Hyaluronidases are enzymes that degrade hyaluronic acid present in the extracellular matrix. It is known that there are six types of hyaluronidases in humans: Hyall, Hyal2, Hyal3, Hyal4, HyalPS1, and PH20/SPAM1. PH20/SPAM1 (hereinafter referred to as PH20) is expressed in the sperm plasma membrane and the acrosomal membrane.

Hyaluronidase hydrolyzes hyaluronic acid, thereby reducing the viscosity of hyaluronic acid in the extracellular matrix and increasing the permeability thereof into tissue (skin). The subcutaneous area of the skin has a neutral pH of about 7.0 to 7.5. Thus, among the various types of hyaluronidases, PH20 is widely used (Bookbinder et al., 2006). In examples in which PH20 is used, PH20 is often co-administered with an antibody therapeutic agent which is injected subcutaneously (Bookbinder et al., 2006).

The stability of a formulation of an antibody and enzyme is complex and confounded by multiple factors such as function of stability of the individual enzyme or antibody in the co-formulation matrix, impact of the presence of the additional excipients, and specific interaction of the antibody and enzyme. Often times, high concentration of antibody formulated with an enzyme, may contribute to other properties of the product which would be undesirable, e.g. low injectability due to increased viscosity and higher than physiological osmolality and increased aggregation. As a consequence, the need exists for stable formulations of an anti-PD-1 antibody and PH20 or PH20 variants for subcutaneous administration. Such stable formulations will preferably exhibit stability over months to years under conditions typical for storage of drugs for self-administration, *i.e.* at refrigerator temperature in a syringe, container or other device, resulting in a long shelf-life for the corresponding drug product.

SUMMARY ~~OF THE INVENTION~~

The ~~invention~~disclosure provides a formulation, comprising:

a) about 20 mg/mL to about 200 mg/mL of an anti-human PD-1 antibody, or antigen binding fragment thereof; b) about 0.0009 – 0.050 mg/ml of a PH20 variant or fragment thereof; c) a buffer; d) a non-reducing dissacharide; e) a non-ionic surfactant; and, optionally f) an anti-oxidant.

In one ~~embodiment~~example, the formulation comprises:

a) about 20 mg/mL to about 200 mg/mL of an anti-human PD-1 antibody, or antigen binding fragment thereof;

b) about 0.0009 – 0.050 mg/ml of a PH20 variant or fragment thereof;

c) about 5 mM to about 20 mM buffer;

d) about 3% to about 10% weight/volume (w/v) of a non-reducing dissacharide selected from the group consisting of sucrose and trehalose;

e) about 0.005 % to about 0.10% non-ionic surfactant; and, optionally

f) about 1 mM to about 30 mM anti-oxidant.

In one aspect, the disclosure provides a formulation comprising:

a) about 50 mg/mL to about 175 mg/mL of an anti-human PD-1 antibody, or antigen binding fragment thereof;

b) about 0.0009 – 0.050 mg/ml of a PH20 variant or fragment thereof;

c) about 5 mM to about 20 mM buffer;

d) about 3% to about 10% weight/volume (w/v) of a non-reducing dissacharide selected from the group consisting of sucrose and trehalose;

e) about 0.005 % to about 0.10% (w/v) non-ionic surfactant; and, optionally

f) about 1 mM to about 30 mM anti-oxidant;

