre-column: TSK-Gel Guard SW_{XL}, 6.0 mm ID × 4.0 cm, 7 μm, 300Å.

Column temperature: 25°C.

Mobile phase flow rate: 0.5 ml/min.

Injection volume: 25 μl.

Sample concentration: 0.5 mg/ml.

Detector wavelength: 214 and 360 nm.

Elution time: 30 min.

Mobile phase: Disodium hydrogen phosphate anhydrous 14.2 mg/ml.
Sodium chloride 11.7 mg/ml.

The mobile phase pH was adjusted to 6.9 with orthophosphoric acid.

14. Evaluation of charged variant profile in capillary on the Caliper LabChip GX II instrument.

Analysis was conducted in accordance with the instructions for the HT Protein charge variant kit. Test samples were adjusted to a protein concentration of 1 mg/ml by diluting or concentrating in 0.5 ml Amicon Ultra 10 kDa centrifuge filters (Millipore) (depending on the initial concentration of samples). Protein content was followed by UV spectrophotometry at a wavelength of 280 nm.

2 μl of carboxypeptidase solution was added to each resulting sample, and the samples were incubated for 2 hours at a temperature of 37 ± 2 °C. After the specified time, the samples were dialyzed against water in Amicon Ultra centrifuge tubes and concentrated to 2 mg/ml.

A 96-well plate was loaded with amounts, as specified in the instructions, of Labelling Buffer solution, Dye Mixture solution and 25 μl of the test sample, the plate was placed in a dark place for 10 minutes, each well was then loaded with 60 μl of water and mixed.

The plate with solutions was centrifuged using a plate centrifuge rotor and placed in the Caliper LabChip GX II instrument. The analysis used a special chip which was filled with Running Buffer solution with pH in accordance with the instructions. The results were processed using LabChip GX software.

15. Determination of charge variant profile by ion exchange high performance liquid chromatography (IE HPLC).

Column: ProPac WCX-10, 4×250 mm, particle size: 10 μm (Thermo Scientific, USA).

Pre-column: ProPac WCX-10G, 4×50 mm, particle size: 10 μm (Thermo Scientific, USA).

Eluent A: Solution of 20 mM 2-(N-morpholino)-ethanesulfonic acid, 4% acetonitrile, pH = 7.0.

Eluent B: Solution of 20 mM sodium phosphate buffer, 95 mM NaCl, 4% acetonitrile, pH = 8.0.

Flow rate: 0.6 ml/min - 1.0 ml/min.

Column temperature: 45 °C.

Autosampler temperature: 5 °C.

Detector: UV, 280 nm.

Reference wavelength: 360 nm, 100 nm bandwidth

Sample volume: 80 μl.

Chromatography time: 43 min.

The test sample was diluted to a concentration of 1.0 mg/ml and treated with carboxypeptidase B in a ratio of 100:1 for 20 minutes at a temperature of 37 ± 2 °C.

Elution mode: Eluent A from 100% to 80%, eluent B from 0% to 20%.

16. Determination of purity and related impurities by capillary polyacrylamide gel electrophoresis in the presence of/without sodium dodecyl sulfate (CE red. and non-red.).

The sample was diluted to a concentration of 4.0 mg/ml. 23 μl of the resulting solution was placed in a 1.5 ml microtube; 70 μl of SDS-MW Sample Buffer, 2 μl of internal standard having a molecular weight of 10 kDa, 5 μl of 0.5M iodoacetamide solution (CE non-red.) or 5 μl of 2-mercaptoethanol (CE red.) were added thereto. The resulting solution was stirred for 15 seconds, centrifuged for 5 seconds at a speed of 2800 rpm and placed in a solid-state thermostat at 70°C for 30 minutes. The solution was cooled to room temperature.

The SDS MW Separation – PA 800 plus.met analysis method was used in 32Karat Software.

Conditions of capillary gel electrophoresis:

Capillary: 50 μm × 30.2 cm

Effective length of capillary: 20.0 cm

Polarity: reverse, inlet on left side (-), outlet on right side (+)

Capillary temperature: 25°C

Analysis time and separation voltage: 35 min, 15 kV

Detection wavelength: 220 nm.

17. Determination of relative specific activity.

Specific activity was determined using a biological test for ability to block PD-L1-dependent inhibition of activation of T-cell-based Jurkat-PD1-NFAT reporter cell line. Samples were processed using the robotic platform Liquid Handling Arm (LiHa); RPMI-1640 with 25 mM HEPES, with 24 mM sodium bicarbonate, containing 2 mM L-glutamine, 10% FBS, 50 μg/ml gentamicin was used as an assay medium (medium for quantitative determination).

The test sample of antibody was diluted using the assay medium (medium for quantitative determination) to a concentration of 1 mg/ml and placed into the robotic platform. The robotic platform Liquid Handling Arm (LiHa) was used to prepare three independent dilutions of the standard and test sample at concentrations of 1 000, 50, 10, 1, 0.5, 0.25, 0.1, 0.025, 0.01, 0.001 μg/ml using the assay medium. We transferred the dilutions and assay medium to culture plates, added Raji-PDL1 cl.3 cell suspension stably expressing PDL-1 at a concentration of (1.00 ± 0.1)×106 cells/ml, Jurkat-NFAT-PD1 cl.1 reporter cell line suspension at a concentration of (1.67 ± 0.1)×106 cells/ml and bispecific antibodies against CD3/CD20. Culture plates were placed in a CO2 incubator, incubated at a temperature of (37 ± 1) °C in humidified air with a carbon dioxide content of 5 % for 22-24 hours.

After the incubation period, the culture plates were kept at room temperature for at least 15 minutes, and BioGlo luciferase substrate was added. The luminescence level was measured in relative luminescence units (RLU) using a microplate reader and Magellan 7.2 software. Using the results of luminescence measurement, we built, for each plate, four-parameter curves optimized using the Levenberg-Marquardt algorithm for standard and test samples, determined the relative specific activity of the test sample relative to the standard sample.

18. Determination of oxidation products by high-performance liquid chromatography of hydrophobic interactions (hydrophobic interaction HPLC).

Column: TSKgel Phenyl-5PW 7.5 × 75 mm, particle size: 10 μm (Tosoh Booscience, Japan).

Eluent A: Solution of 5 mM sodium phosphate buffer, 2% acetonitrile, pH = 7.0.

Eluent B: Solution of 5 mM sodium phosphate buffer, 400 mM ammonium sulfate, 4% acetonitrile, pH = 6.9.

Flow rate: 0.5 ml/min.

Column temperature: 30 °C.

Autosampler temperature: 5 °C.

Detector: UV, 280 nm, bandwidth: 16 nm.

Reference wavelength: 360 nm, 100 nm bandwidth

Sample volume: 25 μl.

Chromatography time: 82 min.

The test sample was diluted to a concentration of 3.0 mg/ml and treated with carboxypeptidase B in a ratio of 100:1 for 20 minutes at a temperature of 37 ± 2 °C.

Elution mode: Eluent A from 0% to 100%, eluent B from 100% to 0%.

19. Determination of stability under oxidation.

Test samples were divided into 2 aliquots of 150 µl each and placed into separate glass vials: 1 vial per formulation was stored in a refrigerator at 5 ± 3 °C, hydrogen peroxide was added to the rest of the samples to final concentration of hydrogen peroxide of 0.1% in samples, the samples were aged for 4 hours at (5 ± 3) °C. Oxidation was quenched by adding an equivalent amount of L-methionine.

20. Determination of photostability.

The test samples were divided into two aliquots and placed in individual glass vials. As dark controls, we used the product in the secondary package, tightly wrapped with aluminum foil. All samples were placed in a climate chamber with a light source, and a photo stress program was launched at 1.2 million lux•h and 200 W•h/m2 (dose ICH×1). Upon reaching the desired stress level, all samples were removed from the chamber and transferred for analysis.

21. Processing of results.

The absolute change in quality indicators when under stresses was calculated by the formula:

Δ = (значение после стресса − значение до стресса)

Absolute change in the charge variant profile was calculated by the formula (abs):

= |acidic fraction content before stress - acidic fraction content following stress|
+ |basic fraction content before stress
- basic fraction content following stress|
+ |dominating fraction content before stress
- dominating fraction content following stress|

Example 1. Selection of buffer system.

In this study, 2 typical buffer systems, acetate and histidine buffer systems, that are suitable for parenteral administration were selected as the basis of the pharmaceutical composition. Study was conducted in full two-factor experiment design with two levels and a center point. The pH level (from 5.0 to 6.5) and the concentration of buffer agents (from 5 to 50 mM) were studied as quantitative factors.

To assess the suitability of buffer systems, we studied the effect of buffer solution's nature on colloidal and conformational stabilities of protein. The aggregation temperature, melting point, diffusion interaction parameter, changes in purity and acid-base profile following thermal stress were determined as a response. The test pharmaceutical compositions are shown in Table 1.

Table 1 – Test formulations

Designation	Formulation	
5 mM Acet pH 6.0	Pembrolizumab Sodium acetate trihydrate Acetic acid glacial	1.5-5 mg/ml 0.644 mg/ml to pH 6.0
50 mM Acet pH 6.0	Pembrolizumab Sodium acetate trihydrate Acetic acid glacial	1.5-5 mg/ml 6.442 mg/ml to pH 6.0
50 mM Acet pH 5.0	Pembrolizumab Sodium acetate trihydrate Acetic acid glacial	1.5-5 mg/ml 0.436 mg/ml to pH 5.0
5 mM Acet pH 5.0	Pembrolizumab Sodium acetate trihydrate Acetic acid glacial	1.5-5 mg/ml 4.355 mg/ml to pH 5.0
27.5 mM Acet pH 5.5	Pembrolizumab Sodium acetate trihydrate Acetic acid glacial	1.5-5 mg/ml 3.177 mg/ml to pH 5.5
5 mM His pH 6.5	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate	1.5-5 mg/ml 0.589 mg/ml 0.252 mg/ml
50 mM His pH 6.5	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate	1.5-5 mg/ml 5.894 mg/ml 2.518 mg/ml
50 mM His pH 5.5	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate	1.5-5 mg/ml 1.864 mg/ml 7.963 mg/ml
5 mM His pH 5.5	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate	1.5-5 mg/ml 0.186 mg/ml 0.796 mg/ml
27.5 mM His pH 6.0	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate	1.5-5 mg/ml 2.133 mg/ml 2.882 mg/ml
Keytruda buf. sol.	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate	1.5-5 mg/ml 0.300 mg/ml 1.700 mg/ml

Study of stability prediction indicators.

The diffusion interaction parameter (k_D) reflects sample's diffusion coefficient as a function of concentration of molecules. If the diffusion coefficient is decreased with increasing concentration ($k_D < 0$), then polydispersity of the given solution is increased and larger particles are formed therein. Such samples have low solubility and tend to aggregate, and formulations thereof are not recommended for use.

The aggregation temperature and melting point make it possible to assess the protein's tendency to aggregation. The most stable samples are those in which particle aggregation begins at a higher temperature and where smaller particles are formed under heating.

Results of the study of aggregation temperature by method 3, melting point by method 4, and diffusion interaction parameter by method 6 are shown in Table 2. The best results have a lighter shade of color.

Table 2 - Results of the study of stability prediction indicators

Formulation designation	Melting point, °C			T agr, °C	k_D
	T ons	T1	T2		
5 mM Acet pH 6.0	54.1	64.3	67.3	62.09	-1.35E-02
50 mM Acet pH 6.0	53.8	64.6	none	58.8	6.44E-03
50 mM Acet pH 5.0	45.7	58.3	65.5	59.8	6.60E-03
5 mM Acet pH 5.0	47.5	60.7	67.3	64.12	3.09E-02
27.5 mM Acet pH 5.5	51.4	62.8	66.1	61.59	-6.64E-03
27.5 mM Acet pH 5.5	52	63.1	65.8	61.55	-6.83E-03
5 mM His pH 6.5	55.6	64.6	none	61.65	1.35E-02
50 mM His pH 6.5	54.1	64.3	none	61.02	-1.24E-02
50 mM His pH 5.5	47.8	57.4	69.4	58.09	-6.86E-03
5 mM His pH 5.5	49	59.5	67.0	63.24	2.19E-02
27.5 mM His pH 6.0	52.9	62.8	66.4	61.35	-8.09E-03
27.5 mM His pH 6.0	52.6	62.8	66.1	61.38	-8.94E-03
Keytruda buf. sol.	48.1	59.5	67.0	63.7	8.06E-03

Determination of thermal stability.

Thermal stability was assessed by method 7. Before and following thermal stress, we determined: purity by SE HPLC by method 13, charge variant profile in capillary by method 14, hydrodynamic radius by method 5. The results are shown in Table 3. The best results have a lighter shade of color.

Table 3 - Results of determination of thermal stability

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Charge variant profile, %			
			Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	TS50 96H	IC	Δ TS50 96H	IC	Δ TS50 96H	IC			TS50 96H
5 mM Acet pH 6.0	5.6	5.9	0.55	0.93	99.42	-1.58	3.65	81.80	14.56	14.05
50 mM Acet pH 6.0	5.2	5.6	0.54	0.92	99.42	-1.54	3.69	81.51	14.79	15.89
50 mM Acet pH 5.0	5.4	5.9	0.52	5.07	99.43	-5.85	3.96	80.00	16.04	23.94
5 mM Acet pH 5.0	4.2	4.2	0.49	0.76	99.44	-1.53	3.83	78.38	17.80	7.17

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Charge variant profile, %			
			Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	TS50 96H	IC	Δ TS50 96H	IC	Δ TS50 96H	IC			TS50 96H
27.5 mM Acet pH 5.5	6	5.8	0.53	1.36	99.41	-2.01	3.91	78.49	17.61	8.27
27.5 mM Acet pH 5.5	5.6	5.8	0.54	1.46	99.40	-2.11	3.89	77.45	18.66	10.60
5 mM His pH 6.5	5.8	4.9	0.55	0.55	99.32	-1.25	3.56	78.51	17.93	18.22
50 mM His pH 6.5	5.7	5.9	0.49	0.60	99.46	-1.45	3.75	78.44	17.81	20.45
50 mM His pH 5.5	5.6	6.3	0.47	4.70	99.44	-5.75	3.70	78.08	18.22	11.32
5 mM His pH 5.5	4.7	4.6	0.47	0.95	99.48	-1.74	3.86	78.11	18.03	5.74
27.5 mM His pH 6.0	5.7	5.7	0.46	0.78	99.50	-1.65	3.53	79.06	17.42	13.77
27.5 mM His pH 6.0	5.6	5.7	0.46	0.80	99.48	-1.68	3.87	78.68	17.45	9.17
Keytruda buf. sol.	6.3	10.8	0.46	0.87	99.52	-1.81	3.68	81.43	14.89	11.32

The resulting data was processed in Minitab software, and models were built to select the formulation that has the most stabilizing effect. Only those responses (quality indicators) that were subject to significant effect by factors (pH and molality of the buffer solution) were used for building of the model. For other responses not considered in the optimization model, it was assumed that there was no statistically significant difference in the studied range of factors. Based on the optimization results, 5 mM histidine buffer solution having pH 5.6 was selected, which is prepared with the following:

L-histidine 0.221 mg/ml

L-histidine hydrochloride monohydrate 0.750 mg/ml

When preparing a solution with specific contents of L-histidine and L-histidine hydrochloride monohydrate, pH may deviate slightly from the desired value and vary in the pH range of 5.5 – 5.7 (pH 5.6 ± 0.1).

