COMPOSITIONS AND METHODS FOR CANCER IMMUNOTHERAPY

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/860,182, filed on June 11, 2019; 62/932,160, filed on November 7, 2019 and 62/924,356, filed on October 22, 2019. The entire teachings of the above applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Interleukin-2 (IL-2) is a cytokine that induces proliferation of antigen-activated T cells and stimulates natural killer (NK) cells. The biological activity of IL-2 is mediated through a multi-subunit IL-2 receptor complex (IL-2R) of three polypeptide subunits that span the cell membrane: p55 (IL-2Rα, the alpha subunit, also known as CD25 in humans), p75 (IL-2R13, the beta subunit, also known as CD122 in humans) and p64 (IL-2Rγ, the gamma subunit, also known as CD132 in humans). T cell response to IL-2 depends on a variety of factors, including: (1) the concentration of IL-2; (2) the number of IL-2R molecules on the cell surface; and (3) the number of IL-2R occupied by IL-2 (i.e., the affinity of the binding interaction between IL-2 and IL-2R). The IL-2:IL-2R complex is internalized upon ligand binding and the different components undergo differential sorting. IL-2Rα is recycled to the cell surface, while IL-2 associated with the IL-2:IL-2Rβγ complex is routed to the lysosome and degraded.

Outcomes of systemic IL-2 administration in cancer patients are poor. While 15 to 20 percent of patients respond objectively to high-dose IL-2, the great majority do not, and many suffer severe, life-threatening side effects. Aldesleukin (recombinant human IL-2 (rhIL-2) also as known as Proleukin), is approved and used for the treatment of metastatic melanoma and RCC.

Among currently used therapies, rhIL-2 is one of the few treatment regimens that elicit a complete and durable response in a subset of patients, up to 12% in melanoma and 7% in RCC. High doses of rhIL-2 are required to stimulate cells that express the intermediate-affinity IL-2 receptor, including memory $CD8^+$ T cells and natural killer (NK) cells, which are the primary cell types mediating anticancer immune responses.

It has been hypothesized that a contributing factor limiting the therapeutic efficacy of rhIL-2 is that it preferentially activates and induces the expansion of immunosuppressive $CD4^+$ T_{regs}, which can counteract anticancer immune responses. This preferential activation is through binding of IL-2 to the high-affinity IL-2 receptor expressed on T_{regs}. Furthermore, it is hypothesized that direct interaction between rhIL-2 with high-affinity IL-2R expressed on vascular and pulmonary endothelial cells contributes to rhIL-2-mediated toxicity via capillary leak syndrome. Despite the poor tolerability of immunotherapy with rhIL-2, it remains one of the few treatment regimens for metastatic melanoma and RCC that elicits a complete and durable response in a subset of patients. Accordingly, novel IL-2 therapies are needed to more effectively combat various cancers.

SUMMARY OF THE INVENTION

The compositions, methods and treatment regimens in accordance with the invention provide numerous advantages for the treatment of cancer using IL-2 immunotherapy as compared to, for example, high dose rhIL-2 therapy (e.g. Aldesleukin). It has been discovered that administration of the fusion protein of SEQ ID NO: 1 when administered at doses of about 6 µg/kg/day to about 70 µg/kg/day and preferably at about 6 µg/kg/day to about 15 µg/kg/day or at a corresponding fixed per day dose based, for example, on an average 60-70 kg adult human (e.g. 0.4 mg/day to about 1-4 mg) or based on a child, for example a child of about 12 kg to 50 kg or more, provides a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in circulating T regulatory (Treg) cells. Preferably, the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) is greater in a patient administered the fusion protein of SEQ ID NO:1 in accordance with the methods of the invention as compared to the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) in a patient receiving, for example, high dose recombinant human IL-2 (rhIL-2) treatment. It has also been discovered that administration of the fusion protein of SEQ ID NO: 1 at doses equivalent to, or higher than, those of high dose IL-2 is not accompanied by the toxicity and side effects, such as capillary leak syndrome (CLS), that often accompany administration of high dose rhIL-2.

Disclosed herein is the use of the fusion protein of SEQ ID NO: 1 in the manufacture of a medicament for treating cancer in a patient, wherein the fusion protein is to be administered at a dose of at least 6 µg/kg/day to 15 µg/kg per day.

Disclosed herein is the use of the fusion protein of SEQ ID NO: 1 in the manufacture

of a medicament for treating cancer in a patient, wherein a dose of at 16 µg/kg/day to 70 µg/kg/day or a corresponding fixed dose based on an 60-70 kg adult or 12 kg to 50 kg or more child is to be intravenously administered to the patient.

Preferably, the invention provides methods of treating cancer in a patient comprising administering to the patient a dose of at least about 6 µg/kg/day to about 15 µg/kg per day, or a corresponding fixed dose thereof based, for example, on a 60-70 kg adult (e.g. ~0.4 mg/day to about ~1.0 mg/day), or a corresponding fixed dose based on a child, for example, a child of about 12 kg to about 50 kg. Preferably the dose of the fusion protein of SEQ ID NO: 1 in terms of µg/kg/day is a dose of about: 6 µg/kg/day, 8 µg/kg/day, 10 µg/kg/day, 12 µg/kg/day, 14 µg/kg/day, or 15 µg/kg/day or a corresponding fixed dose thereof based on, for example, a 60-70 kg adult or based on a child, for example a child of about 12 kg to 50 kg or more. Preferably administration of the fusion protein of SEQ ID NO: 1 results in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in T regulatory (Treg) cells. Preferably the increase in circulating NK cells and CD8+ cells is at least 2-fold over baseline prior to administration of the fusion protein of SEQ ID NO: 1 to the patient. Preferably the increase in circulating NK cells and CD8+ cells is greater relative to the increase in circulating Treg cells. Preferably an increase in circulating NK cells and CD8+ cells is greater relative to the increase in circulating Treg cells as compared to the increase in circulating NK cells and CD8+ cells relative to the increase in circulating Treg cells in a patient receiving high dose rhIL-2 treatment.

Preferably the patient has an improved safety profile as compared to a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment. Preferably the patient has a lower risk of capillary leak syndrome or cytokine release syndrome. Preferably the resulting dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in circulating T regulatory (Treg) cells. Preferably the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) is greater as compared to the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) in a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment and the patient has a lower risk of capillary leak syndrome. Preferably the dose the fusion protein of SEQ ID NO: 1 is administered by intravenous injection or infusion.

Preferably, the fusion protein of SEQ ID NO: 1 is administered by intravenous injection or infusion at a dose of at least about 6 µg/kg to about 15 µg/kg per day for 1 to about 5 consecutive or non-consecutive days followed by a rest period of at least about 9

consecutive days. Preferably, the rest period is at least about 16 days. Preferably the fusion protein of SEQ ID NO: 1 is administered in at least two courses of treatment, the first course of treatment comprising administration by intravenous injection or infusion at a dose of at least about 6 µg/kg to about 15 µg/kg per day for a period 1 to about 5 consecutive or non-consecutive days followed by a rest period of at least about 9 consecutive days resulting in a total 14 day course followed by a second course of treatment comprising administering by intravenous injection or infusion at a dose of at least about 6 µg/kg to about 15 µg/kg per day for 1 to about 5 consecutive or non-consecutive days, followed by a rest period of at least about 16 consecutive days resulting in a total 21 day course. Preferably the second course of treatment begins within about 24 hours or more after completion of the first course of treatment. Preferably a third 21-day course of treatment follows the second course of administration. Preferably, the third course of treatment begins within about 24 hours or more after completion of the second course of treatment. Preferably a fourth 21 day course of treatment follows the third course of administration. Preferably, the fourth course of treatment begins within about 24 hours or more after completion of the third course of treatment.

Preferably administering the fusion protein of SEQ ID NO: 1 further comprises co-administering to the patient a therapeutically effective amount of a therapeutic agent. Preferably the therapeutic agent is an immune checkpoint inhibitor or a PARP inhibitor. Preferably the therapeutic agent is an immune checkpoint inhibitor. Preferably the immune checkpoint inhibitor inhibits the interaction of PD-1 and PD-L. Preferably the immune checkpoint inhibitor is pembrolizumab.

Preferably the fusion protein of SEQ ID NO: 1 is administered in at least two courses of treatment, the first course of treatment comprising administration by intravenous injection or infusion at a dose of at least about 6 µg/kg to about 15 µg/kg per day for 1 to about 5 consecutive or non-consecutive days followed by a rest period of at least about 16 consecutive days resulting in a total 21 day course followed by a second course of treatment comprising administering by intravenous injection or infusion at a dose of at least about 6 µg/kg to about 15 µg/kg per day for a period of at least 5 consecutive days, followed by a rest period of at least about 16 consecutive days resulting in a total 21 day course and wherein the pembrolizumab is co-administered on the first day of the first course of and on the first day of the second course; wherein the pembrolizumab is co-administered prior to, simultaneously with, or subsequent to, administration of the fusion protein of SEQ ID NO:1 preferably wherein the pembrolizumab is co-administered in a separate composition from the

fusion protein of SEQ ID NO: 1. Preferably pembrolizumab is co-administered in an amount of 200 mg by I.V. injection or infusion.

Preferably all methods of the invention result in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in T regulatory (Treg) cells. Preferably the patient has an improved safety profile as compared to a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment; and preferably the patient has a lower risk of cytokine release syndrome as compared to a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment. Preferably the patient has a lower risk of capillary leak syndrome as compared to a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment.

The invention also provides methods of treating cancer in a patient comprising administering to the patient a dose of at least about 6 µg/kg to about 15 µg/kg per day of the fusion protein of SEQ ID NO: 1 wherein the dose is administered by intravenous injection or infusion for 1 to about 5 consecutive or non-consecutive days followed by a rest period of at least about 9 consecutive days resulting in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in T regulatory (Treg) cells; and wherein the increase in circulating NK cells and CD8+ cells is at least 2 fold over baseline prior to administration of the fusion protein of SEQ ID NO: 1 to the patient; preferably wherein the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) is greater as compared to the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) in a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment.

The invention also provides methods of treating cancer in a patient comprising administering to the patient a dose of at least about 6 µg/kg to about 15 µg/kg per day of the fusion protein of SEQ ID NO: 1 wherein the dose is administered by intravenous injection or infusion for 1 to about 5 consecutive or non-consecutive days followed by a rest period of at least about 9 consecutive days resulting in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in T regulatory (Treg) cells and wherein the patient has an improved safety profile as compared to a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment; preferably wherein the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) is greater as compared to the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) in a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment.

The invention also provides methods of treating cancer in a patient comprising administering to the patient a dose of at least about 6 µg/kg to about 15 µg/kg per day of the fusion protein of SEQ ID NO: 1 wherein the dose is administered by intravenous injection or infusion for 1 to about 5 consecutive or non-consecutive days followed by a rest period of at least about 9 consecutive days resulting in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in T regulatory (Treg) cells and wherein the patient has a lower risk of cytokine release syndrome as compared to a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment ; preferably wherein the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) is greater as compared to the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) in a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment.

The invention also provides methods of treating cancer in a patient comprising administering to the patient a dose of at least about 6 µg/kg to about 15 µg/kg per day of the fusion protein of SEQ ID NO: 1 wherein the dose is administered by intravenous injection or infusion for 1 to about 5 consecutive or non-consecutive days followed by a rest period of at least about 9 consecutive days resulting in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in T regulatory (Treg) cells and lower risk of cytokine release syndrome as compared to a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment preferably wherein the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) is greater as compared to the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) in a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment.

The invention also provides pharmaceutical compositions comprising about 6 µg/kg to about 70 µg/kg and preferably about 6 µg/kg to about 15 µg/kg of the fusion protein of SEQ ID NO: 1; preferably wherein the pharmaceutical compositions comprise about 8 µg/kg to about 15 µg/kg of the fusion protein of SEQ ID NO: 1, preferably wherein the pharmaceutical composition comprises about 8 µg/kg of the fusion protein of SEQ ID NO: 1; preferably wherein the pharmaceutical composition comprises about 10 µg/kg of the fusion protein of SEQ ID NO: 1; preferably wherein the pharmaceutical composition comprises about 12 µg/kg of the fusion protein of SEQ ID NO: 1; preferably wherein the fusion protein comprises about 14 µg/kg of the fusion protein of SEQ ID NO: 1; and preferably wherein the fusion protein comprises about 15 µg/kg of the fusion protein of SEQ ID NO: 1.

The invention also provides a method of treating cancer in a patient comprising administering to the patient a dose of at least about 50 µg/kg to about 60 µg/kg per day or at a corresponding fixed per day dose based on an average 60-70 kg adult human (e.g. ~3.0 mg/day to about ~3.6 mg/day) of the fusion protein of SEQ ID NO: 1 wherein the dose is administered by intravenous injection or infusion one day per week resulting in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in circulating T regulatory (Treg) cells and wherein the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) is greater compared to the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) in a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment and wherein the patient has a lower risk of capillary leak syndrome as compared to a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment.

Disclosed herein is the use of the fusion protein of SEQ ID NO: 1 in the manufacture of a medicament for treating cancer in a patient, wherein the fusion protein is to be administered to the patient at a dose of at least 50 µg/kg to 60 µg/kg per day once weekly, resulting in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in circulating T regulatory (Treg) cells, and wherein the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) is greater compared to the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) in a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment, and wherein the patient has a lower risk of capillary leak syndrome as compared to a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment, optionally wherein the dose is to be administered by intravenous injection or infusion.

The invention also provides a pharmaceutical composition comprising at least about 6 µg/kg to about 70 µg/kg and preferably at least about 6 µg/kg to about 15 µg/kg, or at a corresponding fixed per day dose based on an average 60-70 kg adult human (e.g. ~0.4 mg to about ~1.0 mg), or a corresponding fixed dose based on a child, for example, a child of about 12 kg to about 50 kg or more, of the fusion protein of SEQ ID NO: 1.

Disclosed herein is a pharmaceutical composition comprising 0.4 to 1 mg of the fusion protein of SEQ ID NO: 1.

The invention also provides a pharmaceutical composition comprising about 40 µg/kg to about 70 µg/kg or at a corresponding fixed dose based on, for example, an average 60-70

kg adult human (e.g. ~3.0 mg to about ~3.6 mg), or a corresponding fixed dose based on a child, for example, a child of about 12 kg to about 50 kg or more, of the fusion protein of SEQ ID NO: 1; preferably wherein the composition comprises about 40 µg/kg to about 70 µg/kg of the fusion protein of SEQ ID NO: 1.

Disclosed herein is the use of the fusion protein of SEQ ID NO: 1 in the manufacture of a medicament for treating cancer in a patient, wherein the fusion protein is to be administered to the patient at a dose of at least 40 µg/kg to 70 µg/kg per day once weekly.

The invention also provides a method of treating cancer in a patient comprising administering to the patient a dose of less than 6 µg/kg/day such as about 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 and 5.9 µg/kg/day, or a corresponding fixed dose based on, for example, a 60-70 kg adult (e.g. ~0.2 mg/day to ~0.4 mg/day) or a corresponding fixed dose based on a child, for example, a child of about 12 kg to about 50 kg or more, of the fusion protein of SEQ ID NO: 1 resulting in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in circulating T regulatory (Treg) cells and wherein the increase in circulating NK cells and CD8+ cells is greater relative to the increase in circulating T regulatory (Treg).

Disclosed herein is the use of the fusion protein of SEQ ID NO: 1 in the manufacture of a medicament for treating cancer in a patient, wherein the fusion protein is to be administered to the patient at a daily dose of at least 3 µg/kg to 5.5 µg/kg, wherein administration results in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in circulating T regulatory (Treg) cells and wherein the increase in circulating NK cells and CD8+ cells is greater relative to the increase in circulating T regulatory cells (Treg).

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

Figure 1 shows diagrams of structural models of the fusion protein of SEQ ID NO: 1 (panel A) and its selective binding intermediate-affinity IL-2 receptor (panel B). The structural models in panels 1A and 1B were generated using the experimentally determined crystal structure of the quaternary complex of human IL-2 bound to the trimeric high-affinity receptor (Wang et al., *Science.* 2005;310(5751):1159-1163. doi: 10.1126/science.1117893).

Figure 2 are diagrams of the treatment regimens for intravenous (I.V.) infusion of the fusion protein of SEQ ID NO: 1 as monotherapy and as combination therapy with pembrolizumab in the first in human (FIH) study described in Example 1.

Figure 3 are graphs showing systemic exposure in human patients to the fusion protein of SEQ ID NO: 1 at various doses in accordance with the invention.

Figure 4 are graphs showing circulating Treg cells, NK cells and CD8+ cells responses in human patients who have been treated with the fusion protein of SEQ ID NO: 1 in accordance with the invention.

Figure 5 is a graph showing the pharmacodynamic response of patients exposed to high dose rhIL-2 as described in Example 2.

Figure 6 is a graph showing the pharmacodynamic response of patients exposed to high dose rhIL-2 as described in Example 2.

Figure 7 is a graph showing duration of treatment and overall response by tumor type in those patients treated with SEQ ID NO: 1 as a monotherapy.

Figure 8 is a graph showing duration of treatment and overall response by tumor type in those patients treated with SEQ ID NO: 1 as a combination therapy with pembrolizumab.

Figure 9 is graph showing change in tumor size from baseline by tumor type in those patients treated with 8 SEQ ID NO: 1 as a combination therapy with pembrolizumab.

Figure 10 is a series of graphs showing levels of inflammatory cytokines TNFα and IL-6 in mice treated with 8, 20, or 30 µg of the mouse ortholog of SEQ ID NO: 1.

Figure 11 is a graph showing the mean (+ standard deviation) serum concentrations (ng/mL) of SEQ ID NO: 1 in patients with advanced solid tumors after the first IV dose (0.1, 0.3, 1, 3, 6 or 8 µg/kg) of SEQ ID NO: 1 (Cycle 1 Day 1 (C1D1)).

Figure 12 are graphs showing the mean (+ standard deviation) maximum serum concentrations (C_{max}) (left) and area under the concentration versus time curve from time 0 to the last measurable concentration (AUC_{last}) (right) in patients with advanced solid tumors after the first IV dose (0.1, 0.3, 1, 3, 6 or 8 µg/kg) of SEQ ID NO: 1 (Cycle 1 Day 1 (C1D1)).

Figure 13 are graphs showing the mean (± standard error) absolute counts (cells/μL blood) of total NK cells (top), total CD8+ T cells (middle) and T_{regs} (bottom) in patients with advanced solid tumors in patients with advanced solid tumors after the first two treatment cycles with IV SEQ ID NO: 1 at a dose of 0.1, 0.3, 1, 3, 6 or 8 μg/kg.

Figure 14 are graphs showing the mean (± standard error) fold change from baseline (FCB). Absolute counts of total NK cells (left), total CD8+ T cells (middle) and T_{regs} cells (right) on cycle 1, day 8 (C1D8) and cycle 2 day 8 (C2D8) in patients with advanced solid tumors after the first two treatment cycles with IV SEQ ID NO: 1 at a dose of 0.1, 0.3, 1, 3, 6 or 8 μg/kg.

Figure 15 are graphs showing the mean (± standard error) maximum serum concentrations (pg/ml) (± standard error) IFNγ (left) and of IL-6 (right) in patients with advanced solid tumors after the first two treatment cycles with IV SEQ ID NO: 1 at a dose of 0.1, 0.3, 1, 3, 6 or 8 μg/kg.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments in accordance with the invention described herein. The scope of the present invention is not intended to be limited to the following description, but rather is as set forth in the appended claims.

In the claims, articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

It is also noted that the term "comprising" is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term "comprising" is used herein, the term "consisting of" is thus also encompassed and disclosed.

Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

As used herein, the term “about” or “approximately” as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

As used herein any form of administration or coadministration of a “combination”, “combined therapy” and/or “combined treatment regimen” refers to at least two therapeutically active agents or compositions which may be administered or co-administered”, simultaneously, in either separate or combined formulations, or sequentially at different times separated by minutes, hours or days. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent.

As used herein, the term “parenteral” refers to dosage forms that are intended for administration as an injection or infusion and includes subcutaneous, intravenous, intra-arterial, intraperitoneal, intracardiac, intrathecal, and intramuscular injection, as well as infusion injections usually by the intravenous route.

The term “therapeutic agent” encompasses any agent administered to treat a symptom or disease in an individual in need of such treatment in addition to, or in combination with, SEQ ID NO: 1. Such additional therapeutic agent may comprise any active ingredients suitable for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other.

The term “chemotherapeutic agent” refers to a compound or a derivative thereof that can interact with a cancer cell, thereby reducing the proliferative status of the cell and/or

killing the cell for example, by impairing cell division or DNA synthesis, or by damaging DNA, effectively targeting fast dividing cells. Examples of chemotherapeutic agents include, but are not limited to, alkylating agents (e.g., cyclophosphamide, ifosfamide); metabolic antagonists (e.g., methotrexate (MTX), 5-fluorouracil or derivatives thereof); a substituted nucleotide; a substituted nucleoside; DNA demethylating agents (also known as antimetabolites; e.g., azacitidine); antitumor antibiotics (e.g., mitomycin, adriamycin); plant-derived antitumor agents (e.g., vincristine, vindesine, TAXOL®, paclitaxel, abraxane); cisplatin; carboplatin; etoposide; and the like. Such agents may further include, but are not limited to, the anti-cancer agents trimethotrexate (TMTX); temozolomide; raltitrexed; S-(4-Nitrobenzyl)-6-thioinosine (NBMPR); 6-benzyguanidine (6-BG); a nitrosoureas a nitrosourea (rabinopyranosyl-N-methyl-N-nitrosourea (Aranose), Carmustine (BCNU, BiCNU), Chlorozotocin, Ethylnitrosourea (ENU), Fotemustine, Lomustine (CCNU), Nimustine, N-Nitroso-N-methylurea (NMU), Ranimustine (MCNU), Semustine, Streptozocin (Streptozotocin)); cytarabine; and camptothecin; or a therapeutic derivative of any thereof.

As used herein a single “course” of treatment such as a first course, second course, third course and so on refers to a treatment regimen wherein the Fusion Protein is administered for a desired period of time such as 1 to about 5 consecutive or non-consecutive days of treatment or a once weekly administration followed by a rest period of a certain amount of consecutive days. Therefore, one course of treatment includes a period of time of consecutive or non-consecutive administration of the Fusion Protein to the patient followed by a rest period of consecutive days wherein there is no administration of the Fusion Protein to the patient.

The term “fusion protein” designates a protein or peptide linked together with another protein or peptide by peptide bond between their respective N- and C-terminal amino acid residues or verse visa, or by insertion of the first protein or peptide into the internal region of the second protein or peptide by two peptide bonds at the N- and C-termini of the inserted protein or peptide. A peptide bond is a covalent chemical bond formed between carboxyl group of one amino acid and the amine group of another amino acid. A fusion protein is produced by expression of the fusion protein gene in an expression host, in which the coding sequence for the first protein or peptide is linked to the coding sequence of the second protein or peptide.

The term “**F**usion **P**rotein” refers to the fusion protein of SEQ ID NO: 1.
The invention also contemplates the use of a variant of the fusion protein of SEQ ID NO: 1 having an amino acid sequence having sequence identity that is about 75%, 80%, 85%, 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher over a contiguous stretch of about 20 amino acids up to the full length of SEQ ID NO: 1. A variant of the SEQ ID NO: 1 may have a defined sequence identity as compared to SEQ ID NO: 1 over a defined length of contiguous amino acids (e.g., a "comparison window"). Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Madison, Wis.), or by manual alignment and visual inspection (see, e.g., Current Protocols in Molecular Biology (Ausubel et al., eds. 1995 supplement)).

As an example, a variant of the fusion protein of SEQ ID NO: 1 can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, amino acid sequence identity to a contiguous stretch of SEQ ID NO: 1 of at least 20 amino acids and preferably from about 20 amino acids to about 40 amino acids, from about 40 amino acids to about 60 amino acids, from about 60 amino acids to about 80 amino acids, from about 80 amino acids to about 100 amino acids, from about 100 amino acids to about 120 amino acids, from about 120 amino acids to about 140 amino acids, from about 140 amino acids to about 150 amino acids, from about 150 amino acids to about 155 amino acids, from about 155 amino acids up to the full-length of SEQ ID NO: 1.

The term" IL-2 therapy" includes administration of immunotherapy based on IL-2 and its associated biological functions as an immunotherapy including but not limited to maintenance of $CD4^+$ regulatory T cells and differentiation of $CD4^+$ T cells into a variety of subsets; promotion of $CD8^+$ T-cell and NK cell cytotoxicity activity, and modulation of T-cell differentiation programs in response to antigen, promoting naive $CD4^+$ T-cell differentiation into T helper-1 (Th1) and T helper-2 (Th2) cells while inhibiting T helper-17 (Th17) differentiation. Therefore "IL-2 therapy" as used herein includes but is not limited to immunotherapy with rhIL-2 or a variant of rhIL-2 such as the Fusion Protein of SEQ ID NO: 1.

The terms "high dose IL-2" and "HD IL-2" include a dose of interleukin-2 (IL-2) of about or at least about 600,000 International Units (IU)/kg of body weight (kg)/dose, or about or at least about 720,000 IU/kg/dose.

The terms "low dose IL-2" and "LD IL-2" include a dose of interleukin-2 (IL-2) of less than about 600,000 IU/kg of body weight/dose, such as about 60,000 or about 72,000 IU/kg/dose, e.g., from about 60,000 to about 72,000 IU/kg/dose.

As used herein, the term “subject” or “patient” refers to any organism to which a composition in accordance with the present disclosure may be administered, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, and humans) and/or plants. Preferably “patient” refers to a human subject who may seek or be in need of treatment, requires treatment, is receiving treatment, will receive treatment, or a subject who is under care by a trained professional for a particular disease or condition. The “patient” can be child (>1-17 years). In still other embodiments, the patient can be an infant (1 year and younger). In yet still other embodiments, the patient can be a pediatric patient, wherein the term “pediatric” is used as understood by those skilled in the art. For example, pediatric patients include infants, children and adolescents.

The phrase “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein, the term “preventing” refers to partially or completely delaying onset of an infection, disease, disorder and/or condition; partially or completely delaying onset of one or more symptoms, features, or clinical manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying onset of one or more symptoms, features, or manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying progression from an infection, a particular disease, disorder and/or condition; and/or decreasing the risk of developing pathology associated with the infection, the disease, disorder, and/or condition.

The term “protein” or “peptide” as used herein refers to a at least two or more amino acid residues linked together by peptide bond. The amino acid sequence in a protein or peptide is shown in the standard format, i.e., from amino terminus (N-terminus) to carboxyl terminus (C-terminus).

The term “recombinant production” refers to the techniques for manipulating and combining two or more DNA sequences together that include recombination, PCR (polymerase chain reaction), *in vitro* mutagenesis, and direct DNA synthesis. These

techniques are described in numerous published books and manuals, including the "Current protocols in molecular biology" (Ausubel eds. 2008. John Wiley & Son).

As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

The phrase "therapeutically effective amount" or an "effective amount refers to the administration of an agent to a subject, either alone or as part of a pharmaceutical composition and either in a single dose or as part of a series of doses, in an amount capable of having any detectable, positive effect on any symptom, aspect, or characteristic of a disease, disorder or condition when administered to the subject. The therapeutically effective amount can be ascertained by measuring relevant physiological effects, and it can be adjusted in connection with the dosing regimen and diagnostic analysis of the subject's condition, and the like. By way of example, measurement of the amount of inflammatory cytokines produced following administration can be indicative of whether a therapeutically effective amount has been used. In reference to cancer or pathologies related to unregulated cell division, a therapeutically effective amount refers to that amount which has the effect of (1) reducing the size of a tumor (i.e. tumor regression), (2) inhibiting (that is, slowing to some extent, preferably stopping) aberrant cell division, for example cancer cell division, (3) preventing or reducing the metastasis of cancer cells, and/or, (4) relieving to some extent (or, preferably, eliminating) one or more symptoms associated with a pathology related to or caused in part by unregulated or aberrant cellular division, including for example, cancer. An "effective amount" is also that amount that results in desirable PD and PK profiles and desirable immune cell profiling upon administration of the therapeutically active compositions of the invention.

The terms "treating" or "treatment" of a disease (or a condition or a disorder) as used herein refer to preventing the disease from occurring in a human subject or an animal subject that may be predisposed to the disease but does not yet experience or exhibit symptoms of the disease (prophylactic treatment), inhibiting the disease (slowing or arresting its development), providing relief from the symptoms or side-effects of the disease (including palliative treatment), and causing regression of the disease. The terms "treat," "treating," "treatment," "therapeutic," and "therapy" do not necessarily mean total cure or abolition of the disease or

condition. Any alleviation of any undesired signs or symptoms of a disease or condition, to any extent can be considered treatment and/or therapy. Furthermore, treatment may include acts that may worsen the patient's overall feeling of well-being or appearance. With regard to cancer, these terms also mean that the life expectancy of an individual affected with a cancer may be increased or that one or more of the symptoms of the disease will be reduced. With regard to cancer, "treating" also includes enhancing or prolonging an anti-tumor response in a subject.

"Progression free survival (PFS)," as used in the context of the cancers described herein, refers to the length of time during and after treatment of the cancer until objective tumor progression or death of the patient. The treatment may be assessed by objective or subjective parameters; including the results of a physical examination, neurological examination, or psychiatric evaluation. In preferred aspects, PFS may be assessed by blinded imaging central review and may further optionally be confirmed by ORR or by blinded independent central review (BICR).

"Overall survival (OS)" may be assessed by OS rate at certain time points (e.g., 1 year and 2 years) by the Kaplan-Meier method and corresponding 95% CI will be derived based on Greenwood formula by study treatment for each tumor type. OS rate is defined as the proportion of participants who are alive at the time point. OS for a participant is defined as the time from the first dosing date to the date of death due to any cause.

As used herein a "complete response" is the disappearance of all signs of cancer in response to treatment. A complete response may also be referred to herein as "total remission".

As used herein the term "partial response" means a decrease in the size of the tumor, or in the extent of cancer in the body in response to treatment. A partial response may also be referred to herein as "partial remission".

The term "cancer", as used herein, shall be given its ordinary meaning, as a general term for diseases in which abnormal cells divide without control.

The term "reducing a tumor" or "tumor regression" as used herein refers to a reduction in the size or volume of a tumor mass, a decrease in the number of metastasized tumors in a subject, a decrease in the proliferative status (the degree to which the cancer cells are multiplying) of the cancer cells, and the like.

The term "enhancing", as used herein, refers to allowing a subject or tumor cell to improve its ability to respond to a treatment disclosed herein. For example, an enhanced response may comprise an increase in responsiveness of at least 5%, 10%, 15%, 20%, 25%,

30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 98% or more. As used herein, "enhancing" can also refer to enhancing the number of subjects who respond to a treatment such as a combination therapy comprising chemotherapy, drug-resistant immunocompetent cells, and immune checkpoint inhibitors. For example, an enhanced response may refer to a total percentage of subjects who respond to a treatment wherein the percentage is of at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 98% or more.

"Immune checkpoint proteins" regulate T cell function in the immune system. T cells play a central role in cell-mediated immunity. Immune checkpoint proteins interact with specific ligands that send a signal into the T cell and essentially switch off or inhibit T cell function. Cancer cells take advantage of this system by driving high levels of expression of immune checkpoint proteins on their surface that results in control of the T cells expressing immune checkpoint proteins on the surface of T cells that enter the tumor microenvironment, thus suppressing the anticancer immune response. As such, inhibition of immune checkpoint proteins by agents referred to herein as "immune checkpoint protein inhibitors" or "immune checkpoint inhibitors" would result in restoration of T cell function and an immune response to the cancer cells. Examples of immune checkpoint proteins include, but are not limited to: CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, OX40, B-7 family ligands or a combination thereof. Preferably, the immune checkpoint inhibitor interacts with a ligand of an immune checkpoint protein which may be CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, OX40, A2aR, B-7 family ligands or a combination thereof. Examples of immune checkpoint inhibitors include but are not limited to: from a PD-l antagonist, PD-L1 antagonist, CTLA-4 antagonist, adenosine A2A receptor antagonist, B7-H3 antagonist, B7-H4 antagonist, BTLA antagonist, KIR antagonist, LAG3 antagonist, TIM-3 antagonist, VISTA antagonist or TIGIT antagonist.

As used herein the term "angiogenesis inhibitor" refers to a drug, compound, antibody or other agent that keeps new blood vessels from forming. In cancer treatment, angiogenesis inhibitors may prevent the growth of new blood vessels that tumors need to grow. Angiogenesis inhibitors include those agents that can target one or more signaling pathways associated with receptor tyrosine kinases (RTK). RTKs include, but are not limited to, vascular endothelial growth factor receptors types 1, 2, and 3 (VEGFR1–3); platelet derived growth factor receptors, types alpha and beta (PDGFRα/β) and fibroblast growth factor

receptors (FGFR), types 1, 2, and 3 (FGFR1–3). Preferred angiogenesis inhibitors in accordance with the invention have broad target selectivity and are capable of simultaneous targeted inhibition of multiple RTKs and are referred to herein as "multiple receptor tyrosine kinase inhibitors".

"Pharmaceutically acceptable salt" refers to a salt of a compound of the disclosure that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound. In particular, such salts are non-toxic may be inorganic or organic acid addition salts and base addition salts. Specifically, such salts include: (1) acid addition salts, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic acid, glucoheptonic acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, and the like; or (2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, N-methylglucamine and the like. Salts further include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the compound contains a basic functionality, salts of non-toxic organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate and the like.

Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in Allen, Jr., L. V., ed., *Remington: The Science and Practice of Pharmacy,* 22nd Edition, Pharmaceutical Press, London, UK (2012).

Fusion Protein of SEQ ID NO: 1

A recombinant human IL-2 variant fusion protein, described in WO 2013/184942, is a circularly permuted (cp) IL-2 variant fused to the extracellular domain of the IL-2Rα portion of the IL-2 receptor and is referred to herein as the "fusion protein of SEQ ID NO: 1" or the "**F**usion **P**rotein" and has the following amino acid sequence: SKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFSQSIISTLTG GSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCL EEELKPLEEVLNLAQGSGGGSELCDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRR IKSGSLYMLCTGNSSHSSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPM QPVDQASLPGHCREPPPWENEATERIYHFVVGQMVYYQCVQGYRALHRGPAESVC KMTHGKTRWTQPQLICTG (SEQ ID NO: 1).

It is contemplated that fusion proteins that are closely related to SEQ ID NO: 1, such as those fusion proteins having sequence identities of about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher identity over the full length of SEQ ID NO: 1 may also be suitable for administration in accordance with the methods of the invention. It is contemplated that fusion proteins that are closely related to SEQ ID NO: 1, such as those fusion proteins having sequence identities of about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher identity over a contiguous sequence of at least about 20 amino acids up to the full length of SEQ ID NO: 1 may also be suitable for administration in accordance with the methods of the invention.

The fusion protein of SEQ ID NO: 1 may be produced using a biological recombinant expression system or any protein synthesizer. Strategies for recombinant protein expression are well known in the art, and typically involve transfecting cells with a DNA vector that contains a genetic template encoding the Fusion Protein of SEQ ID NO: 1 and then culturing the cells so that they transcribe and translate the Fusion Protein. Typically, the cells are then lysed to extract the expressed protein for subsequent purification. Both prokaryotic and eukaryotic *in vivo* protein expression systems are widely used. Preferably, the fusion protein of SEQ ID NO: 1 is produced in CHO cells.

The invention provides pharmaceutical compositions of a dose of at least about 6 µg/kg to about 70 µg/kg of the fusion protein of SEQ ID NO: 1 and preferably pharmaceutical compositions of at least about 6 µg/kg to at least about 15 µg/kg of the fusion protein of SEQ ID NO: 1 and a pharmaceutically acceptable excipient or a corresponding fixed dose based on an average 60-70 kg adult human (e.g. ~0.4 mg to about ~1.0 mg) or based on a child, for example, a child of about 12 kg to about 50 kg or more. The invention

also provides pharmaceutical compositions of a dose of at least about 6, 6.5, 7, 7,5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, or 15µg/kg or at a corresponding fixed dose based on, for example, an average 60-70 kg adult or based on, for example, a child of about 12 kg to about 50 kg or more.

The invention also provides pharmaceutical compositions of a dose of at least about 3 µg/kg to at least about 5.5 µg/kg of the fusion protein of SEQ ID NO: 1 and a pharmaceutically acceptable excipient or a corresponding fixed dose based on an average 60-70 kg adult human (e.g. ~0.2 mg to ~0.4 mg). The invention also provides pharmaceutical compositions of a dose of at least about 3, 3.5, 4, 4.5, 5, 5.5 µg/kg or a corresponding fixed dose based on an average 60-70 kg adult human or based on a child, for example a child of about 12 kg to about 50 kg or more.

The invention also provides pharmaceutical compositions of a dose of at least about 40 µg/kg to at least about 70 µg/kg of the fusion protein of SEQ ID NO: 1 and a pharmaceutically acceptable excipient or a corresponding fixed dose based on an average 60-70 kg adult human (e.g. about 3.0 mg to about 3.6 mg) or based on a child, for example a child of about 12 kg to about 50 kg or more.

A pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, Md., 2006; incorporated herein by reference) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof.

Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this present disclosure.

Liquid dosage forms for oral and parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms may comprise inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate,

benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, oral compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and/or perfuming agents. In certain embodiments for parenteral administration, compositions are mixed with solubilizing agents such as CREMOPHOR®, alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and/or combinations thereof.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing agents, wetting agents, and/or suspending agents. Sterile injectable preparations may be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid can be used in the preparation of injectables.

Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use. General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, Md., 2006; incorporated herein by reference).

The fusion protein of SEQ ID NO: 1 (Figure 1A) is designed to selectively bind to and activate the intermediate-affinity IL-2R, but not the high-affinity IL-2R. The IL-2Rα domain of the fusion protein of SEQ ID NO: 1 serves to sterically impede the binding of the fusion protein of SEQ ID NO: 1 to the high-affinity IL-2R yet still allow binding to the intermediate-affinity IL-2R.

In vitro and *in vivo* nonclinical pharmacodynamic (PD) data support selective signaling through the intermediate-affinity IL-2 receptor by the fusion protein of SEQ ID NO: 1, leading to the activation and expansion of effector cells such as NK cells and CD8+

cells, while minimizing the activation and expansion of immunosuppressive T_{regs}. Additionally, *in vivo* in mice, the fusion protein of SEQ ID NO: 1 displays improved tolerability relative to rhIL-2 at doses that elicit equivalent or greater expansion of effector cells relative to T_{regs}.

Additional nonclinical data demonstrate that IV or SC administration of the fusion protein of SEQ ID NO: 1 results in equivalent tumor growth inhibition in a mouse syngeneic tumor model, as well as similar peripheral expansion of NK and $CD8^+$ T cells after either route of administration to cynomolgus monkeys.

First in human clinical data described in Example 1, indicates that the fusion protein of SEQ ID NO: 1 activates expansion of CD8+ cells and NK cells in a dose dependent manner in the absence of dose dependent activation of Tregs. Therefore, the fusion protein of SEQ ID NO: 1 can be dosed in human patients at a concentration that is comparative to high dose rhIL-2 to elicit equivalent or greater expansion of NK cells and CD8+ cells as compared to high dose rhIL-2 but with far less (at least two fold less) relative expansion of immunosuppressive Tregs as compared to high dose rhIL-2 (Table 2). This result was unexpected.