wherein the anti-human PD-1 antibody or antigen binding fragment thereof comprises a light chain variable region comprising three light chain CDRs comprising CDRL1 of SEQ ID NO:1, CDRL2 SEQ ID NO:2 and CDRL3 of SEQ ID NO:3 and a heavy chain variable region comprising three heavy chain CDRs of CDRH1 of SEQ ID NO:6, CDRH2 of SEQ ID NO:7 and CDRH3 SEQ ID NO:8; wherein the PH20 variant has amino acid residue substitutions including M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, and I361T in SEQ ID NO: 21, and the fragment thereof has either an N-terminus deletion of amino acid residues 1-36, 1-37, 1-38, 1-39, 1-40, 1-41, or 1-42 of SEQ ID NO: 21 and/or a C-terminus deletion of amino acid residues 455-509, 456-509, 457-509, 458-509, 459-509, 460-509, 461-509, 462-509, 463-509, 464-509, 465-509, 466-509, 467-509, 468-509, 469-509, 470-509, 471-509, 472-509, 473-509, 474-509, 475-509, 476-509, 477-509, 478-509, 479-509, 480-509, 481-509, 482-509, 483-509, 484-509, 485-509, 486-509, 487-509, 488-509, 489-509, 490-509, 491-509, 492-509, 493-509, 494-509, 495-509, 496-509, 497-509, 498-509, 499-509, 500-509, 501-509, 502-509, 503-509, 504-509, 505-509, 506-509, 507-509, 508-509, or 509, wherein the numbering is by reference to SEQ ID NO: 21.

Surprisingly, certain ~~embodiment~~examples of the formulations of the ~~invention~~disclosure have increased hyaluronidase activity of the PH20 variant or fragment thereof compared to the corresponding PH20 variant or fragment thereof alone in the same formulation after 1 or 3 months storage at 25 °C; after 6 months at 5 °C and after 3 months at 25 °C under stainless steel stress; and under light stress. The ~~invention~~disclosure can be a liquid formulation or a liquid formulation that is reconstituted from a lyophilized formulation.

In specific ~~embodiment~~examples of the ~~invention~~disclosure, the anti-PD-1 antibody is pembrolizumab or an antigen binding fragment of pembrolizumab. In specific embodiments of the ~~invention~~disclosure, the PH20 variant or fragment is PH20 variant fragment 2 set forth in the amino acid sequence of SEQ ID NO: 23.

Also provided herein are methods of treating cancer and methods of treating chronic infection in a human patient in need thereof comprising: administering an effective amount of the formulations of the ~~invention~~disclosure to the patient. In another aspect, the disclosure provides use of the formulation as defined therein in the manufacture of a medicament for the treatment of cancer. In another aspect, the disclosure provides the formulation as defined herein for use in the treatment of cancer in a human patient.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Temperature-dependent viscosity profiles at 5 and 25 °C for formulations with AK03 (pembrolizumab only at 100 mg/ml) and AK10-AK15 (pembrolizumab and PH20 variant fragment 2 at different concentrations)

Figure 2. % High Molecular Weight Species (HMWS) as measured by UP-SEC of formulations AK03 (pembrolizumab only at 100 mg/ml) and AK10-AK15 (pembrolizumab and PH20 variant fragment 2 at different concentrations) at 5, 25 and 40 °C at initial, 1 month, 3 months and 6 months, respectively.

Figure 3. Acidic Variants as measured by HP-IEX of formulations AK03 (pembrolizumab only at 100 mg/ml) and AK10-AK15 (pembrolizumab and PH20 variant fragment 2 at different concentrations) at 5, 25 and 40 °C at initial, 1 month, 3 months and 6 months, respectively.

Figure 4. Main Variants as measured by HP-IEX of formulations AK03 (pembrolizumab only at 100 mg/ml) and AK10-AK15 (pembrolizumab and PH20 variant fragment 2 at different concentrations) at 5, 25 and 40 °C at initial, 1 month, 3 months and 6 months, respectively.

Figure 5. Basic Variants as measured by HP-IEX of formulations AK03 (pembrolizumab only at 100 mg/ml) and AK10-AK15 (pembrolizumab and PH20 variant fragment 2 at different concentrations) at 5, 25 and 40 °C at initial, 1 month, 3 months and 6 months, respectively.

Figure 6. Met[105] Oxidation species (the % of pre-peak 1 +2) as measured by HP-HIC in pembrolizumab of formulations AK03 (pembrolizumab only at 100 mg/ml) and AK10-AK15