This formulation showed the best stabilizing properties among all the test samples. According to the results of the model, a minimal change in the content of the monomer (protein) and aggregates is expected in the histidine buffer solution under thermal exposure, and high values of the aggregation temperature and diffusion interaction parameter are also expected. The optimal composition, as compared to the Keytruda buffer solution, exhibits a smaller change in the monomer content under thermal exposure, a smaller change in the aggregate content and an increased diffusion interaction parameter, which indicates greater colloidal stability.

Example 2. Selection of osmotic agent.

Test formulations.

Excipients suitable for parenteral administration were studied to be used as osmotic agents. The test formulations are shown in Table 4.

Table 4 – Test formulations

Designation	Formulation	
5 mM His, pH 5.6	Pembrolizumab	1.5–30 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
His + Tre 100 mg/ml	Pembrolizumab	1.5–30 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	Trehalose dihydrate	100 mg/ml
His + Sorb 50 mg/ml	Pembrolizumab	1.5–30 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	Sorbitol	50 mg/ml
His + Prol 30 mg/ml	Pembrolizumab	1.5–30 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	L-proline	30 mg/ml
His + NaCl 9.0 mg/ml	Pembrolizumab	1.5–30 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	Sodium chloride	9.0 mg/ml
Keytruda buf. sol. + Sucr	Pembrolizumab	1.5–30 mg/ml
	L-histidine	0.300 mg/ml
	L-histidine hydrochloride monohydrate	1.700 mg/ml
	Sucrose	70.0 mg/ml

Study of stability prediction indicators.

Results of the study of aggregation temperature by method 3, melting point by method 4, and diffusion interaction parameter by method 6 for selection of osmotic agent are shown in Table 5. The best results have a lighter shade of color.

Table 5 – Results of the study of stability prediction indicators

Formulation designation	Melting point, °C			T agr, °C	k_D
	T ons	T1	T2		
5 mM His Buf, pH 5.6	50.8	60.4	67.3	64.33	1.89E+02
His + Tre 100 mg/ml	52.9	62.2	68.2	66.02	2.74E-02
His + Sorb 50 mg/ml	51.7	61.3	67.9	65.81	2.51E-02

His + Prol 30 mg/ml	50.5	61.6	67.6	65.18	3.05E-02
His + NaCl 9.0 mg/ml	46.3	58.3	65.2	58.24	-8.56E-03
Keytruda buf. sol. + Sucr	50.5	59.2	67.0	63.44	9.22E-03

Determination of thermal stability.

Thermal stability was assessed by method 7. Before and following thermal stress, we determined: purity by SE HPLC by method 13, charge variant profile in capillary by method 14, hydrodynamic radius by method 6. The results are shown in Table 6. The best results have a lighter shade of color.

Table 6 – Results of determination of thermal stability

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Charge variant profile, %			
			Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	TS50 96H	IC	Δ TS50 96H	IC	Δ TS50 96H	IC			TS50 96H
5 mM His Buf, pH 5.6	4.7	4.8	0.44	0.58	99.53	-1.56	2.81	83.30	13.90	9.62
His + Tre 100 mg/ml	6.1	5.7	0.43	0.22	99.50	0.03	2.77	84.15	13.09	10.72
His + Sorb 50 mg/ml	5.2	5.4	0.44	0.41	99.53	-1.47	2.66	83.28	14.06	14.06
His + Prol 30 mg/ml	5.1	5.1	0.43	0.49	99.55	-1.51	2.59	84.19	13.23	11.30
His + NaCl 9.0 mg/ml	5.7	7.4	0.45	2.80	99.53	-4.11	2.73	83.05	14.23	11.46
Keytruda buf. sol. + Sucr	6.2	5.8	0.42	0.94	99.54	-2.12	2.73	85.04	12.24	13.41

Determination of stability under shaking.

Stability under shaking was assessed by method 8. Before and following shaking, we determined: purity by SE HPLC by method 13, charge variant profile in capillary by method 14, hydrodynamic radius by method 6. The results are shown in Table 7. The best results have a lighter shade of color.

Table 7 – Results of determination of stability under shaking

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Charge variant profile, %			
			Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	SH800 96H	IC	Δ SH800 96H	IC	Δ SH800 96H	IC			SH800 96H
5 mM His Buf, pH 5.6	4.7	4.7	0.44	-0.01	99.53	0.02	2.81	83.30	13.90	1.35

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Charge variant profile, %			
			Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	SH800 96H	IC	Δ SH800 96H	IC	Δ SH800 96H	IC			SH800 96H
His + Tre 100 mg/ml	6.1	5.7	0.43	-0.01	99.50	-0.02	2.77	84.15	13.09	0.50
His + Sorb 50 mg/ml	5.2	5.2	0.44	-0.01	99.53	0.03	2.66	83.28	14.06	2.39
His + Prol 30 mg/ml	5.1	4.9	0.43	-0.02	99.55	0.02	2.59	84.19	13.23	2.37
His + NaCl 9.0 mg/ml	5.7	5.5	0.45	-0.03	99.53	0.04	2.73	83.05	14.23	1.81
Keytruda buf. sol. + Sucr	6.2	23.3	0.42	0.00	99.54	-0.02	2.73	85.04	12.24	2.13

Determination of stability during freezing and thawing.

Stability under freeze-thaw was assessed by method 9. Before and following stress, we determined: purity by SE HPLC by method 13, charge variant profile in capillary by method 14, hydrodynamic radius by method 6. The results are shown in Table 8. The best results have a lighter shade of color.

Table 8 – Results of determination of stability under freeze-thaw

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Charge variant profile, %			
			Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	FT(-20) *5	IC	Δ FT(-20) *5	IC	Δ FT(-20) *5	IC			FT(-20) *5
5 mM His Buf, pH 5.6	4.7	6.1	0.44	0.60	99.53	-0.58	2.81	83.30	13.90	1.88
His + Tre 100 mg/ml	6.1	6.1	0.43	0.20	99.50	-0.22	2.77	84.15	13.09	1.43
His + Sorb 50 mg/ml	5.2	6.3	0.44	0.19	99.53	-0.18	2.66	83.28	14.06	1.05
His + Prol 30 mg/ml	5.1	5.7	0.43	0.06	99.55	-0.06	2.59	84.19	13.23	1.26
His + NaCl 9.0 mg/ml	5.7	5.8	0.45	0.20	99.53	-0.20	2.73	83.05	14.23	2.59
Keytruda buf. sol. + Sucr	6.2	7.5	0.42	0.13	99.54	-0.12	2.73	85.04	12.24	2.36

The formulations comprising trehalose dihydrate and L-proline showed the best stabilizing properties among all the test samples. The sample comprising trehalose dihydrate showed the best results in terms of aggregation temperature and melting point. Also, this sample showed the smallest change in

quality indicators under thermal exposure, minor changes under shaking were observed. The sample comprising L-proline also showed one of the best results for aggregation temperature and an average result for melting point. The composition with L-proline showed average results under thermal exposure, small change in quality indicators under shaking, and the best result under freeze-thaw.

Introduction of trehalose dihydrate or L-proline, selected for the next step, reduces the formation of aggregates under freeze-thaw and thermal stress. Also, the selected formulations exhibit a slight change in quality under shaking.

The selected osmotic agents, when compared with the formulation containing sucrose, provide increased thermal stability; in particular, we observed increased melting point/aggregation temperature, a lower rate of formation of aggregates under thermal exposure and a smaller change in the monomer content, and we also observed a smaller absolute change in the acid-base profile. The sample containing trehalose dihydrate in the excipients showed better results in terms of the diffusion interaction parameter, which indicates increased stability and less tendency to aggregation during concentration and diafiltration. As compared with the formulation containing sucrose, the studied formulations containing trehalose dihydrate, sorbitol or L-proline show a significantly smaller increase in the particle hydrodynamic radius under shaking and freezing, which indicates the formation of large high-molecular particles.

Excipient formulations selected for the next step:

His + Tre 100 mg/ml:	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	Trehalose dihydrate	100 mg/ml
His + Prol 30 mg/ml:	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	L-proline	30 mg/ml

Example 3. Screening of osmotic agents and stabilizers.

For the screening of osmotic agents and stabilizers, excipients suitable for parenteral administration were used. The test formulations are shown in Table 9. Pharmaceutical compositions containing pembrolizumab in the test formulations were prepared in accordance with method 2.

Table 9 – Test formulations

Designation	Formulation

His+Tre	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate Trehalose dihydrate	1.5–30 mg/ml 0.221 mg/ml 0.750 mg/ml 100 mg/ml
His+Tre + Arginine h/c 100 mM	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate Trehalose dihydrate Arginine hydrochloride monohydrate	1.5–30 mg/ml 0.221 mg/ml 0.750 mg/ml 100 mg/ml 21.07 mg/ml
His+Tre + Glycine 100 mM	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate Trehalose dihydrate Glycine	1.5–30 mg/ml 0.221 mg/ml 0.750 mg/ml 100 mg/ml 7.51 mg/ml
His+Tre + Methionine 10 mM	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate Trehalose dihydrate Methionine	1.5–30 mg/ml 0.221 mg/ml 0.750 mg/ml 100 mg/ml 1.490 mg/ml
His+Prol	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate L-proline	1.5–30 mg/ml 0.221 mg/ml 0.750 mg/ml 30 mg/ml
His+Prol + Arginine h/c 100 mM	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate L-proline Arginine hydrochloride monohydrate	1.5–30 mg/ml 0.221 mg/ml 0.750 mg/ml 30 mg/ml 21.07 mg/ml
His+Prol + Glycine 100 mM	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate L-proline Glycine	1.5–30 mg/ml 0.221 mg/ml 0.750 mg/ml 30 mg/ml 7.51 mg/ml
His+Prol + Methionine 10 mM	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate L-proline Methionine	1.5–30 mg/ml 0.221 mg/ml 0.750 mg/ml 30 mg/ml 1.490 mg/ml
Keytruda buf. sol. + Sucr	Pembrolizumab L-histidine L-histidine h/c m/h Sucrose	1.5–30 mg/ml 0.3 mg/ml 1.7 mg/ml 70 mg/ml
Keytruda pl	Pembrolizumab L-histidine	1.5–30 mg/ml 0.3 mg/ml

	L-histidine h/c m/h Sucrose Polysorbate 80	1.7 mg/ml 70 mg/ml 0.2 mg/ml
His+Tre + Polysorbate 80 0.5 mg/ml	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate Trehalose dihydrate Polysorbate 80	1.5–30 mg/ml 0.221 mg/ml 0.750 mg/ml 100 mg/ml 0.5 mg/ml
His+Tre + Poloxamer 188 0.5 mg/ml	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate Trehalose dihydrate Poloxamer 188	1.5–30 mg/ml 0.221 mg/ml 0.750 mg/ml 100 mg/ml 0.5 mg/ml
His+Prol + Polysorbate 80 0.5 mg/ml	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate L-proline Polysorbate 80	1.5–30 mg/ml 0.221 mg/ml 0.750 mg/ml 30 mg/ml 0.5 mg/ml
His+Prol + Poloxamer 188 0.5 mg/ml	Pembrolizumab L-histidine L-histidine hydrochloride monohydrate L-proline Poloxamer 188	1.5–30 mg/ml 0.221 mg/ml 0.750 mg/ml 30 mg/ml 0.5 mg/ml

Study of stability prediction indicators.

Results of the study of aggregation temperature by method 3, melting point by method 4, and diffusion interaction parameter by method 6 for selection of osmotic agent are shown in Table 10. The best results have a lighter shade of color.

Table 10 – Results of study of stability prediction indicators

Formulation designation	Melting point, °C			T agr, °C	k_D
	T ons	T1	T2		
His+Tre	51.7	61.9	68.2	66.05	8.23E-03
His+Tre + Arginine h/c 100 mM	47.2	59.5	64.9	60.59	-9.31E-03
His+Tre + Glycine 100 mM	52.3	62.5	68.5	66.42	1.10E-02
His+Tre + Methionine 10 mM	51.4	61.9	68.5	66.12	1.13E-02
His+Prol	51.1	61.3	67.3	64.17	2.97E-02
His+Prol + Arginine h/c 100 mM	47.5	59.2	64.6	59.56	-5.11E-03
His+Prol + Glycine 100 mM	50.8	61.9	67.9	64.61	3.36E-02

His+Prol + Methionine 10 mM	50.8	61.3	67.3	64.34	3.24E-02
Keytruda buf. sol. + Sucr	48.7	59.2	67.0	63.63	9.22E-03
Keytruda pl	n/a	n/a	n/a	n/a	n/a
His+Tre + Polysorbate 80 0.5 mg/ml	n/a	n/a	n/a	n/a	n/a
His+Tre + Poloxamer 188 0.5 mg/ml	n/a	n/a	n/a	n/a	n/a
His+Prol + Polysorbate 80 0.5 mg/ml	n/a	n/a	n/a	n/a	n/a
His+Prol + Poloxamer 188 0.5 mg/ml	n/a	n/a	n/a	n/a	n/a

Determination of thermal stability.

Thermal stability was assessed by method 7. Before and following thermal stress, we determined: purity by SE HPLC by method 13, charge variant profile in capillary by method 14, hydrodynamic radius by method 6. The results are shown in Table 11. The best results have a lighter shade of color.