Dosing Regimens

Preferably, the fusion protein of SEQ ID NO: 1 is administered to a cancer patient in accordance with the methods and dosing regimens of the invention. Preferred routes of administration are intravenous, e.g., intravenous injection and intravenous infusion, e.g., via central venous access. Additional routes of administration include subcutaneous, intramuscular, oral, nasal, and pulmonary administration. Preferably the Fusion Protein may be administered as part of a pharmaceutical composition comprising at least one excipient.

Preferably, the invention provides pharmaceutical compositions for intravenous (I.V.) administration comprising a dose of the fusion protein of SEQ ID NO: 1 in terms of µg/kg as is often preferred for calculating dose in pediatric patients but is also useful for calculating dose for adults, preferably at a dose of: about 0.1 µg/kg to about 70 µg/kg; about 1 µg/kg to about 70 µg/kg; about 1 µg/kg to about 50 µg/kg; about 1 µg/kg to about 30 µg/kg; about 1 µg/kg to about 25 µg/kg; about 1 µg/kg to about 15 µg/kg; about 1 µg/kg to about 10 µg/kg; about 1 µg/kg to about 5 µg/kg; about 1 µg/kg to about 3 µg/kg; about 6 µg/kg to about 70 µg/kg; about 6 µg/kg to about 50 µg/kg; about 6 µg/kg to about 30 µg/kg; about 6 µg/kg to about 25 µg/kg; about 6 µg/kg to about 15 µg/kg; about 6 µg/kg to about 10 µg/kg; about 6 µg/kg to about 8 µg/kg; about 8 µg/kg to about 70 µg/kg; about 8 µg/kg to about 50 µg/kg;

about 8 µg/kg to about 30 µg/kg; about 8 µg/kg to about 25 µg/kg; about 8 µg/kg to about 15 µg/kg; about 8 µg/kg to about 10 µg/kg; about 10 µg/kg to about 70 µg/kg; about 10 µg/kg to about 50 µg/kg; about 10 µg/kg to about 30 µg/kg; about 10 µg/kg to about 25 µg/kg; about 10 µg/kg to about 15 µg/kg; about 12 µg/kg to about 12 µg/kg; about 12 µg/kg to about 50 µg/kg; about 12 µg/kg to about 30 µg/kg; about 12 µg/kg to about 25 µg/kg; about 12 µg/kg to about 15 µg/kg; about 14 µg/kg to about 70 µg/kg; about 14 µg/kg to about 50 µg/kg; about 14 µg/kg to about 30 µg/kg; about 14 µg/kg to about 25 µg/kg; about 30 µg/kg to about 70 µg/kg; about 30 µg/kg to about 50 µg/kg; about 40 µg/kg to about 70 µg/kg; about 40 µg/kg to about 50 µg/kg; about 50 µg/kg to about 70 µg/kg; about 50 µg/kg to about 60 µg/kg; or a corresponding fixed dose thereof based on, for example, a 60-70 kg adult (e.g. about 1 mg to about 4 mg) or a corresponding fixed dose based a child, for example, a child of about 12 kg to about 50 kg or more.

Preferably, the fusion protein of SEQ ID NO: 1 is administered by I.V infusion to a patient at a dose of at least about 6 µg/kg per day to at least about 15 µg/kg/day or at a corresponding fixed per day dose based on, for example, an average 60-70 kg adult human (e.g. ~0.4 mg/day to about ~1.0 mg/day) or a corresponding fixed per day dose based on a child for example, a child of about 12 kg to about 50 kg or more. Preferably the dose is about 6 µg/kg per day. Preferably the dose is about 8 µg/kg per day. Preferably the dose is about 10 µg/kg per day. Preferably the dose is about 12 µg/kg per day. Preferably the dose is about 14 µg/kg per day. Preferably the dose is about 15 µg/kg per day. Preferably the dose is about 6 µg/kg/day, 6.5 µg/kg/day, 7 µg/kg/day, 7.5 µg/kg/day, 8 µg/kg/day, 8.5 µg/kg/day, 9 µg/kg/day, 9.5 µg/kg/day, 10 µg/kg/day, 10.5 µg/kg/day, 11 µg/kg/day, 11.5 µg/kg/day, 12 µg/kg/day, 12.5 µg/kg/day, 13 µg/kg/day, 13.5 µg/kg/day, 14 µg/kg/day, 14.5 µg/kg/day, 15 µg/kg/day or a corresponding fixed per day dose based on an average 60-70 kg adult or a corresponding fixed per day dose based on a child, for example, a child of about 12 kg to about 50 kg or more.

Even higher doses may be contemplated for administration to a patient, such as doses of 16 µg/kg/day, 18 µg/kg/day, 20 µg/kg/day, 22 µg/kg/day, 24 µg/kg/day, 26 µg/kg/day, 28 µg/kg/day, 30 µg/kg/day, 40 µg/kg/day, 50 µg/kg/day, 60 µg/kg/day, 70 µg/kg/day or a corresponding fixed per day dose based, for example, on an average 60-70 kg adult (e.g. ~1 mg to ~4 mg) or a corresponding fixed dose based, for example on a child of about 12 kg to about 50 kg or more.

Preferably the fusion protein of SEQ ID NO: 1 is administered as a single I.V. infusion per day. A single I.V. infusion may take from 5 minutes to 2 hours.

Preferably the dosing regimen for administration of the fusion protein provides for one or more treatment courses. A single treatment course may take place over a period of days ranging from 1-90 days. Preferably a single treatment course extends for a period of 14 days or 21 days. A treatment course may involve multiple consecutive days wherein the Fusion Protein as administered to the patient once per day via I.V. infusion, followed by multiple consecutive days wherein the Fusion Protein is not administered to the patient also referred to herein as a "rest period". It is contemplated that the days of administration may be non-consecutive if, for example, a patient experiences discomfort with consecutive daily administrations. Preferably, the Fusion protein is administered to the patient for at least about 1, 2, 3, 4, or 5 consecutive days. Preferably, administration of the Fusion Protein need not be consecutive and may take place over the course of 1, 2, 3, 4, or 5 non-consecutive days. Preferably the fusion protein is administered to the patient as a once a day by I.V. infusion for 1 to about 5 consecutive days. Preferably the Fusion Protein is administered to the patient once a day for 1-5 non-consecutive days, e.g. infusions on day 1 with one or two days in between before the next infusion and so on for the desired total number of infusion days prior to the rest period.

Preferably the first course of treatment comprises administering the Fusion Protein by I.V. infusion once a day for five (5) consecutive days followed by a rest period of 9 days for a first treatment course that lasts about 14 days. Preferably a second course of treatment follows the first course of treatment. The second course of treatment may begin at any time after the first course of treatment but preferably begins within about 24 hours or more after the first course of treatment has ended.

Preferably, the second course of treatment comprises administering the Fusion Protein of SEQ ID NO: 1 by I.V. infusion once a day for five (5) consecutive days followed by a rest period of at least about 16 consecutive days for a treatment course that lasts about 21 days. Additional treatment courses such as a third, fourth and fifth treatment courses, may follow the second treatment course, preferably starting within about 24 hours after the previous course is finished. Preferably, all treatment courses after the first treatment course are at least about 21-day treatment courses.

Preferably, Fusion Protein is administered with another therapeutic and/or anti-cancer agent as described *infra*. Preferably the therapeutic agent is the immune checkpoint inhibitor, pembrolizumab. Preferably pembrolizumab is administered in a separate composition from the Fusion Protein and is preferably administered by I.V. infusion prior to, subsequent to, or simultaneously infusion of the Fusion Protein. Preferably, pembrolizumab is administered in

a once a day dose of about 200 µg or as per the standard prescribing recommendations. Preferably, pembrolizumab is administered on the first day of each course of treatment with the Fusion Protein. An exemplary treatment regimen is shown in Figure 2. Preferably, when co- administering the Fusion Protein with pembrolizumab, the first course of treatment with the fusion protein and all subsequent courses of treatment (e.g. Treatment courses 2, 3, 4 and 5) are generally about 21 day courses wherein the Fusion Protein is administered by I.V. infusion once a day for five (5) consecutive days followed by a rest period of at least about 16 consecutive days prior to the next course of administration. As discussed above, the days of administration need not be consecutive and may take place over the course of 1, 2, 3, 4, or 5 non-consecutive days.

The invention also provides a dosing regimen wherein the fusion protein of SEQ ID NO: 1 is administered periodically such as only once every about 3 days to once every about 60 days. Preferably the periodic dosing is once every about 3 days to once every about 21 days. Preferably, the periodic dosing is once every 3 days, once every 4 days, once every 7 days, once every 14 days or once every 21 days. In accordance with this periodic dosing e.g. once weekly dosing regimen, the dose of Fusion Protein is administered on a once weekly basis, for example, wherein the dose is about 40µg/kg to about 70 µg/kg; preferably wherein the dose is about 50 µg/kg to about 60 µg/kg; or a corresponding fixed dose, thereof based on, for example a 60 kg to 70 kg adult or based on a child, for example, a child of about 12 kg to about 50 kg or more.

Preferably the fusion protein of SEQ ID NO: 1 is administered by I.V. infusion on day 1, day 7, day 14 and day 21 during each treatment cycle. Preferably the fusion protein of SEQ ID NO: 1 is administered by I.V. infusion on day 1, and day 14 during each treatment cycle. Preferably the fusion protein of SEQ ID NO: 1 is administered by I.V. infusion on day 1, and day 21 during each treatment cycle. Preferably the fusion protein of SEQ ID NO: 1 is administered by I.V. infusion on day 1, day 7 and day 14 during each treatment cycle. Preferably the fusion protein of SEQ ID NO: 1 is administered by I.V. infusion on day 1, day 7 and day 21 during each treatment cycle.

Preferably the fusion protein of SEQ ID NO: 1 is administered periodically, for example, the fusion protein of SEQ ID NO: 1 is administered to the patient by I.V. infusion on one or more days during a treatment cycle wherein each I.V. administration during the treatment cycle is separated by about 7 days, about 14 days, or about 21 days or any combination thereof so long as the administration of SEQ ID NO: 1 does not occur on consecutive days at doses of about 6 µg/kg per day to at about 15 µg/kg/day or at doses of

about 16 µg/kg per day to about 70 µg/kg/day or a corresponding fixed per day dose based, for example, on an average 60-70 kg adult (e.g. ~1 mg to ~4 mg) or a corresponding fixed per day dose based on a child, for example, a child of about 12 kg to about 50 kg or more.

Preferably the fusion protein of SEQ ID NO: 1 is administered periodically, for example, the patient is administered to the patient by I.V. infusion on one or more days during a treatment cycle wherein each I.V. administration during the treatment cycle is separated by about 7 days, about 14 days, or about 21 days or any combination thereof so long as the administration of SEQ ID NO: 1 does not occur on consecutive days preferably at a dose of about 16 µg/kg per day to about 70 µg/kg/day; preferably at dose of about 16 µg/kg per day to about 50 µg/kg/day; preferably at a dose of about 16 µg/kg per day to about 30 µg/kg/day; preferably at a dose of about 16 µg/kg per day to about 20 µg/kg/day; preferably at about a dose of 30 µg/kg per day to about 50 µg/kg/day; or a corresponding fixed per day dose based, for example, on an average 60-70 kg adult (e.g. ~1 mg to ~4 mg) or a corresponding fixed dose based, for example, on a child of about 12 kg to about 50 kg or more.

Preferably the periodic dosing is administered by I.V. infusion. Preferably a second therapeutic/anti-cancer agent such as pembrolizumab is co-administered with the fusion protein of SEQ ID NO: 1, preferably by I.V. infusion prior to, subsequent to, or simultaneously with, or on the same day as, administration of the fusion protein of SEQ ID NO: 1.

The methods of the invention also contemplate a dosing regimen wherein the fusion protein of SEQ ID NO: 1 is administered once a day at a dose of less than about 6 µg/kg/day or a fixed dose equivalent thereof based on a 60-70 kg human or based on a child of about 12kg to about 50 kg or more. For example, a patient may be administered a dose of about 3, 3.5, 4, 4.5, 5, 5.5 µg/kg/per day or a corresponding fixed per day dose based on, for example, an average 60-70 kg adult human or a corresponding fixed dose based on a child, for example, a child of about 12 kg to about 50 kg or more. A once a day dosing regimen of less than about 6 µg/kg/day or a fixed dose equivalent thereof based on a 60-70 kg human may be administered in accordance with any of the dosing regimens described above for in relation to doses between 6 µg/kg/day and 15 µg/kg/day.

All of the dosing regimens of the invention described above preferably result in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in T regulatory (Treg) cells and preferably result in an increase in circulating NK cells and CD8+ cells that is greater relative to the increase in circulating Treg

cells in the patient. As compared to high dose or low dose rhIL-2 therapy, all dosing regimens of the invention preferably require less frequent dosing e.g., once daily dosing of the fusion protein of SEQ ID NO: 1 as compared to dosing 3 times per day dosing of high dose or low dose rhIL-2.

Preferably the increase in circulating CD8+ T-cells resulting from administration of the fusion protein of SEQ ID NO: 1 is at least about a 2-fold, at least about a 3-fold, at least about a 4-fold, at least about a 5-fold, at least about a 6-fold, at least about a 7-fold, at least about an 8-fold, about, a 9-fold, about a 10-fold, or more as compared to baseline. Preferably the ratio of increase in circulating CD8+ T cells resulting from administration of the fusion protein of SEQ ID NO: 1 is greater relative to the ratio of increase in circulating T regulatory cells.

Preferably the fusion protein of SEQ ID NO: 1 and pharmaceutical compositions thereof, in combination with one or more immune checkpoint inhibitors to treat and/or prevent various diseases, disorders and conditions (e.g., cancers) is affected by utilizing particular dosing parameters that serve to minimize any adverse effects associated with administration of the individual therapies by themselves. By way of example, the addition of the administration of the fusion protein of SEQ ID NO: 1 in a treatment regimen comprising an immune checkpoint inhibitor (e.g. pembrolizumab) might allow a reduction of the amount of an immune checkpoint inhibitor needed to achieve the therapeutic goal, thus reducing (or even eliminating) severe and fatal immune-mediated adverse reactions that prompted the FDA to require a “black box” warning on certain immune checkpoint inhibitors (e.g. pembrolizumab).

In general, dosing parameters of monotherapy with the fusion protein of SEQ ID NO: 1 or any of the combination therapies described herein dictate that the dosage amount be less than an amount that could be irreversibly toxic to the subject (i.e., the maximum tolerated dose, “MTD”) and not less than an amount required to produce a measurable effect on the subject. Such amounts are determined by, for example, the pharmacokinetic and pharmacodynamic parameters associated with ADME, taking into consideration the route of administration and other factors.

An effective dose (ED) is the dose or amount of an agent that produces a therapeutic response or desired effect in some fraction of the subjects taking it. The “median effective dose” or ED50 of an agent is the dose or amount of an agent that produces a therapeutic response or desired effect in 50% of the population to which it is administered. Although the ED50 is commonly used as a measure of reasonable expectance of an agent's effect, it is not

necessarily the dose that a clinician might deem appropriate taking into consideration all relevant factors. Thus, in some situations the effective amount can be more than the calculated ED50, in other situations the effective amount can be less than the calculated ED50, and in still other situations the effective amount can be the same as the calculated ED50.

In addition, an effective dose of the fusion protein of SEQ ID NO: 1 can be an amount that, when administered in one or more doses to a subject, produces a desired result relative to a healthy subject. For example, for a subject experiencing a particular disorder, an effective dose can be one that improves a diagnostic parameter, measure, marker and the like of that disorder by at least about 5%, at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more than 90%, where 100% is defined as the diagnostic parameter, measure, marker and the like exhibited by a normal subject.

Preferably, the patient is administered the fusion protein of SEQ ID NO: 1 again if after initial treatment the cancer reoccurs. For example, if the patient is initially treated for a solid tumor, and the tumor returns or more tumors develop, the patient is administered SEQ ID NO: 1, as, for example, another course or series of courses of SEQ ID NO: 1.

Preferably, the fusion protein of SEQ ID NO: 1 is administered to a cancer patient in accordance with the methods and dosing regimens of the invention. Preferred routes of administration are intravenous, e.g., intravenous injection and intravenous infusion, e.g., via central venous access. Additional preferred routes of administration include subcutaneous, intramuscular, oral, nasal, and pulmonary administration.

The treatment regimens of the invention are administered to the patient until the patient is cured or until the patient is no longer benefiting from the treatment regimen.

Improved Safety Profile

The toxicity of rhIL-2 in humans and animals is well documented. At the high doses of rhIL-2 used in most cancer trials, considerable toxicity has been documented with only occasional tumor responses. One major dose-limiting toxicity of human recombinant interleukin-2 (rhIL-2) is capillary leak syndrome (CLS) also referred to herein as vascular leak syndrome (VLS). CLS is characterized by an increase in vascular permeability accompanied by extravasation of fluids and proteins resulting in interstitial edema and organ failure. Manifestations of CLS include fluid retention, increase in body weight, peripheral edema, pleural and pericardial effusions, ascites, anasarca and, in severe form, signs of

pulmonary and cardiovascular failure. Symptoms are highly variable among patients and the causes are poorly understood.

The pathogenesis of endothelial cell (EC) damage is complex and can involve activation or damage to ECs and leukocytes, release of cytokines and of inflammatory mediators, alteration in cell–cell and cell–matrix adhesion and in cytoskeleton function. CLS restricts the doses of IL-2 which can be administered to humans and, in some cases, necessitates the cessation of therapy.

The methods of the invention reduce the risk of side effects often associated with high dose therapy while maintaining the desired therapeutic activity of IL-2 therapy including, but not limited to, CLS as well as cytokine release syndrome (CRS), another syndrome associated with immune therapy with cytokines that often accompanies and/or overlaps with CLS.

Dose escalation studies in human clinical trials as described in the Examples have surprisingly revealed that the administration of the Fusion Polypeptide of SEQ ID NO: 1 to a patient at concentrations that were equivalent to those of high dose rh-IL-2 did not result in frequency and severity of certain side effects, for example, capillary leak syndrome, often associated with high dose rhIL-2 therapy. As described in the Examples outlining human clinical studies, dose limiting toxicities (DLTs) have not yet been reached doses of 6 ug/kg/day and higher even though the EC50 values for natural killer cell and CD8+ T cell activation have been exceeded. Therefore, the methods of the invention may result in an improved safety profile for patients having cancer and in need of IL-2 therapy as compared to, for example, standard rhIL-2 therapy and particularly as compared to high dose rhIL-2 therapy.

As used herein, an" improved safety profile", or a "lower risk of a side effects", or "reduced frequency or severity of a side effect" associated with, for example standard rhIL-2 therapy and particularly as compared to high dose rhIL-2 therapy can be assessed in several ways. A side effect or symptom of IL-2 therapy may be quantified. A side effect or symptom of IL-2 therapy may be quantified on a semi-quantitative scale, for example 0 to 5, where 0 represents absence, 1 to 4 represent identifiable increases in severity, and 5 represents maximum severity. Clinical trials often use a 1 to 5 scale where: 1 represents a mild adverse event (side effect); 2 represents a moderate adverse event (side effect) ; 3 represents a severe adverse event (side effect) ; 4 represents a life-threatening or disabling adverse event (side effect); and 5 represents death related to adverse event (side effect) . Alternatively, a side effect or symptom of IL-2 therapy may be quantified as a binary event,

i.e. presence or absence, 0 or 1. Other semi-quantitative scales will be readily apparent to the person skilled in the art. In another embodiment, a side effect or symptom of IL-2 therapy may be quantified on a quantitative scale, for instance: mass per volume such as mass of cytokine per volume of tissue fluid; temperature; duration; rate; enzyme activity; oxygen saturation; and so on. The person skilled in the art will readily understand how to assess ad quantify any side effect or symptom of IL-2 therapy and be able to do so without difficulty or undue burden. For example, the person skilled in the art will be able to measure: a cytokine concentration in plasma or serum; temperature (fever); heart rate (tachycardia); blood pressure (hypotension); cardiac dysfunction; renal impairment; serum or plasma enzyme concentrations (hepatic function); and so on. Any quantification of a side effect or symptom of IL-2 therapy may be compared to a control, for example a healthy control subject not receiving IL-2 therapy, or comparing to other control subjects receiving, for example, standard high dose rhIL-2 therapy.

A “lowered risk” of a side effect of IL-2 therapy may be about a 1% decrease, about a 2% decrease, about a 3% decrease, about a 4% decrease, about a 5% decrease, about a 6% decrease, about a 7% decrease, about an 8% decrease, about a 9% decrease, about a 10% decrease, about a 20% decrease, about a 30% decrease, about a 40% decrease, about a 50% decrease, about a 60% decrease, about a 70% decrease, about an 80% decrease, about a 90% decrease, about a 100%, decrease in the manifestation of side effects or symptom of IL-2 therapy as compared to, for example high dose rhIL-2 therapy. Alternatively, treating a side effect or symptom of IL-2 therapy may be about a 2-fold, about a 3-fold, about a 4-fold, about a 5-fold, about a 6-fold, about a 7-fold, about an 8-fold, about, a 9-fold, about a 10-fold, or more decrease in the side effect or symptom of IL-2 therapy. It follows that "less severe side effects " refers to such a decrease in the side effect or symptom of IL-2 therapy.

Preferably the dosing regimen of the Fusion Protein in accordance with the invention reduces the frequency and severity of capillary leak syndrome (CLS) also referred to herein as vascular leak syndrome (VLS).

The risks of other side effects often associated with rhIL-2 immunotherapy that may be also be lowered by the treatment regimens of the invention include but are not limited to cytokine-release syndrome (CRS). CRS is a serious side effect of immunotherapy having symptoms that may overlap clinically with those of CLS and yet may cause symptoms that are entirely different from CRS. CRS is thought to result from proliferating T cells that release large quantities of cytokines, including IL-6, IFN-γ, TNF, IL-2, IL-2-receptor a, IL-8, IL-10, and GMCSF. Patients with CRS may experience any one or more of fever,

cardiovascular symptoms including tachycardia, hypotension, arrhythmias, decreased cardiac ejection fraction, pulmonary symptoms including edema, hypoxia, dyspnea, and pneumonitis, acute renal injury usually caused by reduced renal perfusion, hepatic and gastrointestinal symptoms including elevated serum transaminases and bilirubin, diarrhea, colitis, nausea, and abdominal pain, hematologic symptoms including cytopenia such as grade 3-4 anemia, thrombocytopenia, leukopenia, neutropenia, and lymphopenia, derangements of coagulation including prolongation of the prothrombin time and activated partial thromboplastin time (PTT), D- dimer elevation, low fibrinogen, disseminated intravascular coagulation, macrophage activation syndrome (MAS), hemorrhage, B-cell aplasia, and hypogammaglobulinemia, infectious diseases including bacteremia, Salmonella, urinary tract infections, viral infections such as influenza, respiratory syncytial virus, and herpes zoster virus, musculoskeletal symptoms including elevated creatine kinase, myalgias and weakness, neurological symptoms including delirium, confusion, and seizure.

Administration of SEQ ID NO: 1 has been shown to induce lower levels of inflammatory cytokines as compared to for example, rhIL-2 in mice. See Example 4 and Figure 10.

Administration of SEQ ID NO: 1 has also been shown to induce higher levels of desirable cytokines such as IFN while inducing lower levels of inflammatory cytokines such as IL-6 in humans. See Example 5 and Figure 15.

MAS overlaps clinically with CRS with subjects potentially experiencing hepatosplenomegaly, lymphadenopathy, pancytopenia, liver dysfunction, disseminated intravascular coagulation, hypofibrinogenemia, hyperferritinemia, and hypertriglyceridemia. Like CRS, subjects with MAS exhibit elevated levels of cytokines, including IFN-γ and GMCSF.

Another side effect of immunotherapy including IL-2 therapy is tumor lysis syndrome (TLS), which occurs when the contents of cells are released as a result of therapy causing cell death, most often with lymphoma and leukemia. TLS is characterized by blood ion and metabolite imbalance, and symptoms include nausea, vomiting, acute uric acid nephropathy, acute kidney failure, seizures, cardiac arrhythmias, and death.

Neurotoxicity may result from immunotherapy including IL-2 therapy and symptoms may include cerebral edema, delirium, hallucinations, dysphasia, akinetic mutism, headache, confusion, alterations in wakefulness, ataxia, apraxia, facial nerve palsy, tremor, dysmetria, and seizure.

Patients undergoing IL-2 immunotherapy may experience one or more side effects or symptoms that are not necessarily caused by CLS, CRS, MAS or TLS including anemia, aphasia, arrhythmia, arthralgia, back pain, blood and bone marrow disorders, blood and lymphatic system disorders, cardiac disorders, chills, coagulation disorders, colitis, confused state, constitutional symptoms, cough, decreased appetite, diarrhea, disorientation, dizziness, dyspnea, encephalopathy, fatigue, fever, gastrointestinal disorders, general cardiovascular disorders, hemorrhage, hepatic disorders, hyperglycemia , hypokalemia, hypothyroidism, increased ALT, increased AST, increased C-reactive protein, infection febrile neutropenia, leukopenia, malaise, abnormal metabolic laboratory-testing results, metabolism nutrition disorders, mucosal inflammation, musculoskeletal disorders, myalgia nausea, nervous system disorders, neurologic disorders, neutropenia edema, pain, palmar-plantar erythrodysesthesia, paresthesia, pneumonia, pruritus, pulmonary disorders, pyrexia, rash, renal genitourinary disorders, respiratory disorders, skin and subcutaneous tissue disorders, somnolence, speech disorders, sweats thoracic mediastinal disorders, thrombocytopenia, tremor, tumor flare, tumor lysis syndrome, vascular disorders, and vomiting.

Cancer Indications

The treatment regimens of the invention using the fusion protein of SEQ ID NO: 1 are useful in the treatment of many types of cancer. The term “cancer”, as used herein, shall be given its ordinary meaning, as a general term for diseases in which abnormal cells divide without control. In particular, and in the context of the embodiments of the present invention, cancer refers to angiogenesis-related cancer. Cancer cells can invade nearby tissues and can spread through the bloodstream and lymphatic system to other parts of the body. There are several main types of cancer, for example, carcinoma is cancer that begins in the skin or in tissues that line or cover internal organs. Sarcoma is cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue. Leukemia is cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the bloodstream. Lymphoma is cancer that begins in the cells of the immune system.

When normal cells lose their ability to behave as a specified, controlled and coordinated unit, a tumor is formed. Generally, a solid tumor is an abnormal mass of tissue that usually does not contain cysts or liquid areas (some brain tumors do have cysts and central necrotic areas filled with liquid). A single tumor may even have different populations of cells within it, with differing processes that have gone awry. Solid tumors may be benign

(not cancerous), or malignant (cancerous). Different types of solid tumors are named for the type of cells that form them. Examples of solid tumors are sarcomas, carcinomas, and lymphomas. Leukemias (cancers of the blood) generally do not form solid tumors.

Representative cancers include, but are not limited to, Acute Lymphoblastic Leukemia, Adult; Acute Lymphoblastic Leukemia, Childhood; Acute Myeloid Leukemia, Adult; Adrenocortical Carcinoma; Adrenocortical Carcinoma, Childhood; AIDS-Related Lymphoma; AIDS-Related Malignancies; Anal Cancer; Astrocytoma, Childhood Cerebellar; Astrocytoma, Childhood Cerebral; Bile Duct Cancer, Extrahepatic; Bladder Cancer; Bladder Cancer, Childhood; Bone Cancer, Osteosarcoma/Malignant Fibrous Histiocytoma; Glioblastoma, Childhood; Glioblastoma, Adult; Brain Stem Glioma, Childhood; Brain Tumor, Adult; Brain Tumor, Brain Stem Glioma, Childhood; Brain Tumor, Cerebellar Astrocytoma, Childhood; Brain Tumor, Cerebral Astrocytoma/Malignant Glioma, Childhood; Brain Tumor, Ependymoma, Childhood; Brain Tumor, Medulloblastoma, Childhood; Brain Tumor, Supratentorial Primitive Neuroectodermal Tumors, Childhood; Brain Tumor, Visual Pathway and Hypothalamic Glioma, Childhood; Brain Tumor, Childhood (Other); Breast Cancer; Breast Cancer and Pregnancy; Breast Cancer, Childhood; Breast Cancer, Male; Bronchial Adenomas/Carcinoids, Childhood: Carcinoid Tumor, Childhood; Carcinoid Tumor, Gastrointestinal; Carcinoma, Adrenocortical; Carcinoma, Islet Cell; Carcinoma of Unknown Primary; Central Nervous System Lymphoma, Primary; Cerebellar Astrocytoma, Childhood; Cerebral Astrocytoma/Malignant Glioma, Childhood; Cervical Cancer; Childhood Cancers; Chronic Lymphocytic Leukemia; Chronic Myelogenous Leukemia; Chronic Myeloproliferative Disorders; Clear Cell Sarcoma of Tendon Sheaths; Colon Cancer; Colorectal Cancer, Childhood; Cutaneous T-Cell Lymphoma; Endometrial Cancer; Ependymoma, Childhood; Epithelial Cancer, Ovarian; Esophageal Cancer; Esophageal Cancer, Childhood; Ewing's Family of Tumors; Extracranial Germ Cell Tumor, Childhood; Extragonadal Germ Cell Tumor; Extrahepatic Bile Duct Cancer; Eye Cancer, Intraocular Melanoma; Eye Cancer, Retinoblastoma; Gallbladder Cancer; Gastric (Stomach) Cancer; Gastric (Stomach) Cancer, Childhood; Gastrointestinal Carcinoid Tumor; Germ Cell Tumor, Extracranial, Childhood; Germ Cell Tumor, Extragonadal; Germ Cell Tumor, Ovarian; Gestational Trophoblastic Tumor; Glioma. Childhood Brain Stem; Glioma. Childhood Visual Pathway and Hypothalamic; Hairy Cell Leukemia; Head and Neck Cancer; Hepatocellular (Liver) Cancer, Adult (Primary); Hepatocellular (Liver) Cancer, Childhood (Primary); Hodgkin's Lymphoma, Adult; Hodgkin's Lymphoma, Childhood; Hodgkin's Lymphoma During Pregnancy; Hypopharyngeal Cancer; Hypothalamic and Visual Pathway Glioma,

Childhood; Intraocular Melanoma; Islet Cell Carcinoma (Endocrine Pancreas); Kaposi's Sarcoma; Kidney Cancer; Laryngeal Cancer; Laryngeal Cancer, Childhood; Leukemia, Acute Lymphoblastic, Adult; Leukemia, Acute Lymphoblastic, Childhood; Leukemia, Acute Myeloid, Adult; Leukemia, Acute Myeloid, Childhood; Leukemia, Chronic Lymphocytic; Leukemia, Chronic Myelogenous; Leukemia, Hairy Cell; Lip and Oral Cavity Cancer; Liver Cancer, Adult (Primary); Liver Cancer, Childhood (Primary); Lung Cancer, Non-Small Cell; Lung Cancer, Small Cell; Lymphoblastic Leukemia, Adult Acute; Lymphoblastic Leukemia, Childhood Acute; Lymphocytic Leukemia, Chronic; Lymphoma, AIDS-Related; Lymphoma, Central Nervous System (Primary); Lymphoma, Cutaneous T-Cell; Lymphoma, Hodgkin's, Adult; Lymphoma, Hodgkin's; Childhood; Lymphoma, Hodgkin's During Pregnancy; Lymphoma, Non-Hodgkin's, Adult; Lymphoma, Non-Hodgkin's, Childhood; Lymphoma, Non-Hodgkin's During Pregnancy; Lymphoma, Primary Central Nervous System; Macroglobulinemia, Waldenstrom's; Male Breast Cancer; Malignant Mesothelioma, Adult; Malignant Mesothelioma, Childhood; Malignant Thymoma; Medulloblastoma, Childhood; Melanoma; Melanoma, Intraocular; Merkel Cell Carcinoma; Mesothelioma, Malignant; Metastatic Squamous Neck Cancer with Occult Primary; Multiple Endocrine Neoplasia Syndrome, Childhood; Multiple Myeloma/Plasma Cell Neoplasm; Mycosis Fungoides; Myelodysplastic Syndromes; Myelogenous Leukemia, Chronic; Myeloid Leukemia, Childhood Acute; Myeloma, Multiple; Myeloproliferative Disorders, Chronic; Nasal Cavity and Paranasal Sinus Cancer; Nasopharyngeal Cancer; Nasopharyngeal Cancer, Childhood; Neuroblastoma; Neurofibroma; Non-Hodgkin's Lymphoma, Adult; Non-Hodgkin's Lymphoma, Childhood; Non-Hodgkin's Lymphoma During Pregnancy; Non-Small Cell Lung Cancer; Oral Cancer, Childhood; Oral Cavity and Lip Cancer; Oropharyngeal Cancer; Osteosarcoma/Malignant Fibrous Histiocytoma of Bone; Ovarian Cancer, Childhood; Ovarian Epithelial Cancer; Ovarian Germ Cell Tumor; Ovarian Low Malignant Potential Tumor; Pancreatic Cancer; Pancreatic Cancer, Childhood', Pancreatic Cancer, Islet Cell; Paranasal Sinus and Nasal Cavity Cancer; Parathyroid Cancer; Penile Cancer; Pheochromocytoma; Pineal and Supratentorial Primitive Neuroectodermal Tumors, Childhood; Pituitary Tumor; Plasma Cell Neoplasm/Multiple Myeloma; Pleuropulmonary Blastoma; Pregnancy and Breast Cancer; Pregnancy and Hodgkin's Lymphoma; Pregnancy and Non-Hodgkin's Lymphoma; Primary Central Nervous System Lymphoma; Primary Liver Cancer, Adult; Primary Liver Cancer, Childhood; Prostate Cancer; Rectal Cancer; Renal Cell (Kidney) Cancer; Renal Cell Cancer, Childhood; Renal Pelvis and Ureter, Transitional Cell Cancer; Retinoblastoma; Rhabdomyosarcoma, Childhood; Salivary Gland Cancer; Salivary

Gland' Cancer, Childhood; Sarcoma, Ewing's Family of Tumors; Sarcoma, Kaposi's; Sarcoma (Osteosarcoma)/Malignant Fibrous Histiocytoma of Bone; Sarcoma, Rhabdomyosarcoma, Childhood; Sarcoma, Soft Tissue, Adult; Sarcoma, Soft Tissue, Childhood; Sezary Syndrome; Skin Cancer; Skin Cancer, Childhood; Skin Cancer (Melanoma); Skin Carcinoma, Merkel Cell; Small Cell Lung Cancer; Small Intestine Cancer; Soft Tissue Sarcoma, Adult; Soft Tissue Sarcoma, Childhood; Squamous Neck Cancer with Occult Primary, Metastatic; Stomach (Gastric) Cancer; Stomach (Gastric) Cancer, Childhood; Supratentorial Primitive Neuroectodermal Tumors, Childhood; T-Cell Lymphoma, Cutaneous; Testicular Cancer; Thymoma, Childhood; Thymoma, Malignant; Thyroid Cancer; Thyroid Cancer, Childhood; Transitional Cell Cancer of the Renal Pelvis and Ureter; Trophoblastic Tumor, Gestational; Unknown Primary Site, Cancer of, Childhood; Unusual Cancers of Childhood; Ureter and Renal Pelvis, Transitional Cell Cancer; Urethral Cancer; Uterine Sarcoma; Vaginal Cancer; Visual Pathway and Hypothalamic Glioma, Childhood; Vulvar Cancer; Waldenstrom's Macro globulinemia; and Wilms' Tumor, among others.

A tumor can be classified as malignant or benign. In both cases, there is an abnormal aggregation and proliferation of cells. In the case of a malignant tumor, these cells behave more aggressively, acquiring properties of increased invasiveness. Ultimately, the tumor cells may even gain the ability to break away from the microscopic environment in which they originated, spread to another area of the body (with a very different environment, not normally conducive to their growth), and continue their rapid growth and division in this new location. This is called metastasis. Once malignant cells have metastasized, achieving a cure is more difficult. Benign tumors have less of a tendency to invade and are less likely to metastasize.

The term “reducing a tumor” as used herein refers to a reduction in the size or volume of a tumor mass, a decrease in the number of metastasized tumors in a subject, a decrease in the proliferative status (the degree to which the cancer cells are multiplying) of the cancer cells, and the like.

The treatment regimens of the invention are particularly suited for treating solid tumors including but not limited to: lymphomas, melanoma, renal cell carcinoma (RCC), advanced solid tumors, tumors that have previously been treated with therapeutic therapy but remain refractory to previous therapies. Preferably, the treatment regimens of the invention are particularly suited for treating solid tumors including but not limited to: lymphomas, melanoma, renal cell carcinoma (RCC), hepatic cell carcinoma (HCC), non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), squamous cell carcinoma of the head and

neck (SCCHN) and including advanced solid tumors and tumors that have previously been treated with anti-cancer therapy but remain refractory to previous therapies.

Complementary Immunotherapies and other Combination Therapies

While the fusion protein of SEQ ID NO: 1 may be used as a monotherapy in the treatment regimens in accordance with the invention, the combination of the fusion protein of SEQ ID NO: 1 with other anticancer treatments in the context of the invention is also contemplated. Other therapeutic treatment regimens include other therapeutic immunotherapies such as adoptive cell transfer regimens, antigen-specific vaccination, inhibition of DNA repair proteins (e.g. inhibitors of the nucleic enzyme poly(adenosine 5′-diphospho-ribose) polymerase ["poly(ADP-ribose) polymerase" PARP inhibitors") and blockade of immune checkpoint inhibitory molecules, for example cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) antibodies.

Immune checkpoint proteins regulate T cell function in the immune system. T cells play a central role in cell-mediated immunity. Immune checkpoint proteins interact with specific ligands that send a signal into the T cell and essentially switch off or inhibit T cell function. Cancer cells take advantage of this system by driving high levels of expression of immune checkpoint proteins on their surface that results in control of the T cells expressing immune checkpoint proteins on the surface of T cells that enter the tumor microenvironment, thus suppressing the anticancer immune response. As such, inhibition of immune checkpoint proteins by agents referred to herein as "immune checkpoint protein (ICP) inhibitors" would result in restoration of T cell function and an immune response to the cancer cells. Examples of immune checkpoint proteins include, but are not limited to: CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, OX40, B-7 family ligands or a combination thereof. Preferably, the immune checkpoint inhibitor interacts with a ligand of an immune checkpoint protein which may be CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, OX40, A2aR, B-7 family ligands or a combination thereof. Preferably, the immune checkpoint inhibitor is a biologic therapeutic or a small molecule. Preferably, the immune checkpoint inhibitor is a monoclonal antibody, a humanized antibody, a fully human antibody, a fusion protein or a combination thereof. Preferably, the PD1 immune checkpoint inhibitor comprises one or more anti-PD-1 antibodies, including nivolumab and pembrolizumab.