Table 11 – Results of determination of thermal stability

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Charge variant profile, %			
			Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	TS50 96H	IC	Δ TS50 96H	IC	Δ TS50 96H	IC			TS50 96H
His+Tre	5.5	5.5	0.47	-0.04	99.49	0.03	2.78	85.19	12.04	1.83
His+Tre + Arginine h/c 100 mM	7.2	6.9	0.51	0.00	99.46	-0.02	2.81	85.41	11.79	1.51
His+Tre + Glycine 100 mM	5.6	5.5	0.47	-0.04	99.49	0.05	2.79	84.99	12.23	1.28
His+Tre + Methionine 10 mM	5.4	5.5	0.47	-0.02	99.49	0.02	2.74	84.70	12.57	1.58
His+Prol	5	5	0.46	-0.01	99.48	0.01	2.78	84.01	13.21	2.83
His+Prol + Arginine h/c 100 mM	6.1	6	0.47	-0.01	99.48	-0.01	2.70	85.16	12.15	1.77
His+Prol + Glycine 100 mM	5.3	4.9	0.47	-0.03	99.49	0.02	2.94	85.17	11.90	3.41
His+Prol + Methionine 10 mM	5.2	4.9	0.46	-0.02	99.53	-0.03	2.79	84.52	12.70	0.57
Keytruda buf. sol. + Sucr	6.1	5.9	0.45	-0.02	99.53	0.01	2.71	84.59	12.71	2.05
Keytruda pl	5.9	n/a	0.46	n/a	99.52	-0.03	3.11	84.03	12.87	1.41

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Charge variant profile, %			
			Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	TS50 96H	IC	Δ TS50 96H	IC	Δ TS50 96H	IC			TS50 96H
His+Tre + Polysorbate 80 0.5 mg/ml	5.5	n/a	0.54	n/a	99.43	n/a	3.23	83.61	13.17	n/a
His+Tre + Poloxamer 188 0.5 mg/ml	5.5	n/a	0.46	n/a	99.51	n/a	3.12	84.62	12.27	n/a
His+Prol + Polysorbate 80 0.5 mg/ml	5.1	n/a	0.52	n/a	99.43	n/a	3.15	84.86	12.00	n/a
His+Prol + Poloxamer 188 0.5 mg/ml	4.9	n/a	0.46	n/a	99.51	n/a	2.96	84.73	12.31	n/a

Determination of stability under acid hydrolysis.

Stability under acid hydrolysis for formulations devoid of poloxamer 188 was assessed by method 10 with adjusting to pH 3.5 and ageing for 1 hour. Before and following hydrolysis, we determined: purity by SE HPLC by method 13, charge variant profile in capillary by method 14, hydrodynamic radius by method 6. The results are shown in Table 12. The best results have a lighter shade of color.

Table 12 - Results of determination of stability under acid hydrolysis

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Charge variant profile, %			
			Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	Acid 3.5 1H	IC	Δ Acid 3.5 1H	IC	Δ Acid 3.5 1H	IC			Acid 3.5 1H
His+Tre	5.5	14.9	0.47	29.91	99.49	-30.34	2.78	85.19	12.04	n/a
His+Tre + Arginine h/c 100 mM	7.2	8.9	0.51	4.99	99.46	-5.01	2.81	85.41	11.79	13.64
His+Tre + Glycine 100 mM	5.6	12.2	0.47	1.77	99.49	-1.77	2.79	84.99	12.23	5.97
His+Tre + Methionine 10 mM	5.4	18.3	0.47	3.24	99.49	-3.24	2.74	84.70	12.57	4.92
His+Prol	5	12.8	0.46	4.48	99.48	-4.49	2.78	84.01	13.21	4.77

His+Prol + Arginine h/c 100 mM	6.1	6.6	0.47	5.27	99.48	-5.26	2.70	85.16	12.15	3.54
His+Prol + Glycine 100 mM	5.3	7.6	0.47	2.64	99.49	-2.63	2.94	85.17	11.90	1.20
His+Prol + Methionine 10 mM	5.2	7	0.46	0.76	99.53	-0.79	2.79	84.52	12.70	4.77
Keytruda buf. sol. + Sucr	6.2	9.5	0.45	62.96	99.53	-64.53	2.71	84.59	12.71	23.15
Keytruda pl	5.9	n/a	0.46	n/a	99.52	n/a	3.11	84.03	12.87	n/a
His+Tre + Polysorbate 80 0.5 mg/ml	5.5	n/a	0.54	n/a	99.43	n/a	3.23	83.61	13.17	n/a
His+Tre + Poloxamer 188 0.5 mg/ml	5.5	n/a	0.46	n/a	99.51	n/a	3.12	84.62	12.27	n/a
His+Prol + Polysorbate 80 0.5 mg/ml	5.1	n/a	0.52	n/a	99.43	n/a	3.15	84.86	12.00	n/a
His+Prol + Poloxamer 188 0.5 mg/ml	4.9	n/a	0.46	n/a	99.51	n/a	2.96	84.73	12.31	n/a

Determination of stability under basic hydrolysis.

Stability under basic hydrolysis for formulations devoid of poloxamer 188 was assessed by method 11 with adjusting to pH 8.5 and ageing for 1 hour. Before and following hydrolysis, we determined: purity by SE HPLC by method 13, charge variant profile in capillary by method 14, hydrodynamic radius by method 6. The results are shown in Table 13. The best results have a lighter shade of color.

Table 13 - Results of determination of stability under basic hydrolysis

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Charge variant profile, %			
			Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	Basic 8.5 1H	IC	Δ Basic 8.5 1H	IC	Δ Basic 8.5 1H	IC			Basic 8.5 1H
His+Tre	5.5	10.5	0.47	9.82	99.49	-9.83	2.78	85.19	12.04	23.46

His+Tre + Arginine h/c 100 mM	7.2	10.1	0.51	9.26	99.46	-9.30	2.81	85.41	11.79	13.95
His+Tre + Glycine 100 mM	5.6	9.4	0.47	6.14	99.49	-6.16	2.79	84.99	12.23	14.71
His+Tre + Methionine 10 mM	5.4	n/a	0.47	10.87	99.49	-10.93	2.74	84.70	12.57	23.38
His+Prol	5	11.3	0.46	2.10	99.48	-2.08	2.78	84.01	13.21	3.05
His+Prol + Arginine h/c 100 mM	6.1	6.7	0.47	4.86	99.48	-4.88	2.70	85.16	12.15	6.32
His+Prol + Glycine 100 mM	5.3	6.1	0.47	1.98	99.49	-1.96	2.94	85.17	11.90	3.74
His+Prol + Methionine 10 mM	5.2	9.2	0.46	3.64	99.53	-3.67	2.79	84.52	12.70	6.18
Keytruda buf. sol. + Sucr	6.2	20.7	0.45	10.36	99.53	-10.45	2.71	84.59	12.71	24.21
Keytruda pl	5.9	n/a	0.46	n/a	99.52	n/a	3.11	84.03	12.87	n/a
His+Tre + Polysorbate 80 0.5 mg/ml	5.5	n/a	0.54	n/a	99.43	n/a	3.23	83.61	13.17	n/a
His+Tre + Poloxamer 188 0.5 mg/ml	5.5	n/a	0.46	n/a	99.51	n/a	3.12	84.62	12.27	n/a
His+Prol + Polysorbate 80 0.5 mg/ml	5.1	n/a	0.52	n/a	99.43	n/a	3.15	84.86	12.00	n/a
His+Prol + Poloxamer 188 0.5 mg/ml	4.9	n/a	0.46	n/a	99.51	n/a	2.96	84.73	12.31	n/a

Determination of stability under shaking.

Stability under shaking was assessed by method 8. Before and following stress, we determined: purity by SE HPLC by method 13, charge variant profile in capillary by method 14, hydrodynamic radius by method 6. The results are shown in Table 14. The best results have a lighter shade of color.

Table 14 – Results of determination of stability under shaking

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Charge variant profile, %			
			Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	SH800 96H	IC	Δ SH800 96H	IC	Δ SH800 96H	IC			SH800 96H
His+Tre	5.5	5.9	0.47	-0.05	99.49	0.04	2.78	85.19	12.04	0.61

His+Tre + Arginine h/c 100 mM	7.2	7	0.51	-0.06	99.46	0.06	2.81	85.41	11.79	0.18
His+Tre + Glycine 100 mM	5.6	5.9	0.47	-0.04	99.49	0.03	2.79	84.99	12.23	1.03
His+Tre + Methionine 10 mM	5.4	9.5	0.47	-0.03	99.49	0.03	2.74	84.70	12.57	2.81
His+Prol	5	4.8	0.46	-0.02	99.48	0.02	2.78	84.01	13.21	2.13
His+Prol + Arginine h/c 100 mM	6.1	5.9	0.47	-0.03	99.48	0.03	2.70	85.16	12.15	0.46
His+Prol + Glycine 100 mM	5.3	4.9	0.47	-0.03	99.49	0.02	2.94	85.17	11.90	0.93
His+Prol + Methionine 10 mM	5.2	4.9	0.46	-0.02	99.53	-0.01	2.79	84.52	12.70	0.85
Keytruda buf. sol. + Sucr	6.2	10.5	0.45	-0.02	99.53	0.01	2.71	84.59	12.71	0.04
Keytruda pl	5.9	5.7	0.46	-0.02	99.52	0.02	3.11	84.03	12.87	0.13
His+Tre + Polysorbate 80 0.5 mg/ml	5.5	5.5	0.54	-0.01	99.43	0.00	3.23	83.61	13.17	0.64
His+Tre + Poloxamer 188 0.5 mg/ml	5.5	5.4	0.46	0.00	99.51	-0.03	3.12	84.62	12.27	0.47
His+Prol + Polysorbate 80 0.5 mg/ml	5.1	4.9	0.52	-0.01	99.43	0.00	3.15	84.86	12.00	5.23
His+Prol + Poloxamer 188 0.5 mg/ml	4.9	4.9	0.46	-0.02	99.51	-0.01	2.96	84.73	12.31	5.28

Determination of stability during freezing and thawing.

Stability under freeze-thaw was assessed by method 9. Before and following stress, we determined: purity by SE HPLC by method 13, charge variant profile in capillary by method 14, hydrodynamic radius by method 6. The results are shown in Table 15. The best results have a lighter shade of color.

Table 15 – Results of determination of stability under freeze-thaw

		Purity, SE HPLC	Charge variant profile, %

Formulation designation	Hydrodynamic radius, nm		Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	FT (-20) *3	IC	Δ FT (-20) *3	IC	Δ FT (-20) *3	IC			FT (-20) *3
His+Tre	5.5	5.9	0.47	0.18	99.49	-0.17	2.78	85.19	12.04	5.94
His+Tre + Arginine h/c 100 mM	7.2	7.1	0.51	0.13	99.46	-0.14	2.81	85.41	11.79	2.84
His+Tre + Glycine 100 mM	5.6	6	0.47	0.15	99.49	-0.14	2.79	84.99	12.23	2.85
His+Tre + Methionine 10 mM	5.4	6	0.47	0.16	99.49	-0.14	2.74	84.70	12.57	3.66
His+Prol	5	5.2	0.46	0.14	99.48	-0.12	2.78	84.01	13.21	1.93
His+Prol + Arginine h/c 100 mM	6.1	6	0.47	0.08	99.48	-0.08	2.70	85.16	12.15	1.84
His+Prol + Glycine 100 mM	5.3	5.3	0.47	0.12	99.49	-0.10	2.94	85.17	11.90	3.34
His+Prol + Methionine 10 mM	5.2	5.3	0.46	0.09	99.53	-0.13	2.79	84.52	12.70	2.04
Keytruda buf. sol. + Sucr	6.2	6.6	0.45	0.14	99.53	-0.15	2.71	84.59	12.71	2.62
Keytruda pl	5.9	5.9	0.46	0.00	99.52	0.00	3.11	84.03	12.87	3.06
His+Tre + Polysorbate 80 0.5 mg/ml	5.5	5.4	0.54	0.03	99.43	-0.04	3.23	83.61	13.17	4.29
His+Tre + Poloxamer 188 0.5 mg/ml	5.5	5.4	0.46	0.02	99.51	-0.02	3.12	84.62	12.27	3.98
His+Prol + Polysorbate 80 0.5 mg/ml	5.1	5.2	0.52	0.02	99.43	-0.03	3.15	84.86	12.00	6.69
His+Prol + Poloxamer 188 0.5 mg/ml	4.9	4.9	0.46	0.01	99.51	0.00	2.96	84.73	12.31	4.46

The following formulations among the test samples may be distinguished in terms of stability:

1. His+Tre + Glycine 100 mM	Pembrolizumab	1.5–30 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	Trehalose dihydrate	100 mg/ml
	Glycine	7.51 mg/ml
2. His+Tre + Poloxamer 188 0.5 mg/ml	Pembrolizumab	1.5–30 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	Trehalose dihydrate	100 mg/ml
	Poloxamer 188	0.5 mg/ml
3. His+Prol	Pembrolizumab	1.5–30 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	L-proline	30 mg/ml
4. His+Prol + Glycine 100 mM	Pembrolizumab	1.5–30 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	L-proline	30 mg/ml
	Glycine	7.51 mg/ml
5. His+Prol + Glycine 100 mM + Poloxamer 188 0.5 mg/ml	Pembrolizumab	1.5–30 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	L-proline	30 mg/ml
	Glycine	7.51 mg/ml
	Poloxamer 188	0.5 mg/ml

Formulations containing trehalose dihydrate, compositions of trehalose dihydrate in combination with glycine, as well as compositions with L-proline, as well as compositions with L-proline in combination with glycine or methionine were selected for further development, since they showed positive stability results under stress.

All formulations with trehalose dihydrate exhibited higher content of the basic fraction as compared to that with L-proline. Among the formulations containing trehalose dihydrate, the formulation with glycine showed the best results. We observed the positive effect of glycine on the aggregation temperature and melting point, as well as on the diffusion interaction parameter. When under thermal stress, the addition of glycine to the formulation showed slight improvements. The addition of glycine to the formulation showed improvements in purity and acid-base profile before and after stress. As compared to the sample in the formulation of Keytruda excipients without added surfactants, we observed a significant increase in the melting point and aggregation temperature, as well as a significantly smaller change in purity and acid-base profile under acid and basic hydrolysis.

Among the formulations containing surfactants, the positive effect of poloxamer 188 was observed. These compositions exhibited a smaller change in the purity and charged variant profile under shaking and freezing. Accordingly, Poloxamer 188 was selected to be used as a surfactant in the next step.