The combination therapy methods described herein include administering at least one immune checkpoint inhibitor in combination with the fusion protein of SEQ ID NO: 1. The invention is not limited to any specific immune checkpoint inhibitor so long as the immune checkpoint inhibitor inhibits one or more activities of the target immune checkpoint proteins when administered in an effective amount as monotherapy or in combination with the fusion protein of SEQ ID NO: 1. In some instances, due to, for example, synergistic effects, minimal inhibition of the immune checkpoint protein by the immune checkpoint inhibitor may be sufficient in the presence of SEQ ID NO: 1. Many immune checkpoint inhibitors are known in the art.

Exemplary PD-1/PD-L1 based immune checkpoint inhibitors include antibody-based therapeutics. Exemplary treatment methods that employ PD-1/PD-L1 based immune checkpoint inhibition are described in U.S. Patent Nos. 8,728,474 and 9,073,994, and EP Patent No. 1537878B1, and, for example, include the use of anti-PD-1 antibodies. Exemplary anti-PD-1 antibodies are described, for example, in U.S. Patent Nos. 8,952,136, 8,779,105, 8,008,449, 8,741,295, 9,205,148, 9,181,342, 9,102,728, 9,102,727, 8,952,136, 8,927,697, 8,900,587, 8,735,553, and 7,488,802. Exemplary anti-PD-1 antibodies include, for example, nivolumab (OPDIVO®, Bristol-Myers Squibb Co.), pembrolizumab (KEYTRUDA®, Merck Sharp & Dohme Corp.), PDR001 (Novartis Pharmaceuticals), and pidilizumab (CT-011, Cure Tech). Exemplary anti-PD-L1 antibodies are described, for example, in U.S. Patent Nos. 9,273,135, 7,943,743, 9,175,082, 8,741,295, 8,552,154, and 8,217,149. Exemplary anti-PD-L1 antibodies include, for example, atezolizumab (TECENTRIQ®, Genentech), durvalumab (AstraZeneca), MEDI4736, avelumab, and BMS 936559 (Bristol Myers Squibb Co.).

In certain embodiments, a method or composition described herein is administered in combination with a CTLA-4 inhibitor. In the CTLA-4 pathway, the interaction of CTLA-4 on a T-cell with its ligands (e.g., CD80, also known as B7-1, and CD86) on the surface of an antigen presenting cells (rather than cancer cells) leads to T-cell inhibition. Exemplary CTLA-4 based immune checkpoint inhibition methods are described in U.S. Patent Nos. 5,811,097, 5,855,887, 6,051,227. Exemplary anti-CTLA-4 antibodies are described in U.S. Patent Nos. 6,984,720, 6,682,736, 7,311,910; 7,307,064, 7,109,003, 7,132,281, 6,207,156, 7,807,797, 7,824,679, 8,143,379, 8,263,073, 8,318,916, 8,017,114, 8,784,815, and 8,883,984, International (PCT) Publication Nos. WO98/42752, WO00/37504, and WO01/14424, and European Patent No. EP 1212422 B1. Exemplary CTLA-4 antibodies include ipilimumab or tremelimumab.

Preferably, a method or composition of the invention is administered in combination with (i) a PD-1 or PD-L1 inhibitor, e.g., a PD-1 or PD-L1 inhibitor disclosed herein, and (ii) CTLA-4 inhibitor, e.g., a CTLA-4 inhibitor disclosed herein.

Examples of FDA approved immune checkpoint protein inhibitors includes:

- ipilimumab (YERVOY®)
- pembrolizumab (KEYTRUDA®)
- atezolizumab (TECENTRIQ®)
- durvalumab (IMFINZ®)
- avelumab (BAVENCIO®)
- nivolumab (OPDIVO®).

A preferred treatment regimen of the invention combines the fusion protein of SEQ ID NO: 1 administered in accordance with the invention with the immune checkpoint inhibitor, pembrolizumab. Preferably, pembrolizumab is administered on the first day of each treatment cycle of the treatment regimen according to the invention. Preferably 200 mg of pembrolizumab is administered in accordance with manufacturer's recommendations, generally once every three weeks or 21 days.

Treatment regimens with the fusion protein of SEQ ID NO: 1 in accordance with the invention may also be combined with other therapeutic agents and/or anti-cancer agents in addition to, or instead of, immune checkpoint inhibitors. Preferably, the therapeutic agent and/or anti-cancer agent is an antibody. Preferably, the therapeutic agent is a therapeutic protein. Preferably, the therapeutic agent is a small molecule. Preferably the anticancer agent is an antigen. Preferably, the therapeutic agent is a population of cells. Preferably, the therapeutic agent is a therapeutic antibody. Preferably the therapeutic agent is another cytotoxic and/or chemotherapeutic agent. The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Chemotherapeutic agent" includes chemical compounds useful in the treatment of cancer.

Antibodies

Preferably the administration of SEQ ID NO: 1 may be combined with a therapeutic antibody. Methods of producing antibodies, and antigen-binding fragments thereof, are well known in the art and are disclosed in, e.g., U.S. Pat. No. 7,247,301, US2008/0138336, and U.S. Pat. No. 7,923,221, all of which are herein incorporated by reference in their entirety.

Therapeutic antibodies that can be used in the methods of the present invention include, but are not limited to, any of the art-recognized therapeutic antibodies that are approved for use, in clinical trials, or in development for clinical use. In some embodiments, more than one therapeutic antibody can be included in the combination therapy of the present invention. Non-limiting examples of therapeutic antibodies include the following, without limitation:

- trastuzumab (HERCEPTIN™. by Genentech, South San Francisco, Calif.), which is used to treat HER-2/neu positive breast cancer or metastatic breast cancer;
- bevacizumab (AVASTIN™ by Genentech), which is used to treat colorectal cancer, metastatic colorectal cancer, breast cancer, metastatic breast cancer, non-small cell lung cancer, or renal cell carcinoma;
- rituximab (RITUXAN™ by Genentech), which is used to treat non-Hodgkin's lymphoma or chronic lymphocytic leukemia;
- pertuzumab (OMNITARG™ by Genentech), which is used to treat breast cancer, prostate cancer, non-small cell lung cancer, or ovarian cancer;
- cetuximab (ERBITUX™ by ImClone Systems Incorporated, New York, N.Y.), which can be used to treat colorectal cancer, metastatic colorectal cancer, lung cancer, head and neck cancer, colon cancer, breast cancer, prostate cancer, gastric cancer, ovarian cancer, brain cancer, pancreatic cancer, esophageal cancer, renal cell cancer, prostate cancer, cervical cancer, or bladder cancer;
- IMC-1C11 (ImClone Systems Incorporated), which is used to treat colorectal cancer, head and neck cancer, as well as other potential cancer targets;
- tositumomab and tositumomab and iodine I^{131} (BEXXAR™ by Corixa Corporation, Seattle, Wash.), which is used to treat non-Hodgkin's lymphoma, which can be CD20 positive, follicular, non-Hodgkin's lymphoma, with and without transformation, whose disease is refractory to Rituximab and has relapsed following chemotherapy;
- In^{111} ibirtumomab tiuxetan; Y^{90} ibirtumomab tiuxetan; I^{111} ibirtumomab tiuxetan and Y^{90} ibirtumomab tiuxetan (ZEVALIN™ by Biogen Idec, Cambridge, Mass.), which is used to treat lymphoma or non-Hodgkin's lymphoma, which can include relapsed follicular lymphoma; relapsed or refractory, low grade or follicular non-Hodgkin's lymphoma; or transformed B-cell non-Hodgkin's lymphoma;
- EMD 7200 (EMD Pharmaceuticals, Durham, N.C.), which is used for treating for treating non-small cell lung cancer or cervical cancer;

- SGN-30 (a genetically engineered monoclonal antibody targeted to CD30 antigen by Seattle Genetics, Bothell, Wash.), which is used for treating Hodgkin's lymphoma or non-Hodgkin's lymphoma;
- SGN-15 (a genetically engineered monoclonal antibody targeted to a Lewisγ-related antigen that is conjugated to doxorubicin by Seattle Genetics), which is used for treating non-small cell lung cancer;
- SGN-33 (a humanized antibody targeted to CD33 antigen by Seattle Genetics), which is used for treating acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS);
- SGN-40 (a humanized monoclonal antibody targeted to CD40 antigen by Seattle Genetics), which is used for treating multiple myeloma or non-Hodgkin's lymphoma;
- SGN-35 (a genetically engineered monoclonal antibody targeted to a CD30 antigen that is conjugated to auristatin E by Seattle Genetics), which is used for treating non-Hodgkin's lymphoma;
- SGN-70 (a humanized antibody targeted to CD70 antigen by Seattle Genetics), that is used for treating renal cancer and nasopharyngeal carcinoma;
- SGN-75 (a conjugate comprised of the SGN70 antibody and an Auristatin derivative by Seattle Genetics); and
- SGN-17/19 (a fusion protein containing antibody and enzyme conjugated to melphalan prodrug by Seattle Genetics), which is used for treating melanoma or metastatic melanoma.

The therapeutic antibodies to be used in the methods of the present invention are not limited to those described herein. For example, the following approved therapeutic antibodies can also be used in the methods of the invention: brentuximab vedotin (ADCETRIS™) for anaplastic large cell lymphoma and Hodgkin lymphoma, ipilimumab (MDX-101; YERVOY™) for melanoma, ofatumumab (ARZERRA™) for chronic lymphocytic leukemia, panitumumab (VECTIBIX™) for colorectal cancer, alemtuzumab (CAMPATH™) for chronic lymphocytic leukemia, ofatumumab (ARZERRA™) for chronic lymphocytic leukemia, gemtuzumab ozogamicin (MYLOTARG™) for acute myelogenous leukemia.

Antibodies for use in accordance with the invention can also target molecules expressed by immune cells, such as, but not limited to, tremelimumab (CP-675,206) and

ipilimumab (MDX-010) which targets CTLA4 and has the effect of tumor rejection, protection from re-challenge, and enhanced tumor-specific T cell responses; OX86 which targets OX40 and increases antigen-specific CD8+ T cells at tumor sites and enhances tumor rejection; CT-011 which targets PD 1 and has the effect of maintaining and expanding tumor specific memory T cells and activates NK cells; BMS-663513 which targets CD137 and causes regression of established tumors, as well as the expansion and maintenance of CD8+ T cells, and daclizumab (ZENAPAX™) which targets CD25 and causes transient depletion of CD4+CD25+FOXP3+Tregs and enhances tumor regression and increases the number of effector T cells. A more detailed discussion of these antibodies can be found in, e.g., Weiner et al., Nature Rev. Immunol 2010; 10:317-27.

Preferably, the antibody is a pro-inflammatory and/or pro-tumorigenic cytokine targeting antibody including, but not limited to, anti-TNF antibodies, anti-IL-1Ra receptor targeting antibodies, anti-IL-1 antibodies, anti-IL-6 receptor antibodies, and anti-IL-6 antibodies. Preferably antibodies include those that target pro-inflammatory T helper type 17 cells (TH17).

The therapeutic antibody can be a fragment of an antibody; a complex comprising an antibody; or a conjugate comprising an antibody. The antibody can optionally be chimeric or humanized or fully human.

Therapeutic Proteins and polypeptides

Preferably the methods of the invention include administration of the fusion protein of SEQ ID NO: 1 in accordance with the treatment regimen of the invention in combination with a therapeutic protein or peptide. Therapeutic proteins that are effective in treating cancer are well known in the art. Preferably, the therapeutic polypeptide or protein is a "suicide protein" that causes cell death by itself or in the presence of other compounds.

A representative example of such a suicide protein is thymidine kinase of the herpes simplex virus. Additional examples include thymidine kinase of varicella zoster virus, the bacterial gene cytosine deaminase (which converts 5-fluorocytosine to the highly toxic compound 5-fluorouracil), p450 oxidoreductase, carboxypeptidase G2, beta-glucuronidase, penicillin-V-amidase, penicillin-G-amidase, beta-lactamase, nitroreductase, carboxypeptidase A, linamarase (also referred to as β-glucosidase), the *E. coli* gpt gene, and the *E. coli* Deo gene, although others are known in the art. In some embodiments, the suicide protein converts a prodrug into a toxic compound.

As used herein, "prodrug" means any compound useful in the methods of the present invention that can be converted to a toxic product, i.e. toxic to tumor cells. The prodrug is converted to a toxic product by the suicide protein. Representative examples of such prodrugs include: ganciclovir, acyclovir, and FIAU (1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil) for thymidine kinase; ifosfamide for oxidoreductase; 6-methoxypurine arabinoside for VZV-TK; 5-fluorocytosine for cytosine deaminase; doxorubicin for beta-glucuronidase; CB 1954 and nitrofurazone for nitroreductase; and N-(Cyanoacetyl)-L-phenylalanine or N-(3-chloropropionyl)-L-phenylalanine for carboxypeptidase A. The prodrug may be administered readily by a person having ordinary skill in this art. A person with ordinary skill would readily be able to determine the most appropriate dose and route for the administration of the prodrug.

Preferably the therapeutic protein or polypeptide, is a cancer suppressor, for example p53 or Rb, or a nude acid encoding such a protein or polypeptide. Those of skill know of a wide variety of such cancer suppressors and how to obtain them and/or the nucleic acids encoding them.

Other examples of anti-cancer/therapeutic proteins or polypeptides include pro-apoptotic therapeutic proteins and polypeptides, for example, p15, p16, or $p21^{WAF-1}$.

Cytokines, and nucleic acid encoding them may also be used as therapeutic proteins and polypeptides. Examples include: GM-CSF (granulocyte macrophage colony stimulating factor); TNF-alpha (Tumor necrosis factor alpha); Interferons including, but not limited to, IFN-alpha and IFN-gamma; and Interleukins including, but not limited to, Interleukin-1 (IL-1), Interleukin-Beta (IL-beta), Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-5 (IL-5), Interleukin-6 (IL-6), Interleukin-7 (IL-7), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Interleukin-12 (IL-12), Interleukin-13 (IL-13), Interleukin-14 (IL-14), Interleukin-15 (IL-15), Interleukin-16 (IL-16), Interleukin-18 (IL-18), Interleukin-23 (IL-23), Interleukin-24 (IL-24), although other embodiments are known in the art.

Additional examples of cytocidal genes includes, but is not limited to, mutated cyclin G1 genes. By way of example, the cytocidal gene may be a dominant negative mutation of the cyclin G1 protein (e.g., WO/01/64870).

Vaccines

Preferably, the therapeutic regimens of the invention include administration of a fusion protein of SEQ ID NO: 1 in combination with administration of a cancer vaccine for stimulating a cancer specific-immune response, e.g., innate and adaptive immune responses,

for generating host immunity against a cancer (see, e.g., Overwijk, et al. Journal of Experimental Medicine 2008; 198:569-80). Illustrative vaccines include, but are not limited to, for example, antigen vaccines, whole cell vaccines, dendritic cell vaccines, and DNA vaccines. Depending upon the particular type of vaccine, the vaccine composition may include one or more suitable adjuvants known to enhance a subject's immune response to the vaccine.

The vaccine may, for example, be cellular based, i.e., created using cells from the patient's own cancer cells to identify and obtain an antigen. Exemplary vaccines include tumor cell-based and dendritic-cell based vaccines, where activated immune cells from the subject are delivered back to the same subject, along with other proteins, to further facilitate immune activation of these tumor antigen primed immune cells. Tumor cell-based vaccines include whole tumor cells and gene-modified tumor cells. Whole tumor cell vaccines may optionally be processed to enhance antigen presentation, e.g., by irradiation of either the tumor cells or tumor lysates). Vaccine administration may also be accompanied by adjuvants such as bacillus calmette-guerin (BCG) or keyhole limpet hemocyanin (KLH), depending upon the type of vaccine employed. Plasmid DNA vaccines may also be used and can be administered via direct injection or biolistically. Also contemplated for use are peptide vaccines, viral gene transfer vector vaccines, and antigen-modified dentritic cells (DCs).

Preferably the vaccine is a therapeutic cancer peptide-based vaccine. Peptide vaccines can be created using known sequences or from isolated antigens from a subject's own tumor(s) and include neoantigens and modified antigens. Illustrative antigen-based vaccines include those where the antigen is a tumor-specific antigen. For example, the tumor-specific antigen may be selected from a cancer-testis antigen, a differentiation antigen, and a widely occurring over-expressed tumor associated antigen, among others. Recombinant peptide vaccines, based on peptides from tumor-associated antigens, when used in the instant method, may be administered or formulated with, an adjuvant or immune modulator. Illustrative antigens for use in a peptide-based vaccine include, but are not limited to, the following, since this list is meant to be purely illustrative. For example, a peptide vaccine may comprise a cancer-testis antigen such as MAGE, BAGE, NY-ESO-1 and SSX-2, encoded by genes that are normally silenced in adult tissues but transcriptionally reactivated in tumor cells. Alternatively, the peptide vaccine may comprise a tissue differentiation associated antigen, i.e., an antigen of normal tissue origin and shared by both normal and tumorous tissue. For example, the vaccine may comprise a melanoma-associated antigen such as gp100, Melan-A/Mart-1, MAGE-3, or tyrosinase; or may comprise a prostate cancer antigen such as PSA or

PAP. The vaccine may comprise a breast cancer-associated antigen such as mammaglobin-A. Other tumor antigens that may be comprised in a vaccine for use in the instant method include, for example, CEA, MUC-1, HER1/Nue, hTERT, ras, and B-raf. Other suitable antigens that may be used in a vaccine include SOX-2 and OCT-4, associated with cancer stem cells or the EMT process.

Antigen vaccines include multi-antigen and single antigen vaccines. Exemplary cancer antigens may include peptides having from about 5 to about 30 amino acids, or from about 6 to 25 amino acids, or from about 8 to 20 amino acids.

As described above, an immunostimulatory adjuvant (different from RSLAIL-2) may be used in a vaccine, in particular, a tumor-associated antigen-based vaccine, to assist in generating an effective immune response. For example, a vaccine may incorporate a pathogen-associated molecular pattern (PAMP) to assist in improving immunity. Additional suitable adjuvants include monophosphoryl lipid A, or other lipopolysaccharides; toll-like receptor (TLR) agonists such as, for example, imiquimod, resiquimod (R-848), TLR3, IMO-8400, and rintatolimod. Additional adjuvants suitable for use include heat shock proteins.

A genetic vaccine typically uses viral or plasmid DNA vectors carrying expression cassettes. Upon administration, they transfect somatic cells or dendritic cells as part of the inflammatory response to thereby result in cross-priming or direct antigen presentation. Preferably, a genetic vaccine is one that provides delivery of multiple antigens in one immunization. Genetic vaccines include DNA vaccines, RNA vaccines and viral-based vaccines.

DNA vaccines for use in the instant methods are bacterial plasmids that are constructed to deliver and express tumor antigen. DNA vaccines may be administered by any suitable mode of administration, e.g., subcantaneous or intradermal injection, but may also be injected directly into the lymph nodes. Additional modes of delivery include, for example, gene gun, electroporation, ultrasound, laser, liposomes, microparticles and nanoparticles.

Preferably, the vaccine comprises a neoantigen, or multiple neoantigens. Preferably, the vaccine is a neoantigen-based vaccine. Preferably a neoantigen-based vaccine (NBV) composition may encode multiple cancer neoantigens in tandem, where each neoantigen is a polypeptide fragment derived from a protein mutated in cancer cells. For instance, a neoantigenic vaccine may comprise a first vector comprising a nucleic acid construct encoding multiple immunogenic polypeptide fragments, each of a protein mutated in cancer cells, where each immunogenic polypeptide fragment comprises one or more mutated amino acids flanked by a variable number of wild type amino acids from the original protein, and

each polypeptide fragment is joined head-to-tail to form an immunogenic polypeptide. The lengths of each of the immunogenic polypeptide fragments forming the immunogenic polypeptide can vary.

Viral gene transfer vector vaccines may also be used; in such vaccines, recombinant engineered virus, yeast, bacteria or the like is used to introduce cancer-specific proteins to the patient's immune cells. In a vector-based approach, which can be tumor lytic or non-tumor lytic, the vector can increase the efficiency of the vaccine due to, for example, its inherent immunostimulatory properties. Illustrative viral-based vectors include those from the poxviridae family, such as vaccinia, modified vaccinia strain Ankara and avipoxviruses. Also suitable for use is the cancer vaccine, PROSTVAC, containing a replication-competent vaccinia priming vector and a replication-incompetent fowlbox-boosting vector. Each vector contains transgenes for PSA and three co-stimulatory molecules, CD80, CD54 and CD58, collectively referred to as TRICOM. Other suitable vector-based cancer vaccines include Trovax and TG4010 (encoding MUC1 antigen and IL-2). Additional vaccines for use include bacteria and yeast-based vaccines such as recombinant *Listeria monocytogenes* and *Saccharomyces cerevisae.*

The foregoing vaccines may be combined and/or formulated with adjuvants and other immune boosters to increase efficacy. Depending upon the particular vaccine, administration may be either intratumoral or non-intratumoral (i.e., systemic).

Other cancer antigens that can be used in vaccinations include, but are not limited to, (i) tumor-specific antigens, (ii) tumor-associated antigens, (iii) cells that express tumor-specific antigens, (iv) cells that express tumor-associated antigens, (v) embryonic antigens on tumors, (vi) autologous tumor cells, (vii) tumor-specific membrane antigens, (viii) tumor-associated membrane antigens, (ix) growth factor receptors, (x) growth factor ligands, and (xi) any other type of antigen or antigen-presenting cell or material that is associated with a cancer.

The cancer antigen may be an epithelial cancer antigen, (e.g., breast, gastrointestinal, lung), a prostate specific cancer antigen (PSA) or prostate specific membrane antigen (PSMA), a bladder cancer antigen, a lung (e.g., small cell lung) cancer antigen, a colon cancer antigen, an ovarian cancer antigen, a brain cancer antigen, a gastric cancer antigen, a renal cell carcinoma antigen, a pancreatic cancer antigen, a liver cancer antigen, an esophageal cancer antigen, a head and neck cancer antigen, or a colorectal cancer antigen.

In another embodiment, the cancer antigen is a lymphoma antigen (e.g., non-Hodgkin's lymphoma or Hodgkin's lymphoma), a B-cell lymphoma cancer antigen, a

leukemia antigen, a myeloma (i.e., multiple myeloma or plasma cell myeloma) antigen, an acute lymphoblastic leukemia antigen, a chronic myeloid leukemia antigen, or an acute myelogenous leukemia antigen. The described cancer antigens are only exemplary, and that any cancer antigen can be targeted in the present invention.

Preferably, the cancer antigen is a mucin-1 protein or peptide (MUC-1) that is found on all human adenocarcinomas: pancreas, colon, breast, ovarian, lung, prostate, head and neck, including multiple myelomas and some B cell lymphomas. Patients with inflammatory bowel disease, either Crohn's disease or ulcerative colitis, are at an increased risk for developing colorectal carcinoma. MUC-1 is a type I transmembrane glycoprotein. The major extracellular portion of MUC-1 has a large number of tandem repeats consisting of 20 amino acids which comprise immunogenic epitopes. In some cancers it is exposed in an unglycosylated form that is recognized by the immune system (Gendler et al., *J Biol Chem* 1990; 265:15286-15293).

In another embodiment, the cancer antigen is a mutated B-Raf antigen, which is associated with melanoma and colon cancer. The vast majority of these mutations represent a single nucleotide change of T-A at nucleotide 1796 resulting in a valine to glutamic acid change at residue 599 within the activation segment of B-Raf. Raf proteins are also indirectly associated with cancer as effectors of activated Ras proteins, oncogenic forms of which are present in approximately one-third of all human cancers. Normal non-mutated B-Raf is involved in cell signaling, relaying signals from the cell membrane to the nucleus. The protein is usually only active when needed to relay signals. In contrast, mutant B-Raf has been reported to be constantly active, disrupting the signaling relay (Mercer and Pritchard, Biochim Biophys Acta (2003) 1653(1):25-40; Sharkey et al., Cancer Res. (2004) 64(5):1595-1599).

Preferably, the cancer antigen is a human epidermal growth factor receptor-2 (HER-2/neu) antigen. Cancers that have cells that overexpress HER-2/neu are referred to as HER-2/neu$^+$ cancers. Exemplary HER-2/neu$^+$ cancers include prostate cancer, lung cancer, breast cancer, ovarian cancer, pancreatic cancer, skin cancer, liver cancer (e.g., hepatocellular adenocarcinoma), intestinal cancer, and bladder cancer.

HER-2/neu has an extracellular binding domain (ECD) of approximately 645 aa, with 40% homology to epidermal growth factor receptor (EGFR), a highly hydrophobic transmembrane anchor domain (TMD), and a carboxyterminal intracellular domain (ICD) of approximately 580 aa with 80% homology to EGFR. The nucleotide sequence of HER-2/neu is available at GENBANK™. Accession Nos. AH002823 (human HER-2 gene, promoter

region and exon 1); M16792 (human HER-2 gene, exon 4): M16791 (human HER-2 gene, exon 3); M16790 (human HER-2 gene, exon 2); and M16789 (human HER-2 gene, promoter region and exon 1). The amino acid sequence for the HER-2/neu protein is available at GENBANK™. Accession No. AAA58637. Based on these sequences, one skilled in the art could develop HER-2/neu antigens using known assays to find appropriate epitopes that generate an effective immune response.

Exemplary HER-2/neu antigens include p369-377 (a HER-2/neu derived HLA-A2 peptide); dHER2 (Corixa Corporation); li-Key MHC class II epitope hybrid (Generex Biotechnology Corporation); peptide P4 (amino acids 378-398); peptide P7 (amino acids 610-623); mixture of peptides P6 (amino acids 544-560) and P7; mixture of peptides P4, P6 and P7; HER2 [9_{754}]; and the like.

Preferably, the cancer antigen is an epidermal growth factor receptor (EGFR) antigen. The EGFR antigen can be an EGFR variant 1 antigen, an EGFR variant 2 antigen, an EGFR variant 3 antigen and/or an EGFR variant 4 antigen. Cancers with cells that overexpress EGFR are referred to as EGFR cancers. Exemplary EGFR cancers include lung cancer, head and neck cancer, colon cancer, colorectal cancer, breast cancer, prostate cancer, gastric cancer, ovarian cancer, brain cancer and bladder cancer.

Preferably, the cancer antigen is a vascular endothelial growth factor receptor (VEGFR) antigen. VEGFR is considered to be a regulator of cancer-induced angiogenesis. Cancers with cells that overexpress VEGFR are called $VEGFR^+$ cancers. Exemplary $VEGFR^+$ cancers include breast cancer, lung cancer, small cell lung cancer, colon cancer, colorectal cancer, renal cancer, leukemia, and lymphocytic leukemia.

Preferably, the cancer antigen is prostate-specific antigen (PSA) and/or prostate-specific membrane antigen (PSMA) that are prevalently expressed in androgen-independent prostate cancers.

Preferably, the cancer antigen is Gp-100 Glycoprotein 100 (gp 100) is a tumor-specific antigen associated with melanoma.

Preferably, the cancer antigen is a carcinoembryonic (CEA) antigen. Cancers with cells that overexpress CEA are referred to as CEA^+ cancers. Exemplary CEA^+ cancers include colorectal cancer, gastric cancer and pancreatic cancer. Exemplary CEA antigens include CAP-1 (i.e., CEA aa 571-579), CAP1-6D, CAP-2 (i.e., CEA aa 555-579), CAP-3 (i.e., CEA aa 87-89), CAP-4 (CEA aa 1-11), CAP-5 (i.e., CEA aa 345-354), CAP-6 (i.e., CEA aa 19-28) and CAP-7.

Preferably, the cancer antigen is carbohydrate antigen 10.9 (CA 19.9). CA 19.9 is an oligosaccharide related to the Lewis A blood group substance and is associated with colorectal cancers.

Preferably, the cancer antigen is a melanoma cancer antigen. Melanoma cancer antigens are useful for treating melanoma. Exemplary melanoma cancer antigens include MART-1 (e.g., MART-1 26-35 peptide, MART-1 27-35 peptide); MART-1/Melan A; pMel17; pMel17/gp100; gp100 (e.g., gp 100 peptide 280-288, gp 100 peptide 154-162, gp 100 peptide 457-467); TRP-1; TRP-2; NY-ESO-1; p16; beta-catenin; mum-1; and the like.

Preferably, the cancer antigen is a mutant or wild type ras peptide. The mutant ras peptide can be a mutant K-ras peptide, a mutant N-ras peptide and/or a mutant H-ras peptide. Mutations in the ras protein typically occur at positions 12 (e.g., arginine or valine substituted for glycine), 13 (e.g., asparagine for glycine), 61 (e.g., glutamine to leucine) and/or 59. Mutant ras peptides can be useful as lung cancer antigens, gastrointestinal cancer antigens, hepatoma antigens, myeloid cancer antigens (e.g., acute leukemia, myelodysplasia), skin cancer antigens (e.g., melanoma, basal cell, squamous cell), bladder cancer antigens, colon cancer antigens, colorectal cancer antigens, and renal cell cancer antigens.

In another embodiment of the invention, the cancer antigen is a mutant and/or wildtype p53 peptide. The p53 peptide can be used as colon cancer antigens, lung cancer antigens, breast cancer antigens, hepatocellular carcinoma cancer antigens, lymphoma cancer antigens, prostate cancer antigens, thyroid cancer antigens, bladder cancer antigens, pancreatic cancer antigens and ovarian cancer antigens.

The cancer antigen can be a cell, a protein, a peptide, a fusion protein, DNA encoding a peptide or protein, RNA encoding a peptide or protein, a glycoprotein, a lipoprotein, a phosphoprotein, a carbohydrate, a lipopolysaccharide, a lipid, a chemically linked combination of two or more thereof, a fusion or two or more thereof, or a mixture of two or more thereof, or a virus encoding two or more thereof, or an oncolytic virus encoding two or more thereof. In another embodiment, the cancer antigen is a peptide comprising about 6 to about 24 amino acids; from about 8 to about 20 amino acids; from about 8 to about 12 amino acids; from about 8 to about 10 amino acids; or from about 12 to about 20 amino acids. In one embodiment, the cancer antigen is a peptide having a MHC Class I binding motif or a MHC Class II binding motif. In another embodiment, the cancer antigen comprises a peptide that corresponds to one or more cytotoxic T lymphocyte (CTL) epitopes.

Cell Therapy

Preferably, the methods of the invention include administration of the fusion protein of SEQ ID NO: 1 in combination with administration of a therapeutic cell therapy. Cell therapies that are useful for treating cancer are well known and are disclosed in, e.g., U.S. Pat. No. 7,402,431. In a preferred embodiment, the cell therapy is T cell transplant. In a preferred method, T cells are expanded ex vivo with IL-2 prior to transplantation into a subject. Methods for cell therapies are disclosed in, e.g., U.S. Pat. No. 7,402,431, US2006/0057121, U.S. Pat. No. 5,126,132, U.S. Pat. No. 6,255,073, U.S. Pat. No. 5,846,827, U.S. Pat. No. 6,251,385, U.S. Pat. No. 6,194,207, U.S. Pat. No. 5,443,983, U.S. Pat. No. 6,040,177, U.S. Pat. No. 5,766,920, and US2008/0279836.

Radiation Therapy

Preferably, the therapeutic regimens of the invention include administration of a fusion protein of SEQ ID NO: 1 in further combination with radiation therapy. The term "radiation therapy" may be used interchangeably with the term "radiotherapy", is a type of cancer treatment that uses beams of intense energy to kill cancer cells. Radiation therapy most often uses X-rays, but gamma rays, electron beams, or protons also can be used. The term "radiation therapy" most often refers to external beam radiation therapy. During this type of radiation, the high-energy beams come from a machine outside of the patient's body that aims the beams at a precise point on the body. Each session is quick and painless, lasting about 15 minutes. As used herein, the term "session" or "session of treatment" refers to each radiotherapy treatment. A radiation therapy "regimen" or "schedule" usually consists of a specific number of treatments given over a set period of time, depending on the type and the stage of the cancer.

Small Molecules

Preferably, the therapeutic regimens of the invention include administration of a fusion protein of SEQ ID NO: 1 in combination with administration of an anticancer small molecule. Small molecules that are effective in treating cancer are well known in the art and include antagonists of factors that are involved in tumor growth, such as EGFR, ErbB2 (also known as Her2) ErbB3, ErbB4, or TNF. Non-limiting examples include small molecule receptor tyrosine kinase inhibitors (RTKIs) that target one or more tyrosine kinase receptors, such as VEGF receptors, FGF receptors, EGF receptors and PDGF receptors.

Many therapeutic small molecule RTKIs are known in the art, including, but are not limited to, vatalanib (PTK787), erlotinib (TARCEVA™), OSI-7904, ZD6474 (ZACTIMA™), ZD6126 (ANG453), ZD1839, sunitinib (SUTENT™), semaxanib (SU5416), AMG706, AG013736, Imatinib (GLEEVEC™), MLN-518, CEP-701, PKC-412, Lapatinib (GSK572016), VELCADE™, AZD2171, sorafenib (NEXAVAR™), XL880, and CHIR-265. Small molecule protein tyrosine phosphatase inhibitors, such as those disclosed in Jiang et al., *Cancer Metastasis Rev.* 2008; 27:263-72 are also useful for practicing the methods of the invention. Such inhibitors can target, e.g., HSP2, PRL, PTP1B, or Cdc25 phosphatases.

Small molecules that target Bcl-2/Bcl-XL, such as those disclosed in US2008/0058322, are also useful for practicing the methods of the present invention. Further exemplary small molecules for use in the present invention are disclosed in Zhang et al. Nature Reviews: Cancer 2009; 9:28-39. In particular, chemotherapeutic agents that lead to immunogenic cell death such as anthracyclins (Kepp et al., *Cancer and Metastasis Reviews* 2011; 30:61-9) will be well suited for synergistic effects with extended-PK IL-2.

Other Cytotoxic and Chemotherapeutic Agents

Preferably, the methods of the invention include administration of the fusion protein of SEQ ID NO: 1 in combination with administration with chemotherapeutic agents including but not limited to, alkylating agents, antitumor antibiotics, antimetabolic agents, other anti-tumor antibiotics, and plant derived agents.

Alkylating agents are drugs which impair cell function by forming covalent bonds with amino, carboxyl, sulfhydryl and phosphate groups in biologically important molecules. The most important sites of alkylation are DNA, RNA and proteins. Alkylating agents depend on cell proliferation for activity but are not cell-cycle-phase-specific. Alkylating agents suitable for use in the present invention include, but are not limited to, bischloroethylamines (nitrogen mustards, e.g. chlorambucil, cyclophosphamide, ifosfamide, mechlorethamine, melphalan, uracil mustard), aziridines (e.g. thiotepa), alkyl alkone sulfonates (e.g. busulfan), nitroso-ureas (e. g. BCNU, carmustine, lomustine, streptozocin), nonclassic alkylating agents (e.g., altretamine, dacarbazine, and procarbazine), and platinum compounds (e.g., carboplastin, oxaliplatin and cisplatin).

Antitumor antibiotics like adriamycin intercalate DNA at guanine-cytosine and guanine-thymine sequences, resulting in spontaneous oxidation and formation of free oxygen radicals that cause strand breakage. Other antibiotic agents suitable for use in the present invention include, but are not limited to, anthracyclines (e. g. doxorubicin, daunorubicin,

epirubicin, idarubicin and anthracenedione), mitomycin C, bleomycin, dactinomycin, and plicatomycin.

Antimetabolic agents suitable for use in the present invention include but are not limited to, floxuridine, fluorouracil, methotrexate, leucovorin, hydroxyurea, thioguanine, mercaptopurine, cytarabine, pentostatin, fludarabine phosphate, cladribine, asparaginase, and gemcitabine.

Plant derived agents include taxanes, which are semisynthetic derivatives of extracted precursors from the needles of yew plants. These drugs have a novel 14-member ring, the taxane. Unlike the *vinca* alkaloids, which cause microtubular disassembly, the taxanes (e.g., taxol) promote microtubular assembly and stability, therefore blocking the cell cycle in mitosis. Other plant derived agents include, but are not limited to, vincristine, vinblastine, vindesine, vinzolidine, vinorelbine, etoposide, teniposide, and docetaxel.

Compositions for Combination Therapy

Preferably, the fusion protein of SEQ ID NO: 1 is administered together (simultaneously or sequentially) with one or more additional therapeutic agents or other therapeutic agents, such as a therapeutic antibody. Preferably, the fusion protein of SEQ ID NO: 1 is administered prior to the administration of one or more therapeutic agents, such as a therapeutic antibody. Preferably, the fusion protein of SEQ ID NO: 1 is administered concurrent with the administration of one or more therapeutic agents, such as a therapeutic antibody. Preferably, the fusion protein of SEQ ID NO: 1 is administered subsequent to the administration of one or more therapeutic agents, such as a therapeutic antibody. Preferably, the SEQ ID NO: 1 and one or more therapeutic agents, such as a therapeutic antibody, are administered simultaneously. In other embodiments, the and one or more therapeutic agents, such as a therapeutic antibody, are administered sequentially. Preferably, the fusion protein of SEQ ID NO: 1 and one or more therapeutic agents, such as a therapeutic antibody, are administered within one, two, or three days of each other.

The one or more therapeutic agents may be those that serve as adjunctive therapy for cancer, such as cytokines, chemotherapeutic agents, small molecules, antigens, or therapeutic antibodies, and are well known in the art and discussed *supra*. Additional non-limiting examples of additional agents include GM-CSF (expands monocyte and neutrophil population), IL-7 (important for generation and survival of memory T-cells), interferon alpha, tumor necrosis factor alpha, IL-12, and therapeutic antibodies, such as anti-PD-1, anti-PD-L, anti-CTLA4, anti-CD40, anti-OX40, and anti-CD137, PARP inhibitors, antibodies. In some

embodiments, the subject receives the fusion protein of SEQ ID NO: 1 and one or more therapeutic agents during a same period of prevention, occurrence of a disorder, and/or period of treatment.

Preferably, the invention provides for separate pharmaceutical compositions comprising the fusion protein of SEQ ID NO: 1 with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant and another pharmaceutical composition comprising one or more therapeutic agents, such as a therapeutic antibody, with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant.

Preferably, the invention provides for pharmaceutical compositions comprising the fusion protein of SEQ ID NO: 1 and one or more therapeutic or anti-cancer agents in the same composition, together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant.

Kits

Also provided are kits comprising a fusion protein of SEQ ID NO: 1 formulated for SC administration, and optionally any other chemotherapeutic or anti-cancer agent. The kits are generally in the form of a physical structure housing various components, as described below, and can be utilized, for example, in practicing the methods described above. A kit can include the fusion protein of SEQ ID NO: 1 (provided in, e.g., a sterile container), which can be in the form of a pharmaceutical composition suitable for administration to a subject. The pharmaceutical composition can be provided in a form that is ready for use or in a form requiring, for example, reconstitution or dilution prior to administration. When the compositions are in a form that needs to be reconstituted by a user, the kit can also include buffers, pharmaceutically acceptable excipients, and the like, packaged with or separately from the fusion protein of SEQ ID NO: 1. When combination therapy (e.g., the fusion protein of SEQ ID NO: 1 and an immune checkpoint inhibitor(s) is contemplated, the kit can contain the several agents separately or they can already be combined in the kit. Similarly, when additional complementary therapy is required (e.g., a fusion protein of SEQ ID NO: 1, an immune checkpoint inhibitor(s), and an additional complementary therapy or agent) is, the kit

can contain the several agents separately or two or more of them can already be combined in the kit.