Example 4. Determination of critical quantitative factors of excipient formulation.

Study was conducted in factorial four-factor experiment design with two levels and a center point. Protein concentration (from 10 to 40 mg/ml), pH (from 5.1 to 6.1), osmotic agent concentration (from 70 to 130 mg/ml), L-glycine concentration (from 1.5 to 15 mg/ml) and poloxamer concentration 188 (from 0.10 to 1.0 mg/ml) were studied as quantitative factors. The test formulations are listed in Table 16.

Table 16 – Test formulations

Designation	Protein conc., mg/ml	Buffer solution	Osmotic agent conc., mg/ml	Conc. L-glycine, mg/ml	Conc. Poloxamer 188 (KOLL), mg/ml
Pembro 10 His 5.1+Tre 70+Gly 15+ KOLL 1.0	10	5 mM His pH 5.1	70 Trehalose dihydrate	15.01	1.00
Pembro 10 His 5.1+Tre 130+Gly 15+ KOLL 0.1	10	5 mM His pH 5.1	130 Trehalose dihydrate	15.01	0.10
Pembro 40 His 5.1+Tre 70+Gly 1.5+ KOLL 1.0	40	5 mM His pH 5.1	70 Trehalose dihydrate	1.50	1.00
Pembro 40 His 5.1+Tre 130+Gly 1.5+ KOLL 0.1	40	5 mM His pH 5.1	130 Trehalose dihydrate	1.50	0.10
Pembro 1 25 His 5.6+Tre 100+Gly 8.25+ KOLL 0.55	25	5 mM His pH 5.6	100 Trehalose dihydrate	8.25	0.55
Pembro 2 25 His 5.6+Tre 100+Gly 8.25+ KOLL 0.55	25	5 mM His pH 5.6	100 Trehalose dihydrate	8.25	0.55
Pembro 40 His 6.1+Tre 70+Gly 15+ KOLL 0.1	40	5 mM His pH 6.1	70 Trehalose dihydrate	15.01	0.10
Pembro 10 His 6.1+Tre 70+Gly 1.5+ KOLL 0.1	10	5 mM His pH 6.1	70 Trehalose dihydrate	1.50	0.10

Designation	Protein conc., mg/ml	Buffer solution	Osmotic agent conc., mg/ml	Conc. L-glycine, mg/ml	Conc. Poloxamer 188 (KOLL), mg/ml
Pembro 40 His 6.1+Tre 130+Gly 15+ KOLL 1.0	40	5 mM His pH 6.1	130 Trehalose dihydrate	15.01	1.00
Pembro 10 His 6.1+Tre 130+Gly 1.5+ KOLL 1.0	10	5 mM His pH 6.1	130 Trehalose dihydrate	1.50	1.00
Pembro 25 His 5.6 +Prol 30 + Gly 8.25	25	5 mM His pH 5.6	30 L-proline	8.25	0
Pembro 25 His 5.6 +Prol 30 + Gly 8.25 + KOLL 0.55	25	5 mM His pH 5.6	30 L-proline	8.25	0.55

In Table 16, buffer solutions refer to the following formulations described in Table 17.

Table 17 – Formulations of buffer solutions

5 mM His, pH 5.1	L-histidine	0.087 mg/ml
	L-histidine hydrochloride monohydrate	0.931 mg/ml
5 mM His, pH 5.6	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
5 mM His, pH 6.1	L-histidine	0.432 mg/ml
	L-histidine hydrochloride monohydrate	0.464 mg/ml

Study of stability prediction indicators.

Results of the study of aggregation temperature by method 3, melting point by method 4, and diffusion interaction parameter by method 6 for selection of osmotic agent are shown in Table 18. The best results have a lighter shade of color.

Table 18 – Results of study of stability prediction indicators

Formulation designation	Melting point, °C			T agr, °C	k_D
	T ons	T1	T2		
Pembro 10 His 5.1+ Tre 70+Gly 15+ KOLL 1.0	46.9	58	68.8	66.03	3.48E-02
Pembro 10 His 5.1+ Tre 130+Gly 15+ KOLL 0.1	48.8	61.3	68.8	67.09	1.97E-02
Pembro 40 His 5.1+ Tre 70+Gly 1.5+ KOLL 1.0	46.3	58.3	67.3	64.33	1.48E-02

Pembro 40 His 5.1+ Tre 130+Gly 1.5+ KOLL 0.10	48.4	58.9	67.9	65.33	1.18E-02
Pembro 1 25 His 5.6 + Tre 100+Gly 8.25+ KOLL 0.55	49.9	61	68.5	66.6	1.70E-02
Pembro 2 25 His 5.6 + Tre 100+Gly 8.25+ KOLL 0.55	51.1	61.3	68.8	65.54	1.56E-02
Pembro 40 His 6.1+ Tre 70+Gly 15+ KOLL 0.1	53.2	64.6	none	66.26	1.20E-02
Pembro 10 His 6.1+ Tre 70+Gly 1.5+ KOLL 0.1	53.2	64	none	n/a	3.99E-02
Pembro 40 His 6.1+ Tre 130+Gly 15+ KOLL 1.0	53.8	65.8	68.8	66.66	1.70E-02
Pembro 10 His 6.1+ Tre 130+Gly 1.5+ KOLL 1.0	53.2	63.7	68.8	66.16	4.79E-02
Pembro 25 His 5.6 +Prol 30 + Gly 8.25	52	62.2	67.9	64.13	2.60E-02
Pembro 25 His 5.6 +Prol 30 + Gly 8.25 + KOLL 0.55	50.8	61	67.9	64.58	3.23E-02

Determination of thermal stability.

Thermal stability was assessed by method 7. Before and following thermal stress, we determined: purity by SE HPLC by method 13, charge variant profile in capillary by method 14, hydrodynamic radius by method 6. The results are shown in Table 19. The best results have a lighter shade of color.

Table 19 – Results of determination of thermal stability

Determination of stability under shaking.

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Charge variant profile, %			
			Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	TS50 96H	IC	Δ TS50 96H	IC	Δ TS50 96H	IC			TS50 96H
Pembro 10 His 5.1+ Tre 70+Gly 15+ KOLL 1.0	4.8	4.9	0.51	0.48	99.47	-0.55	3.08	81.29	15.64	8.12
Pembro 10 His 5.1+ Tre 130+Gly 15+ KOLL 0.1	5.3	5.5	0.52	0.25	99.46	-0.30	2.44	81.33	16.24	12.40
Pembro 40 His 5.1+ Tre 70+Gly 1.5+ KOLL 1.0	3.4	3.5	0.57	1.55	99.39	-1.62	3.77	81.31	14.93	10.62

Pembro 40 His 5.1+ Tre 130+Gly 1.5+ KOLL 0.10	3.8	4.1	0.57	0.85	99.39	-0.92	3.09	82.03	14.88	9.60
Pembro 1 25 His 5.6 + Tre 100+Gly 8.25+ KOLL 0.55	4.4	4.5	0.63	0.45	99.33	-0.50	2.84	84.05	13.11	13.83
Pembro 2 25 His 5.6 + Tre 100+Gly 8.25+ KOLL 0.55	4.4	4.5	0.60	0.62	99.36	-0.67	2.87	84.12	13.01	12.79
Pembro 40 His 6.1+ Tre 70+Gly 15+ KOLL 0.1	4.7	5.0	0.64	0.64	99.33	-0.71	2.63	84.03	13.34	15.98
Pembro 10 His 6.1+ Tre 70+Gly 1.5+ KOLL 0.1	5.0	5.1	0.60	0.25	99.39	-0.28	2.85	83.68	13.47	14.08
Pembro 40 His 6.1+ Tre 130+Gly 15+ KOLL 1.0	5.2	5.5	0.63	0.72	99.34	-0.78	2.38	84.78	12.84	21.10
Pembro 10 His 6.1+ Tre 130+Gly 1.5+ KOLL 1.0	5.6	5.6	0.60	0.25	99.37	-0.30	2.58	84.50	12.92	18.44

Stability under shaking was assessed by method 8. Before and following stress, we determined: purity by SE HPLC by method 13, charge variant profile in capillary by method 14, hydrodynamic radius by method 6. The results are shown in Table 20. The best results have a lighter shade of color.

Table 20 – Results of determination of stability under shaking

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Charge variant profile, %			
			Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	SH800 96H	IC	Δ SH800 96H	IC	Δ SH800 96H	IC			SH800 96H
Pembro 10 His 5.1+ Tre 70+Gly 15+ KOLL 1.0	4.8	4.8	0.51	0.01	99.47	-0.01	3.08	81.29	15.64	2.10
Pembro 10 His 5.1+ Tre	5.3	5.4	0.52	-0.01	99.46	0.01	2.44	81.33	16.24	1.08

130+Gly 15+ KOLL 0.1										
Pembro 40 His 5.1+ Tre 70+Gly 1.5+ KOLL 1.0	3.4	3.6	0.57	0.04	99.39	-0.04	3.77	81.31	14.93	1.89
Pembro 40 His 5.1+ Tre 130+Gly 1.5+ KOLL 0.10	3.8	4.0	0.57	-0.01	99.39	0.00	3.09	82.03	14.88	3.70
Pembro 1 25 His 5.6 + Tre 100+Gly 8.25+ KOLL 0.55	4.4	4.4	0.63	-0.07	99.33	-0.02	2.84	84.05	13.11	0.60
Pembro 2 25 His 5.6 + Tre 100+Gly 8.25+ KOLL 0.55	4.4	4.4	0.60	-0.01	99.36	0.01	2.87	84.12	13.01	1.46
Pembro 40 His 6.1+ Tre 70+Gly 15+ KOLL 0.1	4.7	4.6	0.64	0.02	99.33	-0.03	2.63	84.03	13.34	0.28
Pembro 10 His 6.1+ Tre 70+Gly 1.5+ KOLL 0.1	5.0	4.9	0.60	0.01	99.39	-0.02	2.85	83.68	13.47	4.40
Pembro 40 His 6.1+ Tre 130+Gly 15+ KOLL 1.0	5.2	5.4	0.63	0.02	99.34	-0.03	2.38	84.78	12.84	1.93
Pembro 10 His 6.1+ Tre 130+Gly 1.5+ KOLL 1.0	5.6	5.5	0.60	0.00	99.37	0.00	2.58	84.50	12.92	2.92

Determination of stability during freezing and thawing.

Stability under freeze-thaw was assessed by method 9. Before and following stress, we determined: purity by SE HPLC by method 13, charge variant profile in capillary by method 14, hydrodynamic radius by method 6. The results are shown in Table 21. The best results have a lighter shade of color.

Table 21 – Results of determination of stability under freeze-thaw

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Charge variant profile, %			
			Aggregates, %		Monomer, %		Alc.	Base	Acid.	Δ abs
	IC	FT (-20) *3	IC	Δ FT (-20) *3	IC	Δ FT (-20) *3	IC			FT (-20) *3
Pembro 10 His 5.1+ Tre 70+Gly 15+ KOLL 1.0	4.8	4.8	0.51	0.03	99.47	-0.03	3.08	81.29	15.64	1.56
Pembro 10 His 5.1+ Tre 130+Gly 15+ KOLL 0.1	5.3	5.3	0.52	0.02	99.46	-0.02	2.44	81.33	16.24	3.43
Pembro 40 His 5.1+ Tre 70+Gly 1.5+ KOLL 1.0	3.4	3.4	0.57	0.01	99.39	-0.01	3.77	81.31	14.93	1.90
Pembro 40 His 5.1+ Tre 130+Gly 1.5+ KOLL 0.10	3.8	3.8	0.57	0.05	99.39	-0.06	3.09	82.03	14.88	2.28
Pembro 1 25 His 5.6 + Tre 100+Gly 8.25+ KOLL 0.55	4.4	4.4	0.63	0.01	99.33	0.00	2.84	84.05	13.11	3.19
Pembro 2 25 His 5.6 + Tre 100+Gly 8.25+ KOLL 0.55	4.4	4.4	0.60	0.02	99.36	-0.01	2.87	84.12	13.01	1.14
Pembro 40 His 6.1+ Tre 70+Gly 15+ KOLL 0.1	4.7	4.7	0.64	0.02	99.33	-0.03	2.63	84.03	13.34	0.82
Pembro 10 His 6.1+ Tre 70+Gly 1.5+ KOLL 0.1	5	5	0.60	0.06	99.39	-0.07	2.85	83.68	13.47	0.50
Pembro 40 His 6.1+ Tre 130+Gly 15+ KOLL 1.0	5.2	5.2	0.63	0.06	99.34	-0.05	2.38	84.78	12.84	2.70
Pembro 10 His 6.1+ Tre 130+Gly 1.5+ KOLL 1.0	5.6	5.9	0.60	0.04	99.37	-0.05	2.58	84.50	12.92	1.12

The results were used to build the model in Minitab software. Concentrations of L-glycine/poloxamer 188 have no statistically significant effect on the stability of pembrolizumab. According to the optimization results, the recommended concentration of L-glycine is 15 mg/ml, the recommended concentration of poloxamer 188 is 1.0 mg/ml, the recommended concentration of trehalose dihydrate is 84.5 mg/ml. However, to ensure the physiological osmolality of the composition, the content of L-glycine was reduced to 1.5 mg/ml, and that of trehalose dihydrate was reduced to 80 mg/ml. The buffer solution, concentration and pH thereof were selected earlier, the results are shown in Example 1. This example confirmed their statistically significant effect on the stability of pembrolizumab. Under the thermal stress, the surfactants may cause the formation of artifact high-molecular weight impurities. If thermal stress in exluded from the model, it can be concluded that the increased concentration of poloxamer 188 has a positive effect on the stability of pembrolizumab. According to the results, the optimal concentration of poloxamer 188 is 1.0 mg/ml.

Thus, the final selected formulation is as follows:

Pembrolizumab	1.5–40 mg/ml
L-histidine	0.221 mg/ml
L-histidine hydrochloride monohydrate	0.750 mg/ml
Trehalose dihydrate	80 mg/ml
Glycine	1.5 mg/ml
Poloxamer 188	1.0 mg/ml

Example 5. Determination of stability of the final formulation under stress conditions

For stressed stability testing, the final formulation of Pembrolizumab and the formulation of Keytruda excipients were selected. The test formulations are shown in Table 22. Pharmaceutical compositions containing pembrolizumab in the test formulations were prepared in accordance with method 2.