A kit of the invention can be designed for conditions necessary to properly maintain the components housed therein (e.g., refrigeration or freezing). A kit can contain a label or packaging insert including identifying information for the components therein and instructions for their use (e.g., dosing parameters, clinical pharmacology of the active ingredient(s), including mechanism(s) of action, pharmacokinetics and pharmacodynamics, adverse effects, contraindications, etc.).

Each component of the kit can be enclosed within an individual container, and all of the various containers can be within a single package. Labels or inserts can include manufacturer information such as lot numbers and expiration dates. The label or packaging insert can be, e.g., integrated into the physical structure housing the components, contained separately within the physical structure, or affixed to a component of the kit (e.g., an ampule, syringe or vial).

Labels or inserts can additionally include, or be incorporated into, a computer readable medium, such as a disk (e.g., hard disk, card, memory disk), optical disk such as CD- or DVD-ROM/RAM, DVD, MP3, magnetic tape, or an electrical storage media such as RAM and ROM or hybrids of these such as magnetic/optical storage media, FLASH media or memory-type cards. In some embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, e.g., via an internet site, are provided.

Equivalents and Scope

Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

In addition, it is to be understood that any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein.

Any particular embodiment of the compositions of the invention; any method of production; any method of use can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

EXAMPLES

Example 1- A Phase 1 Study of the Fusion Protein of SEQ ID NO: 1 Administered Intravenously as Monotherapy and in Combination with Pembrolizumab in Subjects with Advanced Solid Tumors.

The Fusion Protein of SEQ ID NO: 1 is a fusion of circularly permuted IL-2 and IL-2 Receptor α (IL-2Rα) designed to selectively activate the intermediate-affinity IL-2R, comprised of IL-2Rβ and γ, for activation of cytotoxic $CD8^+$ T cells and NK cells. The intermediate-affinity IL-2R is expressed predominantly on effector lymphocytes, which play an important role in driving antitumor immune responses. Wild-type IL-2 activates the high-affinity IL-2R, comprised of IL-2Rα, β, and γ_c, driving the expansion of immunosuppressive $CD4^+$ regulatory T (T_{reg}) cells at concentrations below those at which intermediate-affinity IL-2R-bearing effector cells are activated. Selective activation of the intermediate affinity IL-2R has the potential to enhance tumor killing and was shown to possess enhanced antitumor activity relative to IL-2 in murine models.

Methods

The fusion protein of SEQ ID NO: 1 is being studied in a Phase 1 study in human subjects with advanced refractory solid tumors. The Fusion Protein was supplied as a sterile, white to off-white, lyophilized powder for IV or SC administration. The excipients included in the Fusion Protein formulation are citric acid monohydrate, sodium citrate tribasic dihydrate, sucrose, and polysorbate 20. For IV administration, sterile water for injection, United States Pharmacopeia (USP), is supplied separately for reconstitution. Citrate buffer containing 1% polysorbate 20 (PS20 Diluent) is supplied separately for dilution. Saline solution (0.9% Sodium Chloride Injection, USP) is sourced separately as needed for additional dilution.

Four different doses (0.1, 0.3, 1, 3 μg/kg/day) of the Fusion Protein was administered to patients as a 30-minute intravenous infusion once daily for 5 consecutive days repeating in treatment cycles of 14 days (first cycle) or 21 days (subsequent cycles). The first part of the study is a dose escalation with the primary objective of investigating the safety and tolerability of the Fusion Protein and to determine the maximum tolerated dose (MTD) and

the recommended phase 2 dose (RP2D). The RP2D will be equal to or less than the MTD and its associated dosing schedule and will be selected based on the safety, PK, pharmacodynamics, and preliminary antitumor activity data observed during dose escalation. Figure 2 shows the treatment regimens followed for this study.

Four different doses (0.1, 0.3, 1, 3 µg/kg/day) of the Fusion Protein was administered as a 30-minute intravenous infusion once daily for 5 consecutive days repeating in treatment cycles of 14 days (first cycle) or 21 days (subsequent cycles). On day 1 of the first cycle, the immune checkpoint inhibitor, pembrolizumab was also administered at a dose of 200 mg. Figure 2 shows the treatment regimens followed for this study.

See below for discussion of higher doses (6 µg/kg/day and 8 µg/kg/day) that were administered as a part of this clinical study protocol (see also Figures 11-15)

Grade 3 Treatment-Related Adverse Events

Consistent with other cytokine therapies, pyrexia and chills were the most common treatment0emergent the Fusion Protein-related adverse events. Overt capillary leak syndrome has not been observed to date. No Grade 4 or 5 adverse events have been reported. At the 3 µg/kg dose level, one incident each of Grade 3 febrile neutropenia and Grade 3 hypoalbuminemia met the protocol definitions for dose-limiting toxicities (DLT). After discussion with the investigators it was determined that these should not be considered DLTs, and DLT definitions were amended, allowing continued dose escalation.

Elevation of Serum Cytokine Levels

Dose-dependent elevations of serum IL-6 and IFN-γ levels were observed in response to treatment with the Fusion Protein. IL-6 levels peaked at 4 hours post-dose and recovered to baseline at 8-10 hours post-dose. Fever coincided with the time of maximum IL-6 and recovered to baseline 8-12 hours post-dose.

Conclusions from dosing with 0.1, 0.3, 1, 3 µg/kg/day.

- Dose proportional increase in the Fusion Protein systemic exposure in patients treated with the Fusion Protein (Figure 3).
- Dose-dependent increase in circulating NK cells and $CD8^+$ T cells (Figure 4) as measured in the peripheral blood of the patient.
- Variable and non-dose dependent increase in T_{reg} (Figure 4).

- Dose related transient elevation of serum IL-6 levels coincided with onset of AE of chills and fever.
- No evidence of capillary leak syndrome.

Cohort 5 Administration of 6 µg/kg/day

Eleven (11) patients of Cohort 5 of the Dose escalation study with advanced solid tumors received 6 µg/kg per day of the Fusion Protein by IV administration daily for 5 days, followed by a period off treatment in repeating cycles. During Cycle 1, the period off treatment was 9 days, resulting in a cycle length of 14 days (2 weeks). Cycle 2 and subsequent cycles had a period off treatment of 16 days, resulting in a cycle length of 21 days (3 weeks). For the first 2 treatment cycles, subjects received the Fusion Protein as inpatients at a medical facility with access to medical support measures and to the intensive care unit, if needed. In the absence of dose limiting toxicities (DLTs), subjects received subsequent doses of the Fusion Protein on an outpatient basis.

Conclusions from Cohort 5 Administration of 6 µg/kg/day

- Dose proportional increase in the Fusion Protein systemic exposure in patients treated with the Fusion Protein (Figure 3).
- Dose-dependent increase in circulating NK cells and $CD8^+$ T cells (Figure 4) as measured in the peripheral blood of the patient.
- Variable and non-dose dependent increase in T_{reg} (Figure 4).
- Dose related transient elevation of serum IL-6 levels coincided with onset of AE of chills and fever.
- No evidence of cytokine release syndrome.
- No evidence of capillary leak syndrome.
- No patients had dose limiting toxicities at the 6 µg/kg/day dosing regimen which is equivalent to high dose rhIL-2 administration (Aldesleukin).
- Given the results from this 6 µg/kg/day dosing regimen, particularly the lack of dose limiting toxicities, it is believed that the maximum tolerated dose (MTD) is higher than a dose of 8 µg/kg/day dosing regimen, 10 µg/kg/day dosing regimen and even 15 µg/kg/day dosing regimen.

Additional Results-Antitumor Activity during monotherapy dose escalation:

A total of 36 patients received SEQ ID NO: 1 at doses up to 6 µg/kg/day and the findings were the same as those reported above. The maximum tolerated dose of SEQ ID NO: 1 was not yet reached. Among the 27 patients with evaluable scans, 14 (52%) had stable disease (Figure 7). One patient with heavily pretreated pancreatic adenocarcinoma had prolonged stable disease and continued receiving SEQ ID NO: 1 monotherapy at 6 µg/kg/day for over 6 months.

Antitumor Activity during combination therapy with pembrolizumab

Twenty-six (26) patients received SEQ ID NO: 1 in combination with pembrolizumab. Among the 26 patients, 18 had evaluable scans. 12 of the 18 patients (67%) has stable disease or better over the course of treatment (Figure 8).

One patient with ovarian cancer had a confirmed partial response. One patient with triple negative breast cancer showed greater than 50% reduction in target lesion size (Figure 9).

Example 2-Peripheral Blood Lymphocyte Responses in Patients with Renal Cell Carcinoma (RCC) treated with high dose IL-2.

Background

Recombinant human interleukin-2 (rhIL-2, aldesleukin) is approved and used for the treatment of metastatic melanoma and renal cell carcinoma.[1-8] However, the use of rhIL-2 is limited to patients with normal cardiac and pulmonary function due to associated capillary leak syndrome and resulting hypotension.[9-12]

Despite the poor tolerability associated with rhIL-2 treatment, it remains one of the few treatment regimens for metastatic melanoma and renal cell carcinoma that elicits a complete and durable response in a subset of patients, up to 12% in melanoma and 7% in renal cell carcinoma.[7,8] It has been hypothesized that rhIL-2 preferentially activates and induces the expansion of immunosuppressive CD4+ Tregs,13 and high-dose IL-2 is required to induce signaling on receptor complexes expressed on potential tumor killing CD8+ T cells and natural killer (NK) cells.

Published data show that the immunosuppressive inducible T cell co-stimulator-positive (ICOS+) Treg cells were significantly expanded in a subset of melanoma patients receiving high dose IL-2 therapy.[14] However, no data are readily available that specifically quantify and compare the levels of expansion of cytotoxic effectors such as CD8+ T cells and

NK cells relative to Treg cells. This study was conducted with the primary goal to assess the pharmacodynamic effects of high-dose IL-2 on numbers of circulating CD8+ T cells, NK cells, and Treg cells.

Methods

- This was a single-center, open-label study.
- Study center: Beth Israel Deaconess Medical Center, Boston, MA.
- Study participants: a cohort of patients with renal cell carcinoma receiving treatment with high-dose aldesleukin (IL-2).
- The study was approved by the Beth Israel Deaconess Medical Center IRB, protocol #06-105.
- Aldesleukin at a dose of 600,000 International Units/kg was administered every 8 hours by a 15-min intravenous infusion for a maximum of 14 doses (cycle 1). Following 9 days of rest, the schedule was repeated (cycle 2) for a maximum of 28 doses, as tolerated.
- Whole blood samples for immunophenotyping by flow cytometry were collected at four time points per patient:
  - Cycle 1 prior to the first dose
  - Cycle 1 within 24 hours after the last dose
  - Cycle 2 prior to the first dose
  - Cycle 2 within 24 hours after the last dose
- CD8+ T cells, NK cells, and Treg cells were quantified by flow cytometry.
- Safety and antitumor activity were monitored throughout the study period.
- Response was assessed clinically based on radiology reports, and best response was recorded.

Results

Baseline Demographic Characteristics:

- Ten patients with renal cell carcinoma were enrolled
- Median age 55 (range 39-62)
- Male/female 6/4
- ECOG PS of 0=9/1=1
- Median number of prior therapies 2 (range 1-3).

Number of Doses Received

- Cycle 1: median 11 (range 8-13)
- Cycle 2: median 6 (range 0-11)
- Total (cycle 1 + cycle 2): median 17 (range 11-23).

Best Clinical Response

- Partial response (PR): 5
- Mixed response: 1
- Progressive disease (PD): 4.

Pharmacodynamic Response

- Administration of high-dose IL-2 resulted in robust expansion of circulating Tregs with a mean maximum expansion of ~4-fold as compared to ~2-fold expansion of circulating total CD8+ T cells and NK cells.
- Minimal or no change to the ratio of NK cells/Tregs and CD8+ T cells/Tregs was observed in response to high-dose IL-2.
- High inter-subject variability in pharmacodynamic response was observed with no apparent correlation to clinical response and number of doses received.

All treatment emergent adverse events (Table 1) seen were consistent with the known adverse event profile of high-dose IL-2.[15]

Table 1: Treatment-Emergent Adverse Events

ADVERSE EVENT	# PATIENTS (%)
	N = 10

Hypotension requiring vasopressors	8 (80%)*
Elevated bilirubin	7 (70%)
Erythematous rash	7 (70%)
Thrombocytopenia	7 (70%)
Diarrhea	7 (70%)
Nausea	6 (60%)
Acute kidney injury	6 (60%)
Vomiting	5 (50%)
Metabolic acidosis	4 (40%)
Rigors	3 (30%)
Toxic encephalopathy	3 (30%)
Dyspnea	2 (20%)
Neutropenia	1 (10%)
Fatigue	1 (10%)
Leukopenia	1 (10%)
Hyponatremia	1 (10%)
Arthralgias	1 (10%)
Gastrointestinal bleeding (prior nivolumab)	1 (10%)

*Including capillary leak syndrome in 5 patients

Conclusions

- The safety profile and clinical response observed in this cohort of patients were similar to previously published data.[15]
- A more robust expansion of T_{regs} over CD8+ T cells and NK cells was observed in patients treated with high-dose IL-2, consistent with the known biological activities of IL-2.
- These results may be useful in the future for evaluating possible differences in immune response observed with novel cytokine therapeutic agents.

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Example 3-Comparison of Pharmacodynamic Response of SEQ ID NO: 1 to High Dose rhIL-2.

The data from Examples 1 and 2 was combined for the purpose of comparing maximum responses to treatment with SEQ ID NO: 1 and High Dose rhIL-2. The Comparisons are shown in Table 2.

Table 2

Fold Change Induced

Example	Drug	Patient #	NK Cell	$CD8^+$ Cell	T_{reg}
1	SEQ ID NO: 1	N=10 at 6 µg/kg/day	Mean 8.5Fold Increase on C2D8	Mean 2.1 fold increase on C2D8	Mean 1.8fold increase on C2D8
		N=2 at 8 µg/kg/day	Mean 4.5 Fold Increase C2D8	Mean 2.0 fold increase on C2D8	Mean 1.7 fold increase on C2D8
2	High Dose rhIL-2	N=10	Mean 2.2 fold increase (C2)	2.2 fold increase (C2)	4.1 fold increase (C2)

The data also shows that the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) is greater compared to the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) in a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment As per Table 2 there was a near 2 fold increase in circulating immunosuppressive T_{regs} when patients are administered the fusion protein of SEQ ID NO: 1 in accordance with the treatment regimens of the invention as compared to an approximately 4 fold increase in circulating immunosuppressive T_{regs} in patients being treated with high dose rhIL-2 therapy.

Example 4 Comparison of Pharmacodynamic Response of SEQ ID NO: 1 to High Dose rhIL-2 in Mice

rhIL-2 treatment induced greater levels of systemic pro-inflammatory cytokines in mice than SEQ ID NO: 1. Serum cytokine production was assayed in female C57BL/6 mice treated SC daily for 4 days with either rhIL-2 (20 µg, 50 µg, and 75 µg) or SEQ ID NO: 1 (8 µg, 20 µg, and 30 µg) at 2, 4, 6 and 24 hours post-dose on Day 1 and Day 4 (n=4 animals per time point). The data Figure 10 shows the cytokine production for IFNγ and IL-6. Elevated levels of TNFα and IL-6 are found in individuals with inflammation that is associated with an infection as in septicemia or are afflicted with a chronic inflammatory disease like rheumatoid arthritis. Antibodies that block TNFα and IL-6 pathways are prescribed for treating rheumatoid arthritis and anti-IL-6 drugs are used to mitigate the effects of inflammation that arise during certain types of anti-cancer treatments like chimeric antigen receptor (CAR) T cell therapy. Therefore, reduced induction of proinflammatory cytokines as

observed in Figure 10 would suggest that treatment with SEQ ID NO: 1 would be better tolerated and potentially safer than treatment with rhIL-2.

Example 5-Continuation of Clinical Trial Study, Part A of Phase 1/2 Expansion Dose to 8 µg/kg/day

List of Abbreviations:

Abbreviation or Term	**Explanation or Definition**
AE	adverse event
ANC	absolute neutrophil count
C2D15	Cycle 2, Day 15
CD	cluster of differentiation
CI	confidence interval
C_{max}	maximum drug concentration in serum
CR	complete response
CSA	Clinical Study Agreement
CTCAE	Common Terminology Criteria for Adverse Events
DCR	disease control rate
DLT	dose-limiting toxicity
DOR	duration of response
ECG	standard 12-lead electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EOT	end of treatment
FIH	first-in-human
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
i-	immune-
iAE	immune adverse event
ICF	informed consent form
ICH	International Council for Harmonisation
iCR	immune complete response
iDCR	immune disease control rate
iDOR	immune duration of response
IFN	interferon
IL-2	interleukin-2
IL-2R	interleukin-2 receptor
iORR	immune overall response rate
iPD	immune progressive disease

iPFS	immune progression-free survival
iPR	immune partial response
IRB	Institutional Review Board
Abbreviation or Term	Explanation or Definition
iRECIST	Immune Response Evaluation Criteria in Solid Tumors
iSD	immune stable disease
IV	intravenous(ly)
MP	memory-phenotype
MTD	maximum tolerated dose
NCI	National Cancer Institute
NK	natural killer [cells]
NSCLC	non–small-cell lung cancer
ORR	overall response rate
PD	progressive disease
PD-1	programmed death receptor-1
PD-L1	programmed death ligand-1
PFS	progression-free survival
PK	pharmacokinetic(s)
PR	partial response
QD	once daily
RCC	renal cell carcinoma
RECIST	Response Evaluation Criteria in Solid Tumors
rhIL-2	recombinant human interleukin-2
RP2D	recommended Phase 2 dose
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	stable disease
SPD	sum of the products of the two longest perpendicular diameters
TID	3 times daily
Tregs	regulatory T cells
ULN	upper limit of normal
USP-NF	United States Pharmacopeia-National Formulary
WOCBP	women of childbearing potential

Overall Study Design and Plan

This is a global, multicenter, open-label, sequential groups Phase 1/2 study which is the same study from which the data and information of Examples 1 and 2 is derived.

The study has three parts: Part A, a dose-escalation monotherapy part; Part B, a dose-expansion monotherapy part; and Part C, a combination therapy part with pembrolizumab (see also Example 1). An overview of the Study is shown in Table 3.

Table 3

Portion of the Study	**Cohort**	**SEQ ID NO: 1 Dose (µg/kg/day)**
Dose Escalation (Part A)[a]	1	0.1
	2	0.3
	3	1
	4	3
	5	6
	6	8
	7	10
	8	12
	9	14
Dose Expansion (Part B)	Melanoma[a]	RP 2D
	RCC	RP 2D
Combination Therapy (Part C)	SEQ ID NO: 1 1 µg/kg/day safety run-in	1
	SEQ ID NO: 1 3 µg/kg/day safety run-in	3
	C1:PD-1/L1 unapproved tumor types	3[b]
	C2: PD-1/L1 approved tumor types (PD-1/L1 pretreated)	3[b]
	C3: PD-1/L1 approved tumor types (PD-1/L1 treatment naive)	3[b]
	C4: Rollover	3[b]
	C5: Melanoma	6[b]
	C6: NSCLC	6[b]
	C7: SCCHN	6[b]

Abbreviations: DLT=dose-limiting toxicity; NSCLC=non–small-cell lung cancer; PD-1=programmed death receptor-1; PD-L1=programmed death ligand-1; RCC=renal cell carcinoma; RP2D=recommended Phase 2 dose; SCCHN=squamous cell carcinoma of the head and neck; TBD=to be determined. [a] No more than 5 ocular melanoma subjects may be enrolled into this cohort. [b] A subject's SEQ ID NO: 1 dose may be reduced by one dose level as needed at the discretion of the Investigator.