Table 22 – Test formulations

Pembro	Pembrolizumab	1.5–30 mg/ml
	L-histidine	0.221 mg/ml
	L-Histidine hydrochloride monohydrate	0.750 mg/ml
	Trehalose d/h	80.0 mg/ml
	Glycine	1.5 mg/ml
	Poloxamer 188	1.0 mg/ml
Keytruda pl	Pembrolizumab	1.5–30 mg/ml
	L-histidine	0.3 mg/ml
	L-histidine h/c m/h	1.7 mg/ml

	Sucrose	70 mg/ml
	Polysorbate 80	0.2 mg/ml

Determination of thermal stability.

Thermal stability was assessed by method 7 for 10 days. Before and after thermal stress, we determined the purity by SE HPLC method (method 13), the charged variant profile by IE HPLC method (method 15), the content of oxidation products by hydrophobic interaction HPLC method (method 18), the hydrodynamic radius by method 5. The results are shown in Table 23. The best results have a lighter shade of color.

Table 23 – Results of determination of thermal stability

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Hydrophobic interaction HPLC			
			Aggregates, %		Monomer, %		Oxid. variants, peak 1+2		Oxid. variants, peak 3.	
	IC	TS50	IC	Δ TS50	IC	Δ TS50	IC	Δ TS50	IC	Δ TS50
Pembro	4.2	4.7	0.15	0.02	99.85	-0.18	7.9	0.74	0	0.50
Keytruda pl	5.0	6.1	0.14	0.07	99.86	-0.25	6.22	12.15	0	1.13

Formulation designation	Charged variant profile, IE HPLC					
	Acid., %		Base, %		Alc., %	
	IC	Δ TS50	IC	Δ TS50	IC	Δ TS50
Pembro	13.81	21.92	73.97	-22.13	12.22	0.21
Keytruda pl	15.59	22.36	72.79	-23.74	11.62	1.38

Determination of stability under shaking.

Stability under shaking was assessed by method 8. Before and after shaking, we determined the purity by SE HPLC method 13, the charged variant profile by IE HPLC method (method 15), the content of oxidation products by hydrophobic interaction HPLC method (method 18), the hydrodynamic radius by method 6. The results are shown in Table 24. The best results have a lighter shade of color.

Table 24 – Results of determination of stability under shaking

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Hydrophobic interaction HPLC			
			Aggregates, %		Monomer, %		Oxid. variants, peak 1+2		Oxid. variants, peak 3.	
	IC	SH800	IC	Δ SH800	IC	Δ SH800	IC	Δ SH800	IC	Δ SH800
Pembro	4.2	0.1	0.15	0.04	99.85	-0.04	7.9	n/a	0	n/a
Keytruda pl	5.0	0.0	0.14	0.04	99.86	-0.04	6.22	n/a	0	n/a

Formulation designation	Charged variant profile, IE HPLC					
	Acid., %		Base, %		Alc., %	
	IC	Δ SH800	IC	Δ SH800	IC	Δ SH800
Pembro	13.81	0.18	73.97	-0.16	12.22	-0.02
Keytruda pl	15.59	-1.28	72.79	1.65	11.62	-0.36

Determination of stability during freezing and thawing.

Stability under freeze-thaw was assessed by method 9. Before and after stress, we determined the purity by SE HPLC method 13, the charged variant profile by IE HPLC method (method 15), the content of oxidation products by hydrophobic interaction HPLC method (method 18), the hydrodynamic radius by method 6. The results are shown in Table 25. The best results have a lighter shade of color.

Table 25 – Results of determination of stability under freeze-thaw

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Hydrophobic interaction HPLC			
			Aggregates, %		Monomer, %		Oxid. variants, peak 1+2		Oxid. variants, peak 3.	
	IC	FT(-20)	IC	Δ FT (-20)	IC	Δ FT (-20)	IC	Δ FT (-20)	IC	Δ FT (-20)
Pembro	4.2	-0.1	0.15	-0.01	99.85	0.01	7.9	n/a	0	n/a
Keytruda pl	5.0	-0.1	0.14	0.01	99.86	-0.01	6.22	n/a	0	n/a

Formulation designation	Charged variant profile, IE HPLC					
	Acid., %		Base, %		Alc., %	
	IC	Δ FT(-20)*3	IC	Δ FT(-20)*3	IC	Δ FT(-20)*3
Pembro	13.81	-0.05	73.97	0.06	12.22	-0.01
Keytruda pl	15.59	-0.09	72.79	0.16	11.62	-0.07

Determination of stability under acid hydrolysis.

Stability under acid hydrolysis was assessed by method 9. Before and after stress, we determined the purity by SE HPLC method 13, the charged variant profile by IE HPLC method (method 15), the content of oxidation products by hydrophobic interaction HPLC method (method 18), the hydrodynamic radius by method 6. The results are shown in Table 26. The best results have a lighter shade of color.

Table 26 – Results of determination of stability under acid hydrolysis

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Hydrophobic interaction HPLC			
			Aggregates, %		Monomer, %		Oxid. variants, peak 1+2		Oxid. variants, peak 3.	
	IC	Acid pH 4.0	IC	Δ Acid pH 4.0	IC	Δ Acid pH 4.0	IC	Δ Acid pH 4.0	IC	Δ Acid pH 4.0

Pembro	4.2	2.00	0.15	0.11	99.85	-0.11	7.9	0.52	0	0.00
Keytruda pl	5.0	1.33	0.14	0.12	99.86	-0.12	6.22	0.19	0	0.00

Formulation designation	Charged variant profile, IE HPLC					
	Acid., %		Base, %		Alc., %	
	IC	Δ Acid pH 4.0	IC	Δ Acid pH 4.0	IC	Δ Acid pH 4.0
Pembro	13.81	-0.11	73.97	0.20	12.22	-0.09
Keytruda pl	15.59	-0.17	72.79	0.22	11.62	-0.04

Determination of stability under basic hydrolysis.

Stability under basic hydrolysis was assessed by method 9. Before and after stress, we determined the purity by SE HPLC method 13, the charged variant profile by IE HPLC method (method 15), the content of oxidation products by hydrophobic interaction HPLC method (method 18), the hydrodynamic radius by method 6. The results are shown in Table 27. The best results have a lighter shade of color.

Table 27 – Results of determination of stability under basic hydrolysis

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Hydrophobic interaction HPLC			
			Aggregates, %		Monomer, %		Oxid. variants, peak 1+2		Oxid. variants, peak 3.	
	IC	Basic pH 8.0	IC	Δ Basic pH 8.0	IC	Δ Basic pH 8.0	IC	Δ Basic pH 8.0	IC	Δ Basic pH 8.0
Pembro	4.2	2.2	0.15	0.18	99.85	-0.24	7.9	0.18	0	0.00
Keytruda pl	5.0	2.1	0.14	0.47	99.86	-0.47	6.22	0.11	0	0.00

Formulation designation	Charged variant profile, IE HPLC					
	Acid., %		Base, %		Alc., %	
	IC	Δ Basic pH 8.0	IC	Δ Basic pH 8.0	IC	Δ Basic pH 8.0
Pembro	13.81	0.28	73.97	-0.07	12.22	-0.20
Keytruda pl	15.59	0.35	72.79	0.06	11.62	-0.42

Determination of stability under oxidation.

Stability under oxidation was assessed by method 9. Before and after stress, we determined the purity by SE HPLC method 13, the charged variant profile by IE HPLC method (method 15), the content of oxidation products by hydrophobic interaction HPLC method (method 18), the hydrodynamic radius by method 6. The results are shown in Table 28. The best results have a lighter shade of color.

Table 28 – Results of determination of stability under oxidation

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Hydrophobic interaction HPLC			
			Aggregates, %		Monomer, %		Oxid. variants, peak 1+2		Oxid. variants, peak 3.	
	IC	Ox 0.1%	IC	Δ Ox 0.1%	IC	Δ Ox 0.1%	IC	Δ Ox 0.1%	IC	Δ Ox 0.1%
Pembro	4.2	2.2	0.31	-0.02	99.63	0.02	7.89	Not subject to marking	0	Not subject to marking
Keytruda pl	5.0	2.1	0.30	-0.01	99.65	0.00	7.12		0	

Formulation designation	Charged variant profile, IE HPLC					
	Acid., %		Base, %		Alc., %	
	IC	Δ Ox 0.1%	IC	Δ Ox 0.1%	IC	Δ Ox 0.1%
Pembro	13.41	-3.07	74.75	-27.21	11.85	30.28
Keytruda pl	15.34	-3.74	73.76	-29.43	10.91	33.17

Determination of photostability.

Stability under oxidation was assessed by method 9. Before and after stress, we determined the purity by SE HPLC method 13, the charged variant profile by IE HPLC method (method 15), the content of oxidation products by hydrophobic interaction HPLC method (method 18), the hydrodynamic radius by method 6. The results are shown in Table 29. The best results have a lighter shade of color.

Table 29 – Results of determination of photostability

Formulation designation	Hydrodynamic radius, nm		Purity, SE HPLC				Hydrophobic interaction HPLC			
			Aggregates, %		Monomer, %		Oxid. variants, peak 1+2		Oxid. variants, peak 3.	
	IC	UV	IC	Δ UV	IC	Δ UV	IC	Δ UV	IC	Δ UV
Pembro	4.3	0.1	0.16	0.17	99.84	-0.16	8.60	1.75	0	0
Keytruda pl	5.0	0.1	0.17	0.18	99.83	-0.18	6.33	5.59	0	0

Formulation designation	Charged variant profile, IE HPLC					
	Acid., %		Base, %		Alc., %	
	IC	Δ UV	IC	Δ UV	IC	Δ UV
Pembro	12.31	3.03	75.71	-6.10	11.98	3.07
Keytruda pl	14.00	2.39	74.78	-6.79	11.23	4.39

According to the results of stressed stability testing, the excipient formulation of Pembrolizumab as compared to that of of Keytruda showed a smaller change in quality indicators under thermal stress, specifically, a smaller change in the hydrodynamic radius, a smaller increase in aggregates and changes in the monomer content, a significantly lower tendency to form oxidized forms, and a smaller change in the charged variant profile.

When under shaking and freezing, a smaller change in the charged variant profile following stress was observed.

In turn, when under acid hydrolysis, a smaller increase in aggregates and change in the monomer content, as well as a smaller change in the basic fraction content were observed. When under basic hydrolysis, the protein within the Pembrolizumab formulation has less tendency to form aggregates, to change the monomer content and to change the charged variant profile.

When under photo stress, a significantly lower tendency to oxidation was observed as compared to the formulation of Keytruda excipients, as well as a smaller change in the monomer content, a smaller increase in aggregates and a smaller change in the charged variant profile.

Example 6. Determination of stability under accelerated ageing at a temperature of 37 ± 2 °C.

To pre-confirm the stability of the selected formulation, it was subjected to accelerated aging at a temperature of 37 ± 2 °C.

Pharmaceutical compositions in the range of protein concentration (from 25 to 50 mg/ml), pH (from 5.1 to 6.1), trehalose dihydrate concentration (from 70 to 90 mg/ml), glycine concentration (from 1.0 to 2.0 mg/ml) and poloxamer 188 concentration (from 0.8 to 1.2 mg/ml) were prepared by method 1 and stored for stability test at a temperature of 37 ± 2 °C.

The test formulations are shown in Table 30.

Table 30 – Test formulations

Pembro 25 mg/ml formul1	Pembrolizumab	25.0 mg/ml
	L-histidine	0.087 mg/ml
	L-histidine hydrochloride monohydrate	0.931 mg/ml
	Trehalose d/h	90 mg/ml
	Glycine	1.0 mg/ml
	Poloxamer 188	0.8 mg/ml
Pembro 25 mg/ml formul2	Pembrolizumab	25.0 mg/ml
	L-histidine	0.087 mg/ml
	L-histidine hydrochloride monohydrate	0.931 mg/ml
	Trehalose d/h	90 mg/ml
	Glycine	2.0 mg/ml
	Poloxamer 188	1.2 mg/ml
Pembro 25 mg/ml formul3	Pembrolizumab	25.0 mg/ml
	L-histidine	0.087 mg/ml
	L-histidine hydrochloride monohydrate	0.931 mg/ml
	Trehalose d/h	70 mg/ml

	Glycine	2.0 mg/ml
	Poloxamer 188	0.8 mg/ml
Pembro 25 mg/ml formul4	Pembrolizumab	25.0 mg/ml
	L-histidine	0.087 mg/ml
	L-histidine hydrochloride monohydrate	0.931 mg/ml
	Trehalose d/h	70 mg/ml
	Glycine	1.0 mg/ml
	Poloxamer 188	1.2 mg/ml
Pembro 50 mg/ml formul5	Pembrolizumab	50.0 mg/ml
	L-histidine	0.087 mg/ml
	L-histidine hydrochloride monohydrate	0.931 mg/ml
	Trehalose d/h	90 mg/ml
	Glycine	2.0 mg/ml
	Poloxamer 188	0.8 mg/ml
Pembro 50 mg/ml formul6	Pembrolizumab	50.0 mg/ml
	L-histidine	0.087 mg/ml
	L-histidine hydrochloride monohydrate	0.931 mg/ml
	Trehalose d/h	70 mg/ml
	Glycine	1.0 mg/ml
	Poloxamer 188	0.8 mg/ml
Pembro 50 mg/ml formul7	Pembrolizumab	50.0 mg/ml
	L-histidine	0.087 mg/ml
	L-histidine hydrochloride monohydrate	0.931 mg/ml
	Trehalose d/h	90 mg/ml
	Glycine	1.0 mg/ml
	Poloxamer 188	1.2 mg/ml
Pembro 50 mg/ml formul8	Pembrolizumab	50.0 mg/ml
	L-histidine	0.087 mg/ml
	L-histidine hydrochloride monohydrate	0.931 mg/ml
	Trehalose d/h	70 mg/ml
	Glycine	2.0 mg/ml
	Poloxamer 188	1.2 mg/ml
Pembro 37.5 mg/ml formul9	Pembrolizumab	37.5 mg/ml
	L-histidine	0.221 mg/ml

	L-histidine hydrochloride monohydrate	0.750 mg/ml
	Trehalose d/h	80.0 mg/ml
	Glycine	1.5 mg/ml
	Poloxamer 188	1.0 mg/ml
Pembro 37.5 mg/ml formul10	Pembrolizumab	37.5 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	Trehalose d/h	80.0 mg/ml
	Glycine	1.5 mg/ml
	Poloxamer 188	1.0 mg/ml
Pembro 25 mg/ml formul11	Pembrolizumab	25.0 mg/ml
	L-histidine	0.432 mg/ml
	L-histidine hydrochloride monohydrate	0.464 mg/ml
	Trehalose d/h	70.0 mg/ml
	Glycine	1.0 mg/ml
	Poloxamer 188	0.8 mg/ml
Pembro 25 mg/ml formul12	Pembrolizumab	25.0 mg/ml
	L-histidine	0.432 mg/ml
	L-histidine hydrochloride monohydrate	0.464 mg/ml
	Trehalose d/h	90.0 mg/ml
	Glycine	2.0 mg/ml
	Poloxamer 188	0.8 mg/ml
Pembro 25 mg/ml formul13	Pembrolizumab	25.0 mg/ml
	L-histidine	0.432 mg/ml
	L-histidine hydrochloride monohydrate	0.464 mg/ml
	Trehalose d/h	70.0 mg/ml
	Glycine	2.0 mg/ml
	Poloxamer 188	1.2 mg/ml
Pembro 25 mg/ml formul14	Pembrolizumab	25.0 mg/ml
	L-histidine	0.432 mg/ml
	L-histidine hydrochloride monohydrate	0.464 mg/ml
	Trehalose d/h	90.0 mg/ml
	Glycine	1.0 mg/ml
	Poloxamer 188	1.2 mg/ml

Pembro 50 mg/ml formul15	Pembrolizumab	50.0 mg/ml
	L-histidine	0.432 mg/ml
	L-histidine hydrochloride monohydrate	0.464 mg/ml
	Trehalose d/h	90.0 mg/ml
	Glycine	1.0 mg/ml
	Poloxamer 188	0.8 mg/ml
Pembro 50 mg/ml formul16	Pembrolizumab	50.0 mg/ml
	L-histidine	0.432 mg/ml
	L-histidine hydrochloride monohydrate	0.464 mg/ml
	Trehalose d/h	70.0 mg/ml
	Glycine	1.0 mg/ml
	Poloxamer 188	1.2 mg/ml
Pembro 50 mg/ml formul17	Pembrolizumab	50.0 mg/ml
	L-histidine	0.432 mg/ml
	L-histidine hydrochloride monohydrate	0.464 mg/ml
	Trehalose d/h	90.0 mg/ml
	Glycine	2.0 mg/ml
	Poloxamer 188	1.2 mg/ml
Pembro 50 mg/ml formul18	Pembrolizumab	50.0 mg/ml
	L-histidine	0.432 mg/ml
	L-histidine hydrochloride monohydrate	0.464 mg/ml
	Trehalose d/h	70.0 mg/ml
	Glycine	2.0 mg/ml
	Poloxamer 188	0.8 mg/ml
Pembro 25 mg/ml formul19	Pembrolizumab	25.0 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	Trehalose d/h	80.0 mg/ml
	Glycine	1.5 mg/ml
	Poloxamer 188	1.0 mg/ml
Pembro 50 mg/ml formul20	Pembrolizumab	50.0 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	Trehalose d/h	80.0 mg/ml

	Glycine	1.5 mg/ml
	Poloxamer 188	1.0 mg/ml

Accelerated ageing.

Pharmaceutical compositions comprising protein at a concentration of 25, 50 and 37.5 mg/ml were prepared by diafiltration according to technique 1 and placed for accelerated storage at a temperature of 37 ± 2 °C in accordance with technique 8. The results of the study are shown in Table 31.

Table 31 – Results of study of stability at a temperature of 37 ± 2 °C

Sample info	Indicator	Initial control	2 weeks	4 weeks	Change
1. Pembro 25 mg/ml formul1 pH5.1 90Tre+ 1Gly+0.8Koll	Concentration, mg/ml	24.30	n/a	24.30	n/a
	pH	5.23	n/a	5.23	n/a
	Osmolality, mOsm/kg	311	n/a	302	n/a
	Aggregate content (SE HPLC), %	0.39	0.45	0.78	0.39
	Monomer content (SE HPLC), %	99.59	99.46	99.16	-0.43
	Acid fractions (IE HPLC), %	16.05	19.58	24.65	8.60
	Basic fractions (IE HPLC), %	69.70	65.81	58.77	-10.93
	Alkaline fractions (IE HPLC), %	14.25	14.61	16.58	2.33
	Absolute change in charged variant profile	n/a	7.78	21.86	n/a
	Monomer content, CE red. cond.	97.74	97.52	97.87	0.13
	Monomer content, CE non-red. cond.	95.81	93.42	93.02	-2.79
	Spec. activity	115	n/a	109	-6
2. Pembro 25 mg/ml formul2 pH5.1 90Tre+ 2Gly+1.2Koll	Concentration, mg/ml	24.80	n/a	23.90	n/a
	pH	5.23	n/a	5.31	n/a
	Osmolality, mOsm/kg	325	n/a	317	n/a
	Aggregate content (SE HPLC), %	0.38	0.44	0.78	0.40
	Monomer content (SE HPLC), %	99.60	99.47	99.12	-0.48
	Acid fractions (IE HPLC), %	16.08	19.59	24.85	8.77
	Basic fractions (IE HPLC), %	69.76	66.08	59.24	-10.52
	Alkaline fractions (IE HPLC), %	14.16	14.33	15.91	1.75
	Absolute change in charged variant profile	n/a	7.36	21.04	n/a

Sample info	Indicator	Initial control	2 weeks	4 weeks	Change
	Monomer content, CE red. cond.	97.63	97.64	97.75	0.12
	Monomer content, CE non-red. cond.	96.00	93.51	93.10	-2.90
	Spec. activity	109	n/a	112	3
3. Pembro 25 mg/ml formul3 pH5.1 70Tre+ 2Gly+0.8Koll	Concentration, mg/ml	24.70	n/a	24.60	n/a
	pH	5.35	n/a	5.35	n/a
	Osmolality, mOsm/kg	247	n/a	246.00	n/a
	Aggregate content (SE HPLC), %	0.39	0.46	0.73	0.34
	Monomer content (SE HPLC), %	99.59	99.46	99.20	-0.39
	Acid fractions (IE HPLC), %	16.13	19.56	24.88	8.75
	Basic fractions (IE HPLC), %	70.15	66.06	59.25	-10.90
	Alkaline fractions (IE HPLC), %	13.73	14.38	15.87	2.14
	Absolute change in charged variant profile	n/a	8.18	21.80	n/a
	Monomer content, CE red. cond.	97.67	97.78	97.8	0.13
	Monomer content, CE non-red. cond.	95.87	93.63	92.82	-3.05
	Spec. activity	100	n/a	104	4
4. Pembro 25 mg/ml formul4 pH5.1 70Tre+ 1Gly+1.2Koll	Concentration, mg/ml	25.10	n/a	24.30	n/a
	pH	5.23	n/a	5.19	n/a
	Osmolality, mOsm/kg	226	n/a	233	n/a
	Aggregate content (SE HPLC), %	0.40	0.45	0.77	0.37
	Monomer content (SE HPLC), %	99.58	99.49	99.14	-0.44
	Acid fractions (IE HPLC), %	16.26	19.45	24.81	8.55
	Basic fractions (IE HPLC), %	70.08	66.38	59.39	-10.69
	Alkaline fractions (IE HPLC), %	13.66	14.18	15.80	2.14
	Absolute change in charged variant profile	n/a	7.41	21.37	n/a
	Monomer content, CE red. cond.	97.57	97.48	97.81	0.24
	Monomer content, CE non-red. cond.	96.29	93.37	93.13	-3.16
	Spec. activity	108	n/a	93	-15
5. Pembro 50 mg/ml formul5 pH5.1 90Tre+ 2Gly+0.8Koll	Concentration, mg/ml	49.90	n/a	48.90	n/a
	pH	5.30	n/a	5.22	n/a

Sample info	Indicator	Initial control	2 weeks	4 weeks	Change
	Osmolality, mOsm/kg	329	n/a	347	n/a
	Aggregate content (SE HPLC), %	0.43	0.54	0.94	0.51
	Monomer content (SE HPLC), %	99.55	99.40	98.95	-0.60
	Acid fractions (IE HPLC), %	16.20	19.76	25.11	8.91
	Basic fractions (IE HPLC), %	70.17	66.25	59.24	-10.93
	Alkaline fractions (IE HPLC), %	13.63	13.99	15.64	2.01
	Absolute change in charged variant profile	n/a	7.83	21.84	n/a
	Monomer content, CE red. cond.	97.64	97.53	97.54	-0.10
	Monomer content, CE non-red. cond.	96.27	93.72	93.13	-3.14
	Spec. activity	116	n/a	104	-12
6. Pembro 50 mg/ml formul6 pH5.1 70Tre+ 1Gly+0.8Koll	Concentration, mg/ml	49.90	n/a	48.60	n/a
	pH	5.29	n/a	5.35	n/a
	Osmolality, mOsm/kg	246	n/a	237	n/a
	Aggregate content (SE HPLC), %	0.44	0.64	0.96	0.52
	Monomer content (SE HPLC), %	99.53	99.31	98.96	-0.57
	Acid fractions (IE HPLC), %	16.16	19.57	24.78	8.62
	Basic fractions (IE HPLC), %	69.51	66.07	58.96	-10.55
	Alkaline fractions (IE HPLC), %	14.32	14.36	16.26	1.94
	Absolute change in charged variant profile	n/a	6.89	21.11	n/a
	Monomer content, CE red. cond.	97.47	97.51	97.79	0.32
	Monomer content, CE non-red. cond.	96.36	93.59	92.94	-3.42
	Spec. activity	91	n/a	121	30
7. Pembro 50 mg/ml formul7 pH5.1 90Tre+ 1Gly+1.2Koll	Concentration, mg/ml	48.70	n/a	48.20	n/a
	pH	5.29	n/a	5.26	n/a
	Osmolality, mOsm/kg	304	n/a	309	n/a
	Aggregate content (SE HPLC), %	0.42	0.59	1.03	0.61
	Monomer content (SE HPLC), %	99.56	99.36	98.88	-0.68
	Acid fractions (IE HPLC), %	16.24	19.74	25.16	8.92

Sample info	Indicator	Initial control	2 weeks	4 weeks	Change
	Basic fractions (IE HPLC), %	69.95	66.92	58.90	-11.05
	Alkaline fractions (IE HPLC), %	13.82	13.34	15.95	2.13
	Absolute change in charged variant profile	n/a	7.01	22.10	n/a
	Monomer content, CE red. cond.	97.48	97.41	97.53	0.05
	Monomer content, CE non-red. cond.	96.32	93.76	92.86	-3.46
	Spec. activity	91	n/a	92	1
8. Pembro 50 mg/ml formul8 pH5.1 70Tre+ 2Gly+1.2Koll	Concentration, mg/ml	49.60	n/a	47.40	n/a
	pH	5.29	n/a	5.43	n/a
	Osmolality, mOsm/kg	261	n/a	253	n/a
	Aggregate content (SE HPLC), %	0.42	0.63	1.03	0.61
	Monomer content (SE HPLC), %	99.53	99.30	98.89	-0.64
	Acid fractions (IE HPLC), %	16.44	19.73	25.46	9.02
	Basic fractions (IE HPLC), %	70.67	66.47	59.57	-11.10
	Alkaline fractions (IE HPLC), %	12.89	13.80	14.98	2.09
	Absolute change in charged variant profile	n/a	8.40	22.21	n/a
	Monomer content, CE red. cond.	97.41	97.42	97.49	0.08
	Monomer content, CE non-red. cond.	96.35	93.82	92.85	-3.50
	Spec. activity	111	n/a	90	-21
9. Pembro 37.5 mg/ml formul9 pH5.6 80Tre+ 1.5Gly+1.0Koll	Concentration, mg/ml	38.80	n/a	38.10	n/a
	pH	5.63	n/a	5.60	n/a
	Osmolality, mOsm/kg	285	n/a	276	n/a
	Aggregate content (SE HPLC), %	0.45	0.56	0.97	0.52
	Monomer content (SE HPLC), %	99.53	99.38	98.95	-0.58
	Acid fractions (IE HPLC), %	16.50	20.05	26.68	10.18
	Basic fractions (IE HPLC), %	69.74	66.34	59.32	-10.42
	Alkaline fractions (IE HPLC), %	13.76	13.61	14.01	0.25
	Absolute change in charged variant profile	n/a	7.10	20.84	n/a

Sample info	Indicator	Initial control	2 weeks	4 weeks	Change
	Monomer content, CE red. cond.	97.70	97.39	97.65	-0.05
	Monomer content, CE non-red. cond.	96.25	93.88	92.85	-3.40
	Spec. activity	100	n/a	86	-14
10. Pembro 37.5 mg/ml formul10 pH5.6 80Tre+ 1.5Gly+1.0Koll	Concentration, mg/ml	38.00	n/a	37.10	n/a
	pH	5.67	n/a	5.59	n/a
	Osmolality, mOsm/kg	288	n/a	276	n/a
	Aggregate content (SE HPLC), %	0.45	0.61	0.96	0.51
	Monomer content (SE HPLC), %	99.53	99.34	98.98	-0.55
	Acid fractions (IE HPLC), %	16.38	20.17	26.58	10.20
	Basic fractions (IE HPLC), %	70.35	67.01	58.80	-11.55
	Alkaline fractions (IE HPLC), %	13.27	12.82	14.62	1.35
	Absolute change in charged variant profile	n/a	7.59	23.11	n/a
	Monomer content, CE red. cond.	97.62	97.58	97.61	-0.01
	Monomer content, CE non-red. cond.	96.38	93.69	93.36	-3.02
	Spec. activity	114	n/a	83	-31
11. Pembro 25 mg/ml formul11 pH6.1 70Tre+ 1Gly+0.8Koll	Concentration, mg/ml	25.50	n/a	25.20	n/a
	pH	6.05	n/a	5.99	n/a
	Osmolality, mOsm/kg	239	n/a	241	n/a
	Aggregate content (SE HPLC), %	0.49	0.60	1.00	0.51
	Monomer content (SE HPLC), %	99.48	99.33	98.93	-0.55
	Acid fractions (IE HPLC), %	16.30	21.95	28.93	12.63
	Basic fractions (IE HPLC), %	68.83	66.24	56.92	-11.91
	Alkaline fractions (IE HPLC), %	14.88	11.81	14.16	-0.72
	Absolute change in charged variant profile	n/a	11.31	25.26	n/a
	Monomer content, CE red. cond.	97.62	97.4	97.52	-0.10
	Monomer content, CE non-red. cond.	96.26	93.58	92.78	-3.48
	Spec. activity	108	n/a	95	-13

Sample info	Indicator	Initial control	2 weeks	4 weeks	Change
12. Pembro 25 mg/ml formul12 pH6.1 90Tre+ 2Gly+0.8Koll	Concentration, mg/ml	27.00	n/a	26.10	n/a
	pH	6.04	n/a	6.03	n/a
	Osmolality, mOsm/kg	324	n/a	313	n/a
	Aggregate content (SE HPLC), %	0.47	0.61	0.99	0.52
	Monomer content (SE HPLC), %	99.50	99.35	98.93	-0.57
	Acid fractions (IE HPLC), %	16.45	22.00	29.67	13.22
	Basic fractions (IE HPLC), %	69.37	65.53	56.93	-12.44
	Alkaline fractions (IE HPLC), %	14.18	12.47	13.40	-0.78
	Absolute change in charged variant profile	n/a	11.10	26.43	n/a
	Monomer content, CE red. cond.	97.42	97.19	97.32	-0.10
	Monomer content, CE non-red. cond.	96.30	93.53	92.51	-3.79
	Spec. activity	141	n/a	91	-50
13. Pembro 25 mg/ml formul13 pH6.1 70Tre+ 2Gly+1.2Koll	Concentration, mg/ml	26.80	n/a	26.20	n/a
	pH	6.05	n/a	6.02	n/a
	Osmolality, mOsm/kg	252	n/a	240	n/a
	Aggregate content (SE HPLC), %	0.48	0.59	1.01	0.53
	Monomer content (SE HPLC), %	99.51	99.37	98.92	-0.59
	Acid fractions (IE HPLC), %	16.70	21.71	29.45	12.75
	Basic fractions (IE HPLC), %	69.76	65.74	57.52	-12.24
	Alkaline fractions (IE HPLC), %	13.54	12.55	13.04	-0.50
	Absolute change in charged variant profile	n/a	10.03	25.50	n/a
	Monomer content, CE red. cond.	97.55	97.41	97.3	-0.25
	Monomer content, CE non-red. cond.	96.37	93.61	92.78	-3.59
	Spec. activity	93	n/a	125	32
14. Pembro 25 mg/ml formul14 pH6.1 90Tre+ 1Gly+1.2Koll	Concentration, mg/ml	27.00	n/a	26.10	n/a
	pH	6.02	n/a	6.06	n/a
	Osmolality, mOsm/kg	304	n/a	288	n/a
	Aggregate content (SE HPLC), %	0.48	0.62	1.03	0.55

Sample info	Indicator	Initial control	2 weeks	4 weeks	Change
	Monomer content (SE HPLC), %	99.50	99.32	98.90	-0.60
	Acid fractions (IE HPLC), %	16.54	21.77	29.19	12.65
	Basic fractions (IE HPLC), %	69.68	65.89	57.49	-12.19
	Alkaline fractions (IE HPLC), %	13.78	12.34	13.33	-0.45
	Absolute change in charged variant profile	n/a	10.45	25.30	n/a
	Monomer content, CE red. cond.	97.71	97.08	97.54	-0.17
	Monomer content, CE non-red. cond.	96.10	93.82	92.70	-3.40
	Spec. activity	108	n/a	117	9
15. Pembro 50 mg/ml formul15 pH6.1 90Tre+ 1Gly+0.8Koll	Concentration, mg/ml	50.80	n/a	52.20	n/a
	pH	6.09	n/a	6.22	n/a
	Osmolality, mOsm/kg	325	n/a	319	n/a
	Aggregate content (SE HPLC), %	0.59	0.79	1.38	0.79
	Monomer content (SE HPLC), %	99.39	99.16	98.55	-0.84
	Acid fractions (IE HPLC), %	16.54	21.86	29.76	13.22
	Basic fractions (IE HPLC), %	70.34	66.38	57.18	-13.16
	Alkaline fractions (IE HPLC), %	13.12	11.76	13.06	-0.06
	Absolute change in charged variant profile	n/a	10.64	26.43	n/a
	Monomer content, CE red. cond.	97.62	97.36	97.33	-0.29
	Monomer content, CE non-red. cond.	96.33	96.50	92.83	-3.50
	Spec. activity	96	n/a	106	10
16. Pembro 50 mg/ml formul16 pH6.1 70Tre+ 1Gly+1.2Koll	Concentration, mg/ml	53.80	n/a	51.40	n/a
	pH	6.07	n/a	6.05	n/a
	Osmolality, mOsm/kg	252	n/a	234	n/a
	Aggregate content (SE HPLC), %	0.54	0.81	1.28	0.74
	Monomer content (SE HPLC), %	99.43	99.15	98.65	-0.78
	Acid fractions (IE HPLC), %	16.33	22.08	29.45	13.12
	Basic fractions (IE HPLC), %	69.70	66.38	57.25	-12.45

Sample info	Indicator	Initial control	2 weeks	4 weeks	Change
	Alkaline fractions (IE HPLC), %	13.97	11.55	13.30	-0.67
	Absolute change in charged variant profile	n/a	11.50	26.25	n/a
	Monomer content, CE red. cond.	97.57	97.39	97.52	-0.05
	Monomer content, CE non-red. cond.	95.15	96.30	92.92	-2.23
	Spec. activity	116	n/a	103	-13
17. Pembro 50 mg/ml formul17 pH6.1 90Tre+ 2Gly+1.2Koll	Concentration, mg/ml	52.90	n/a	52.50	n/a
	pH	6.10	n/a	6.08	n/a
	Osmolality, mOsm/kg	336	n/a	326	n/a
	Aggregate content (SE HPLC), %	0.58	0.75	1.35	0.77
	Monomer content (SE HPLC), %	99.34	99.20	98.58	-0.76
	Acid fractions (IE HPLC), %	16.54	22.18	29.86	13.32
	Basic fractions (IE HPLC), %	70.27	65.86	56.95	-13.32
	Alkaline fractions (IE HPLC), %	13.19	11.97	13.19	0.00
	Absolute change in charged variant profile	n/a	11.27	26.64	n/a
	Monomer content, CE red. cond.	97.66	97.33	97.33	-0.33
	Monomer content, CE non-red. cond.	96.17	96.31	93.02	-3.15
	Spec. activity	91	n/a	106	15
18. Pembro 50 mg/ml formul 18 pH6.1 70Tre+ 2Gly+0.8Koll	Concentration, mg/ml	52.10	n/a	51.20	n/a
	pH	6.10	n/a	6.07	n/a
	Osmolality, mOsm/kg	269	n/a	247	n/a
	Aggregate content (SE HPLC), %	0.53	0.79	1.52	0.99
	Monomer content (SE HPLC), %	99.45	99.15	98.41	-1.04
	Acid fractions (IE HPLC), %	16.59	22.11	30.11	13.52
	Basic fractions (IE HPLC), %	70.19	65.63	56.76	-13.43
	Alkaline fractions (IE HPLC), %	13.22	12.26	13.13	-0.09
	Absolute change in charged variant profile	n/a	11.04	27.04	n/a
	Monomer content, CE red. cond.	97.65	97.51	97.40	-0.25

Sample info	Indicator	Initial control	2 weeks	4 weeks	Change
	Monomer content, CE non-red. cond.	94.04	96.32	93.03	-1.01
	Spec. activity	103	n/a	97	-6
19. Pembro 25 mg/ml formul19 pH5.6 80Tre+ 1.5Gly+1.0Koll IC	Concentration, mg/ml	26.00	n/a	25.40	n/a
	pH	5.59	n/a	5.55	n/a
	Osmolality, mOsm/kg	281	n/a	260	n/a
	Aggregate content (SE HPLC), %	0.43	0.53	0.89	0.46
	Monomer content (SE HPLC), %	99.55	99.43	99.01	-0.54
	Acid fractions (IE HPLC), %	16.08	19.88	26.16	10.08
	Basic fractions (IE HPLC), %	70.71	66.90	58.73	-11.98
	Alkaline fractions (IE HPLC), %	13.21	13.22	15.11	1.90
	Absolute change in charged variant profile	n/a	7.62	23.96	n/a
	Monomer content, CE red. cond.	97.67	97.04	97.76	0.09
	Monomer content, CE non-red. cond.	93.98	96.50	93.22	-0.76
	Spec. activity	100	n/a	104	4
20. Pembro 50 mg/ml formulation 20 pH5.6 80Tre+ 1.5Gly+1.0Koll	Concentration, mg/ml	51.70	n/a	51.30	n/a
	pH	5.65	n/a	5.61	n/a
	Osmolality, mOsm/kg	287	n/a	282	n/a
	Aggregate content (SE HPLC), %	0.50	0.64	1.08	0.58
	Monomer content (SE HPLC), %	99.48	99.32	98.82	-0.66
	Acid fractions (IE HPLC), %	16.57	20.04	26.43	9.86
	Basic fractions (IE HPLC), %	70.47	66.97	59.07	-11.40
	Alkaline fractions (IE HPLC), %	12.97	13.00	14.51	1.54
	Absolute change in charged variant profile	n/a	7.00	22.80	n/a
	Monomer content, CE red. cond.	97.71	97.07	97.55	-0.16
	Monomer content, CE non-red. cond.	94.18	96.36	93.60	-0.58
	Spec. activity	97	n/a	92.00	-5

All test formulations exhibited an acceptable change in quality parameters (purity determined by SE HPLC, CE under reducing and non-reducing conditions, charged variant profile and specific

activity), which was confirmed by Design Space using MODDE software. According to the results of the experiment space reflecting the effect of factors (buffer solution pH and excipient component content) on the responses (quality parameters), the pharmaceutical composition is stable in the provided range of concentrations, pH and excipients under accelerated aging at 37 ± 2 °C for 4 weeks.

Example 7. Determination of stability under long-term accelerated ageing.

To confirm the stability of the selected formulation, it was subjected to accelerated ageing at a temperature of 25 ± 2 °C.

Pharmaceutical compositions in the range of protein concentration (from 25 to 50 mg/ml), pH (from 5.1 to 6.1), trehalose dihydrate concentration (from 70 to 90 mg/ml), glycine concentration (from 1.0 to 2.0 mg/ml) and poloxamer 188 concentration (from 0.8 to 1.2 mg/ml) were prepared by method 1 and stored for stability test at a temperature of 25 ± 2 °C. Further, we selected, among all compositions, the most critical cases, with lowest and highest excipient content and the maximum protein content, as well as formulations with excipient content being at the central point of the range in question.

The test formulations are shown in Table 32.

Table 32 – Test formulations

Designation	Formulation	
Pembro 50 mg/ml formul6	Pembrolizumab	50.0 mg/ml
	L-histidine	0.087 mg/ml
	L-histidine hydrochloride monohydrate	0.931 mg/ml
	Trehalose d/h	70 mg/ml
	Glycine	1.0 mg/ml
	Poloxamer 188	0.8 mg/ml
Pembro 37.5 mg/ml formul9	Pembrolizumab	37.5 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	Trehalose d/h	80.0 mg/ml
	Glycine	1.5 mg/ml
	Poloxamer 188	1.0 mg/ml
Pembro 37.5 mg/ml formul10	Pembrolizumab	37.5 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	Trehalose d/h	80.0 mg/ml
	Glycine	1.5 mg/ml
	Poloxamer 188	1.0 mg/ml

Pembro 50 mg/ml formul17	Pembrolizumab	50.0 mg/ml
	L-histidine	0.432 mg/ml
	L-histidine hydrochloride monohydrate	0.464 mg/ml
	Trehalose d/h	90.0 mg/ml
	Glycine	2.0 mg/ml
	Poloxamer 188	1.2 mg/ml
Pembro 25 mg/ml formul19	Pembrolizumab	25.0 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	Trehalose d/h	80.0 mg/ml
	Glycine	1.5 mg/ml
	Poloxamer 188	1.0 mg/ml
Pembro 50 mg/ml formul20	Pembrolizumab	50.0 mg/ml
	L-histidine	0.221 mg/ml
	L-histidine hydrochloride monohydrate	0.750 mg/ml
	Trehalose d/h	80.0 mg/ml
	Glycine	1.5 mg/ml
	Poloxamer 188	1.0 mg/ml

Accelerated ageing.

Pharmaceutical compositions comprising protein at a concentration of 25, 50 and 37.5 mg/ml were prepared by diafiltration according to technique 1 and placed for accelerated storage at a temperature of 25 ± 2 °C in accordance with technique 8. The results of the study are shown in Table 33 and in Fig. 1, 2, 3, 4, 5 and 6.

Table 33 – Results of study of stability

Sample info	Indicator	Initial control	1 month	3 months	4 months	6 months
6. Pembro 50 mg/ml formul6 pH5.1 70Tre+ 1Gly+0.8Koll	Concentration, mg/ml	49.90	n/a	49.59	49.53	50.00
	pH	5.29	n/a	5.38	n/a	n/a
	Osmolality, mOsm/kg	246	n/a	261	n/a	n/a
	Aggregate content (SE HPLC), %	0.44	0.592	0.60	0.592	0.59
	Monomer content (SE HPLC), %	99.53	99.36	99.36	99.36	99.33
	Acid fractions (IE HPLC), %	16.16	17.28	21.17	22.69	26.63

Sample info	Indicator	Initial control	1 month	3 months	4 months	6 months
	Basic fractions (IE HPLC), %	69.51	66.89	63.35	63.87	59.40
	Alkaline fractions (IE HPLC), %	14.32	15.83	15.48	13.44	13.97
	Absolute change in charged variant profile	n/a	5.24	12.32	13.06	20.93
	Monomer content, CE red. cond.	97.47	97.76	97.47	97.58	96.95
	Monomer content, CE non-red. cond.	96.36	93.52	96.95	96.041	96.48
	Spec. activity	91	n/a	96	n/a	110
9. Pembro 37.5 mg/ml formul9 pH5.6 80Tre+ 1.5Gly+1.0Koll	Concentration, mg/ml	38.80	n/a	38.69	38.24	38.50
	pH	5.63	n/a	5.74	n/a	n/a
	Osmolality, mOsm/kg	285	n/a	287.00	n/a	n/a
	Aggregate content (SE HPLC), %	0.45	0.59	0.61	0.59	0.60
	Monomer content (SE HPLC), %	99.53	99.34	99.34	99.34	99.32
	Acid fractions (IE HPLC), %	16.50	17.24	22.21	22.77	28.21
	Basic fractions (IE HPLC), %	69.74	67.36	63.91	63.11	59.62
	Alkaline fractions (IE HPLC), %	13.76	15.40	13.88	14.12	12.17
	Absolute change in charged variant profile	n/a	4.76	11.67	13.26	23.42
	Monomer content, CE red. cond.	97.70	97.83	97.45	97.62	96.87
	Monomer content, CE non-red. cond.	96.25	93.40	96.86	97.41	96.42
	Spec. activity	100	n/a	92	n/a	114
10. Pembro 37.5 mg/ml formul10 pH5.6 80Tre+ 1.5Gly+1.0Koll -	Concentration, mg/ml	38.00	n/a	35.37	38.05	38.00
	pH	5.67	n/a	5.68	n/a	n/a
	Osmolality, mOsm/kg	288	n/a	280.00	n/a	n/a
	Aggregate content (SE HPLC), %	0.45	0.625	0.61	0.625	0.61
	Monomer content (SE HPLC), %	99.53	99.15	99.35	99.15	99.32

Sample info	Indicator	Initial control	1 month	3 months	4 months	6 months
	Acid fractions (IE HPLC), %	16.38	17.54	22.17	23.76	28.19
	Basic fractions (IE HPLC), %	70.35	67.36	64.24	64.12	59.51
	Alkaline fractions (IE HPLC), %	13.27	15.10	13.59	12.12	12.30
	Absolute change in charged variant profile	n/a	5.99	12.23	14.77	23.62
	Monomer content, CE red. cond.	97.62	97.82	97.43	97.57	97.08
	Monomer content, CE non-red. cond.	96.38	93.64	96.92	97.85	96.18
	Spec. activity	114	n/a	87	n/a	93
17. Pembro 50 mg/ml formul17 pH6.1 90Tre+ 2Gly+1.2Koll	Concentration, mg/ml	52.90	n/a	52.36	52.69	52.00
	pH	6.10	n/a	6.16	n/a	n/a
	Osmolality, mOsm/kg	336	n/a	337.00	n/a	n/a
	Aggregate content (SE HPLC), %	0.58	0.76	0.86	0.76	0.90
	Monomer content (SE HPLC), %	99.34	99.18	99.10	99.18	99.03
	Acid fractions (IE HPLC), %	16.54	18.22	24.92	27.67	32.81
	Basic fractions (IE HPLC), %	70.27	65.97	62.69	61.04	57.28
	Alkaline fractions (IE HPLC), %	13.19	15.81	12.39	11.30	9.91
	Absolute change in charged variant profile	n/a	8.61	16.76	22.25	32.55
	Monomer content, CE red. cond.	97.66	97.79	97.20	97.47	96.78
	Monomer content, CE non-red. cond.	96.17	93.29	96.72	96.17	95.84
	Spec. activity	91	n/a	110	n/a	82
19. Pembro 25 mg/ml formul19 pH5.6 80Tre+ 1.5Gly+1.0Koll IC	Concentration, mg/ml	26.00	n/a	26.00	25.76	25.65
	pH	5.59	n/a	5.60	n/a	n/a
	Osmolality, mOsm/kg	281	n/a	290.00	n/a	n/a
	Aggregate content (SE HPLC), %	0.43	0.53	0.53	0.53	0.50

Sample info	Indicator	Initial control	1 month	3 months	4 months	6 months
	Monomer content (SE HPLC), %	99.55	99.41	99.44	99.41	99.42
	Acid fractions (IE HPLC), %	16.08	17.30	22.15	23.85	28.08
	Basic fractions (IE HPLC), %	70.71	67.01	64.15	64.31	59.79
	Alkaline fractions (IE HPLC), %	13.21	15.69	13.70	11.85	12.13
	Absolute change in charged variant profile	n/a	7.40	13.13	15.53	24.00
	Monomer content, CE red. cond.	97.67	97.62	97.36	97.31	96.98
	Monomer content, CE non-red. cond.	93.98	93.28	96.86	96.56	96.25
	Spec. activity	100	n/a	125	n/a	117
20. Pembro 50 mg/ml formulation 20 pH5.6 80Tre+ 1.5Gly+1.0Koll	Concentration, mg/ml	51.70	n/a	52.59	51.8	52.20
	pH	5.65	n/a	5.65	n/a	n/a
	Osmolality, mOsm/kg	287	n/a	304.00	n/a	n/a
	Aggregate content (SE HPLC), %	0.50	0.692	0.69	0.692	0.71
	Monomer content (SE HPLC), %	99.48	99.25	99.26	99.25	99.22
	Acid fractions (IE HPLC), %	16.57	17.32	22.11	24.24	28.31
	Basic fractions (IE HPLC), %	70.47	67.58	64.30	63.90	59.97
	Alkaline fractions (IE HPLC), %	12.97	15.10	13.59	11.86	11.72
	Absolute change in charged variant profile	n/a	5.77	12.33	15.35	23.49
	Monomer content, CE red. cond.	97.71	97.63	97.39	97.34	96.92
	Monomer content, CE non-red. cond.	94.18	92.83	96.78	96.03	96.19
	Spec. activity	97	n/a	109	n/a	99

All pharmaceutical compositions demonstrated an acceptable level of changes during accelerated storage.

A pharmaceutical composition containing histidine buffer solution in the pH range from 5.1 to 6.1, trehalose dihydrate from 70 to 90 mg/ml, L-glycine from 1.0 to 2.0 mg/ml and poloxamer 188 from 0.8 to 1.2 mg/ml demonstrated an acceptable level of aggregation (increase in aggregates over 6 months at 25 ± 2 °C was no more than 0.32%), as well as a small change in the acid-base profile (change in the basic fraction content of no more than 13.0%) and a slight change in specific activity under accelerated aging both at a concentration of the monoclonal antibody Pembrolizumab of 25 mg/ml and at an increased concentration of up to 50 mg/ml.

The addition of trehalose dihydrate contributed to an increase in the aggregation temperature and melting point of pembrolizumab. We also observed the least change in quality parameters under thermal stress, slight changes in quality parameters following shaking and freezing. Further, glycine has a positive effect on aggregation temperature and melting point, as well as on diffusion interaction parameter. The positive effect of glycine on protein quality parameters under acid and basic hydrolysis was observed. The addition of poloxamer 188 to the formulation contributes to stabilization of the test protein under shaking and freezing.

According to the results of stress stability testing, the developed formulation of Pembrolizumab excipients showed significant advantages over the Keytruda formulation under thermal stress, acid and basic hydrolysis; also, we observed a significantly lower tendency to oxidation under photo stress, which has a significant effect on the structure and specific activity of the protein, and an increased tendency to aggregation.

We provided examples of studies using aqueous pharmaceutical compositions of pembrolizumab. The aqueous pharmaceutical composition used for further study is described in Table 34.

Table 34 – Aqueous pharmaceutical composition of pembrolizumab.

Component	In 1.0 ml	In vial
Pembrolizumab	25 mg	100 mg
L-histidine	0.221 mg	0.884 mg
L-histidine hydrochloride monohydrate	0.750 mg	3.0 mg
Trehalose dihydrate	80 mg	320 mg
Glycine	1.5 mg	6.0 mg
Poloxamer 188	1.0 mg	4.0 mg
Water for injection	to 1 ml	to 4 ml

The experiments revealed that the formulation of the Keytruda product has several disadvantages as follows: 1) insufficient colloidal stability of antibody, 2) insufficient stability under thermal stress, 3) low stability of charged variant profile under mechanical stress and freezing.

Within the framework of the present invention, pharmaceutical compositions have been produced that have sufficient colloidal and thermal stability, as well as high stability of charged variant profile under mechanical stress and freezing. In addition, pharmaceutical compositions containing trehalose and poloxamer 188 in formulations thereof (for example, the formulation indicated in Table 34) are further characterized by a low tendency to form high-molecular weight impurities under long-term storage, as well as a low tendency to oxidation, loss of specific activity and increase in impurities under photo stress and long-term storage.

The resulting data related to the stability of pharmaceutical compositions indicate the compatibility of excipients with each other and with the active substance.

Claims:

1. A pharmaceutical composition of pembrolizumab comprising:

(i) pembrolizumab;

(ii) histidine

(iii) histidine hydrochloride monohydrate;

(iv) glycine;

(v) trehalose and poloxamer 188,
or proline; and

(vi) water for injection.

2. The pharmaceutical composition according to claim 1, wherein pembrolizumab is present at a concentration of 5-50 mg/ml.

3. The pharmaceutical composition according to claim 1, wherein histidine is present at a concentration of 0.087-0.432 mg/ml.

4. The pharmaceutical composition according to claim 1, wherein histidine hydrochloride monohydrate is present at a concentration of 0.464-0.931 mg/ml.

5. The pharmaceutical composition according to claim 1, wherein glycine is present at a concentration of 1-2 mg/ml.

6. The pharmaceutical composition according to claim 1, wherein trehalose is present at a concentration of 70-130 mg/ml.

7. The pharmaceutical composition according to claim 1, wherein poloxamer 188 is present at a concentration of 0.8-1.2 mg/ml.

8. The pharmaceutical composition according to claim 1, wherein proline is present at a concentration of 20-34 mg/ml.

9. The pharmaceutical composition according to claim 1, comprising:

(i) 5-50 mg/ml pembrolizumab;

(ii) 0.087-0.432 mg/ml histidine;

(iii) 0.464-0.931 mg/ml histidine hydrochloride monohydrate;

(iv) 1-2 mg/ml glycine;

(v) 70-130 mg/ml trehalose and 0.8-1.2 mg/ml poloxamer 188, or
20-34 mg/ml of proline; and

(vi) water for injection to 1 ml.

10. The pharmaceutical composition according to claim 9, wherein pembrolizumab is present at a concentration of 20-30 mg/ml.

11. The pharmaceutical composition according to claim 10, wherein pembrolizumab is present at a concentration of 25 mg/ml.

12. The pharmaceutical composition according to claim 9, wherein histidine is present at a concentration of 0.200-0.319 mg/ml.

13. The pharmaceutical composition according to claim 12, wherein histidine is present at a concentration of 0.221 mg/ml.

14. The pharmaceutical composition according to claim 9, wherein histidine hydrochloride monohydrate is present at a concentration of 0.650-0.850 mg/ml.

15. The pharmaceutical composition according to claim 14, wherein histidine hydrochloride monohydrate is present at a concentration of 0.750 mg/ml.

16. The pharmaceutical composition according to claim 9, wherein glycine is present at a concentration of 1.5 mg/ml.

17. The pharmaceutical composition according to claim 9, wherein trehalose is present at a concentration of 70-100 mg/ml.

18. The pharmaceutical composition according to claim 17, wherein trehalose is present at a concentration of 75-85 mg/ml.

19. The pharmaceutical composition according to claim 18, wherein trehalose is present at a concentration of 80 mg/ml.

20. The pharmaceutical composition according to claim 1, wherein trehalose is trehalose dihydrate.

21. The pharmaceutical composition according to claim 9, wherein poloxamer 188 is present at a concentration of 1.0 mg/ml.

22. The pharmaceutical composition according to claim 9, wherein proline is present at a concentration of 24-30 mg/ml.

23. The pharmaceutical composition according to claim 22, wherein proline is present at a concentration of 27 mg/ml.

24. The pharmaceutical composition according to claim 1, wherein the composition has pH 5.1-6.1.

25. The pharmaceutical composition according to claim 24, wherein the composition has pH 5.6.

26. The pharmaceutical composition according to claim 1, comprising:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 80 mg/ml trehalose and 1.0 mg/ml poloxamer 188;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7.

27. The pharmaceutical composition according to claim 20, comprising:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 80 mg/ml trehalose dihydrate and 1.0 mg/ml poloxamer 188;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7.

28. The pharmaceutical composition according to claim 1, comprising:

(i) 25 mg/ml pembrolizumab;

(ii) 0.221 mg/ml histidine;

(iii) 0.750 mg/ml histidine hydrochloride monohydrate;

(iv) 1.5 mg/ml glycine;

(v) 27 mg/ml proline;

(vi) water for injection to 1 ml; and

wherein the composition has pH 5.5 – 5.7.

29. The pharmaceutical composition according to any of claims 26-28, wherein the composition has pH 5.6.

30. A pharmaceutical composition of pembrolizumab produced by lyophilization of the pharmaceutical composition of pembrolizumab according to any of claims 1-29.

31. Use of the pharmaceutical composition of pembrolizumab according to any of claims 1-30 for treating a malignant neoplasm or infectious disease.

32. The use according to claim 31, wherein the malignant neoplasm is selected from the group: melanoma, non-small cell lung cancer, small cell lung cancer, head and neck cancer, primary mediastinal large B-cell lymphoma, urothelial cancer, stomach cancer, high microsatellite instability/DNA mismatch repair deficient (MMR) malignant neoplasms, hepatocellular cancer, cervical cancer, Merkel cell carcinoma, renal cell carcinoma, endometrial cancer, esophageal cancer, squamous cell skin cancer, basal cell carcinoma, breast cancer, colorectal cancer, prostate cancer, thyroid cancer, bladder cancer, non-Hodgkin lymphoma, Hodgkin lymphoma, pancreatic cancer, ovarian cancer, gallbladder cancer, malignant brain tumor, glioblastoma, tumor with high mutational burden.

33. Use of the pharmaceutical composition of pembrolizumab according to any of claims 1-30 for producing a medicinal product intended for treating a malignant neoplasm or infectious disease.

34. The use according to claim 33, wherein the malignant neoplasm is selected from the group: melanoma, non-small cell lung cancer, small cell lung cancer, head and neck cancer, primary mediastinal large B-cell lymphoma, urothelial cancer, stomach cancer, high microsatellite instability/DNA mismatch repair deficient (MMR) malignant neoplasms, hepatocellular cancer, cervical cancer, Merkel cell carcinoma, renal cell carcinoma, endometrial cancer, esophageal cancer, squamous cell skin cancer, basal cell carcinoma, breast cancer, colorectal cancer, prostate cancer, thyroid cancer, bladder cancer, non-Hodgkin lymphoma, Hodgkin lymphoma, pancreatic cancer, ovarian cancer, gallbladder cancer, malignant brain tumor, glioblastoma, tumor with high mutational burden.