In Part A of the study, subjects with advanced solid tumors received SEQ ID NO: 1 by IV administration daily for 5 days, followed by a period off treatment in repeating cycles. During Cycle 1, the period off treatment was 9 days, resulting in a cycle length of 14 days (2 weeks). Cycle 2 and subsequent cycles will have a period off treatment of 16 days, resulting in a cycle length of 21 days (3 weeks). For the first 2 treatment cycles, subjects received SEQ ID NO: 1 as inpatients at a medical facility with access to medical support measures and to the intensive care unit, if needed. In the absence of DLTs, subjects who remain in the study received subsequent doses of SEQ ID NO: 1 on an outpatient basis.

In dose escalation, cohorts in the study used a standard 3+3 study design with 3 to 6 subjects per cohort to receive SEQ ID NO: 1 at dose levels as shown in Table 3. The starting dose of 0.1 µg/kg/day was selected based on minimal anticipated biological effect level. Doses in subsequent cohorts are being increased according Table 3 until stopped for DLTs or an MTD is reached. Additional dose levels will be considered if the RP2D or MTD has not been reached within the proposed dose range.

During dose escalation, each cohort is evaluated for safety and tolerability using a 3+3 study design with allowance for over-enrollment with 4 to 7 subjects and a minimum of 3 evaluable subjects per cohort to receive IV SEQ ID NO: 1 at the specified dose and schedule. If none of the 3 subjects experiences a DLT, then the next dose level will open for enrollment. If 1 of the 3 subjects experiences a DLT, then 3 additional subjects will be enrolled at the same dose level. If no additional DLTs are observed, then the next dose level will open for enrollment.

If two or more subjects experience DLTs at a dose level, no further dose escalations will occur. One or more lower dose level(s) may be tested in search of the MTD, defined as the dose level immediately below that in which ≥2 of 6 evaluable subjects experience DLTs. Prior to any dose escalation, a teleconference of the Safety Review Committee (SRC), to include at a minimum the study investigators who have enrolled subjects and the Sponsor's Medical Monitor, will be convened to review the safety data from the current cohort and to decide if dose escalation is warranted.

Dose-limiting toxicity will be defined by any of the following events described in Example 1, Table 1.

After the RP2D was determined as described in Example 1, the second part of the study (i.e., Part B) commenced. In this part of the study, up to 41 subjects with melanoma and up to 41 subjects with RCC are being enrolled to receive SEQ ID NO: 1 at the RP2D.

Enrollment to these cohorts will follow a partial response (unconfirmed) Simon's two-stage design enrollment. Response assessments will be based on the RECIST guidelines.

In the third part of the study (Part C), subjects received SEQ ID NO: 1 in combination with pembrolizumab (see Example 1). Part C is running independently of and concurrently with monotherapy of Part A and Part B.

A 3- to 6-subject run-in phase is being utilized to assess the safety of SEQ ID NO: 1 in combination with pembrolizumab. During the safety run-in phase, subjects were enrolled with any tumor type. Rollover subjects (Cohort 4) were not eligible to participate in the safety run-in phase of Part C.

During the safety run-in phase, the first 3 subjects received SEQ ID NO: 1 at the dose level of 1 µg/kg/day (Example 1). As all 3 subjects tolerated therapy their first 21-day cycle as assessed by the SRC, then the study progressed to the 3 µg/kg/day dose level. As the first 6 subjects tolerate therapy adequately for their first 21-day cycle as assessed by the SRC, expansion Cohorts C1, C2, C3, and C4 were opened.

Up to 20 subjects are being enrolled into each of Cohorts C1, C2, C3 based on their tumor type and prior treatment with PD-1/PD-L1 pathway inhibitors as described in the inclusion criteria (Example 1). Subjects with RCC or melanoma were not eligible for enrollment in Cohorts C1, C2, or C3. Subjects on SEQ ID NO: 1 monotherapy in Part A or Part B who have experienced disease progression after a minimum of 2 cycles or SD after a minimum of 4 cycles and who are expected to tolerate treatment with combination therapy were eligible for treatment in Part C, Cohort C4. Subjects who have PR or CR on monotherapy were ineligible to rollover unless subsequently demonstrating progressive disease.

Subjects received 200 mg of pembrolizumab every 3 weeks in combination with SEQ ID NO: 1 by IV administration daily for 5 days, followed by a period off treatment of 16 days, resulting in a cycle length of 21 days (3 weeks) for each cycle (Example 1).

After the RP2D was determined for monotherapy as is described in Example, enrollment into Cohorts C5, C6, and C7 was opened. Because monotherapy doses above 6 µg/kg/day SEQ ID NO: 1 be shown to be tolerated, then the dose of SEQ ID NO: 1 in the combination arms was also increased. In Cohorts C5, C6, and C7, up to 53 subjects with melanoma, up to 42 subjects with NSCLC, and up to 36 subjects with squamous cell carcinoma of the head and neck may be enrolled to receive SEQ ID NO: 1 in combination with pembrolizumab at the RP2D. Enrollment to these cohorts will follow a PR (unconfirmed) Simon's two-stage enrollment as outlined below. Response assessments will

be based on RECIST and iRECIST guidelines. Given the RP2D was determined to be 6 µg/kg/day as per Example 1, dose escalation was considered for subjects in Cohorts C1, C2, C3, and C4 who had been assigned to the 1 µg/kg/day or 3 µg/kg/day dose levels and had adequately tolerated the combination therapy.

Tumor Assessments

Antitumor activity was determined by the measurement of extent of known disease at baseline and approximately every 5 to 6 weeks, following each even-numbered treatment cycle.

Appropriate radiological procedures (computed tomography scanning, magnetic resonance imaging, radionuclide imaging) was conducted to evaluate areas of disease. Superficial skin tumors were measured with calipers and photographed for evaluation. The determination of response was conducted according to the standard RECIST and iRECIST criteria for Parts A, B, and C. According to the guidelines for RECIST, tumors are assessed as CR, PR, SD, or PD. According to the guidelines for iRECIST, tumors are assessed as immune CR (iCR), immune PR (iPR), immune SD (iSD), or immune PD (iPD). For purposes of this study, subjects must meet the definition for SD/iSD for a minimum of 12 weeks before this assessment can be determined.

In studies with immunotherapeutic agents, CR, PR, or SD have been shown to occur after an increase in tumor burden characterized as PD by RECIST criteria. The conventional response criteria such as RECIST may not adequately assess the activity of immunotherapeutic agents. PD evaluated radiologically may not mean therapeutic failure, as responses to immune therapies may occur after conventional PD. The appearance of measureable antitumor activity may take longer for immune therapies than for cytotoxic therapies. With immunotherapeutic agents, there should be allowance for clinically insignificant PD, defined as small new lesions in the presence of other responsive lesions, which may occur even though the subject is responding to the immunotherapy. Stable disease may also represent antitumor activity with iRECIST. Therefore, RECIST and iRECIST were used to ensure a more comprehensive evaluation of tumor response for SEQ ID NO: 1.

The ORR/iORR is the number of subjects exhibiting a CR/iCR or PR or iPR divided by the number of subjects evaluable for antitumor activity. Duration of response was also be determined. The ORR/iORR was calculated separately for subjects in the dose-escalation portion of the study (Part A), in the dose-expansion part of the study (Part B), and in the combination therapy part of the study (Part C). Tumor images were collected and stored

centrally. Centralized readings may be used to assess scans beginning in the second stage (N2) of Part B cohorts and Part C C5, C6, and C7 cohorts. Antitumor activity will be expressed as the following:

- ORR based on RECIST
- iORR based on iRECIST
- DCR per RECIST
- iDCR per iRECIST
- DOR per RECIST
- iDOR per iRECIST
- PFS per RECIST
- Immune PFS (iPFS) per iRECIST
- DRR per RECIST (Part B and Part C5, C6, C7)
- iDRR per iRECIST (Part B and Part C5, C6, C7).

Assessment of Pharmacokinetics, Pharmacodynamics and Immunogenicity

Pharmacokinetics

Serum samples for evaluation of SEQ ID NO: 1 PK was obtained from each subject at predetermined time points. A validated electrochemiluminescence method using the Meso Scale Discovery platform will be used for the quantitation of SEQ ID NO: 1 in human serum. Noncompartmental PK analysis was performed to estimate the PK parameters for SEQ ID NO: 1.

Immunogenicity

Serum samples for evaluation of anti-SEQ ID NO: 1 antibody induction were obtained from each subject at predetermined time points. A validated electrochemiluminescence method using the Meso Scale Discovery platform was used for the detection of antidrug antibodies to SEQ ID NO: 1 in human serum. The assessment of immune-response induction for each study subject was based on the comparison of the pre-dose and post-dose sample results.

Pharmacodynamics and Biomarkers

The pharmacodynamic response of various biomarkers were assessed in blood and serum samples collected from all subjects in the study. Additional biomarker analyses were performed on tumor tissue samples, which are optional for study subjects.

Blood-Based Biomarkers

The pharmacodynamic effect of SEQ ID NO: 1 was assessed by measuring circulating $CD8^+$ T cells, T_{regs}, and NK cells in peripheral blood by flow cytometry from each subject at predetermined time points. In addition, serum samples were obtained from each subject at predetermined time points. Concentration of multiple proinflammatory cytokines including interferon-γ, tumor necrosis factor-α, IL-1β, IL-6, and IL-10 were determined. Circulating tumor DNA (ctDNA) was also be measured at predetermined time points.

Tumor Tissue Biomarkers

Tumor Biopsies

Collection of fresh tumor samples via biopsy were collected at baseline from consenting subjects during the study. These samples were analyzed by immunohistochemistry and/or immunofluorescence for markers of immune activation. They were also be used for gene expression analysis using method such as NanoString. Comparison of on-treatment versus baseline results was used to demonstrate the pharmacologic impact to tumor microenvironment. The analysis of the baseline tumor tissues was used for correlative analysis.

General Statistical Methodology

The statistical analysis methods are described below. In general, summary statistics (n, mean, standard deviation, median, minimum, and maximum values for continuous variables and number and percentage of subjects in each category for categorical variables) were provided for evaluated variables. Data was summarized for Part A, Part B, and Part C separately. Baseline is defined as the last value prior to the first dose of study treatment administration for each part separately.

Overall Response Rate

The evaluation of ORR was based on Investigator review of the radiographic or photographic images, as defined according to RECIST 1.1. Overall response rate is defined as the proportion of subjects with objective evidence of CR or PR among the number of subjects evaluable for antitumor activity.

At the analysis stage, the best ORR was assigned for each subject as the best response recorded after initiation of study treatment, taking into account any requirement for confirmation. If applicable, responses recorded after disease progression or initiation of new anticancer treatment were excluded.

The ORR was calculated separately for those subjects in the dose-escalation portion of the study (Part A), in the dose-expansion portion of the study (Part B), and in the combination therapy part of the study (Part C). Summarization of ORR was presented by frequency, percentage, and 95% confidence interval (CI). The CI were obtained using an exact approach given the small sample size. Sum of the Diameters of all lesions reported at each visit were graphed by spider plot (% change over time) and waterfall plot (best % change). The swimmer plot was used to display the characteristics of the responses in subjects.

Immune Overall Response Rate

Responses assigned using iRECIST have a prefix of "i" (ie, immune) to differentiate them from responses assigned using RECIST 1.1. The principles used to establish objective tumor response are largely unchanged from RECIST 1.1, but the major change for iRECIST is the concept of resetting the bar if RECIST 1.1 progression is followed at the next assessment by tumor shrinkage. iRECIST defines iUPD (immune unconfirmed progressive disease) on the basis of RECIST 1.1 principles. If the criteria for iUPD have never been met, principles follow RECIST 1.1. However, if the criteria for iUPD have been met, the next timepoint response could be iUPD, iSD, iPR, or iCR, or iCPD (immune confirmed progressive disease). For iRECIST, the best overall response (iBOR) is the best timepoint response recorded from the start of the study treatment until the end of the study treatment, taking into account any requirement for confirmation. Immune overall response rate was based on iBOR. The iBOR was calculated separately for those subjects in the dose-escalation portion of the study (Part A), in the dose-expansion portion of the study (Part B), and in the combination therapy part of the study (Part C). A spider plot, waterfall plot, and swimmer plot were used to display the characteristics of the responses in subjects.

Disease Control Rate

Disease control rate is defined as the proportion of subjects with objective evidence of CR, PR, or SD at Cycle 4 or later. The DCR was calculated separately for those subjects in the dose-escalation portion of the study (Part A), in the dose-expansion portion of the study (Part B), and in the combination therapy part of the study (Part C). Summarization of DCR was presented by frequency, percentage, and 95% CI. The CI was obtained using an exact approach given the small sample size.

Immune Disease Control Rate

Immune disease control rate is defined as the proportion of subjects with objective evidence of iCR, iPR, or iSD at Cycle 4 or later. The iDCR was calculated separately for those subjects in the dose-escalation portion of the study (Part A), in the dose-expansion portion of the study (Part B), and in the combination therapy part of the study (Part C). Summarization of iDCR was presented by frequency, percentage, and 95% CI. The CI was obtained using an exact approach given the small sample size.

Duration of Response (Part B and Part C)

Duration of response, defined as the time from the first documentation of response (CR or PR) to the first documentation of objective tumor progression or death due to any cause. Subjects who are alive and progression free as of the analysis cut-off date will be censored at their last evaluable tumor response assessment before initiation of any new anticancer treatment. Subjects with two or more consecutive missing response assessments prior to death or a visit with documented progression will be censored at the last date of tumor assessment when the subject was documented to be progression free. Subjects who never achieve CR or PR prior to starting any new anticancer treatment at a lesion site will be excluded from the analysis. The rate of response was calculated for RECIST responders. For Part B, the DOR was calculated as follows (in weeks): (date of PD/death in Part B – date of first response (CR or PR) in Part B + 1)/7 For Part C, the DOR was calculated as follows (in weeks): (date of PD/death in Part C – date of first response (CR or PR) in Part C + 1)/7. The distribution of DOR was estimated for Part B and Part C using Kaplan-Meier methodology. The median point estimate DOR was provided along with the two-sided 95% CIs based on the antitumor evaluable population with subjects who experienced CR or PR. Kaplan-Meier curves were provided.

Immune Duration of Response (Part B and Part C)

Immune duration of response, defined as the time from the first documentation of response (iCR or iPR) to the first documentation of objective tumor progression or death due to any cause. Subjects who are alive and progression free as of the analysis cut-off date were censored at their last evaluable tumor response assessment before initiation of any new anticancer treatment. Subjects with two or more consecutive missing response assessments prior to death or a visit with documented progression were censored at the last date of tumor assessment when the subject was documented to be progression free. Subjects who never achieve iCR or iPR prior to starting any new anticancer treatment at a lesion site were excluded from the analysis. The rate of response were calculated for iRECIST responders. For Part B, the iDOR were calculated as follows (in weeks): (date of iPD/death in Part B – date of first response (iCR or iPR) in Part B + 1)/7 For Part C, the iDOR were calculated as follows (in weeks): (date of iPD/death in Part C – date of first response (iCR or iPR) in Part C + 1)/7. The distribution of iDOR was estimated for Part B and Part C using Kaplan-Meier methodology. The median point estimate iDOR was provided along with the two-sided 95% CIs based on the antitumor evaluable population with subjects who experienced iCR or iPR. Kaplan-Meier curves were provided.

Durable Response Rate (Part B and Part C)

Durable response rate is defined as the percentage of subjects with an objective response (complete or partial response per RECIST1.1) lasting continuously for 6 months and starting any time within 12 months of initiating the study drug. The DRR was summarized by each tumor type. Summary of DRR was presented by frequency, percentage, and 95% CI. The CI was obtained using an exact approach given the small sample size.

Immune durable response rate (iDRR) is defined as the percentage of subjects with an objective response (complete or partial response per iRECIST) lasting continuously for 6 months and starting any time within 12 months of initiating the study drug. The iDRR was summarized by each tumor type. Summary of iDRR was presented by frequency, percentage, and 95% CI. The CI was obtained using an exact approach given the small sample size.

Progression-free Survival (Part B and Part C)

Progression-free survival, defined as the time from the first dose of SEQ ID NO: 1 to the first documentation of objective tumor progression or death due to any cause. Subjects who do not have disease progression or have not died were censored at the last known time

that the subject was progression free. If a subject begins a new anticancer treatment (either systemic or local) prior to documented progression or death, or a subject is removed from the study due to undocumented clinical disease progression, then the subject was censored at the last assessment where the subject was documented as progression free prior to the intervention. Subjects with two or more consecutive missing response assessments prior to a visit with documented progression (or death) were censored at the last date of tumor assessment when the subject was documented to be progression free. For Part B, the PFS was calculated as follows (in weeks): (date of PD/death in Part B – first dose date in Part B + 1)/7 For Part C, the PFS were calculated as follows (in weeks): (date of PD/death in Part C– first dose date in Part C + 1)/7. The survival distribution of PFS was estimated using Kaplan-Meier methodology. The median PFS was provided along with the two-sided 95% CIs based on the antitumor evaluable population. In addition, Kaplan-Meier curves were provided. The 6-month and one-year PFS rate was estimated using the Kaplan-Meier estimate.

Immune Progression-free Survival (Part B and Part C)

Immune progression-free survival, defined as the time from the first dose to the first documentation of objective tumor progression or death due to any cause. Subjects who do not have disease progression or have not died were censored at the last known time that the subject was progression free. If a subject begins a new anticancer treatment (either systemic or local) prior to documented progression or death, or a subject is removed from the study due to undocumented clinical disease progression, then the subject was censored at the last assessment where the subject was documented as progression free prior to the intervention. Subjects with two or more consecutive missing response assessments prior to a visit with documented progression (or death) were censored at the last date of tumor assessment when the subject was documented to be progression free. For Part B, the iPFS was calculated as follows (in weeks): (date of iPD/death in Part B – first dose date in Part B + 1)/7 For Part C, the PFS was calculated as follows (in weeks): (date of iPD/death in Part C– first dose date in Part C + 1)/7. The survival distribution of iPFS will be estimated using Kaplan-Meier methodology. The median iPFS was provided along with the two-sided 95% CIs based on the antitumor evaluable population. In addition, Kaplan-Meier curves were provided. The 6-month and one-year iPFS rate was estimated using the Kaplan-Meier estimate.

Pharmacokinetic Analyses

Individual serum concentrations and concentration-time data were presented and summarized both graphically and in tabular form using descriptive statistics. Pharmacokinetic parameters were summarized using descriptive statistics. A subject listing of individual PK concentration was provided. Concentration data was summarized according to nominal (protocol-specified) sampling times. Pharmacokinetic parameters were calculated by noncompartmental analysis method using Phoenix WinNonlin Professional (version 6.1 or later, Pharsight Corporation); actual elapsed time from dosing will be used to estimate individual serum PK parameters. Dose proportionality and additional PK analyses were performed, as appropriate.

Pharmacodynamic Analyses

Pharmacodynamic data was summarized descriptively. Where possible, the relationship between serum PK parameters or concentration of SEQ ID NO: 1 and pharmacodynamic responses was evaluated by correlation analysis or visual inspections.

Immunogenicity Analysis

The presence of anti-SEQ ID NO: 1 antibodies were determined and the data was summarized by cohort/dose level.

Tissue Biomarker Analysis

The baseline values, post-treatment values, and the changes in density of TILs, ratio of cytotoxic TILs, immunosuppressive TILs, and density of signals of immune-cell–mediated killing were summarized. The correlation between antitumor efficacy endpoints (best overall response, progression free survival) and baseline status (or values) for endpoints derived from tumor tissues was estimated. The efficacy endpoints were summarized separately based on baseline status, or low and high values. The correlation between antitumor efficacy

endpoints (best overall response, progression free survival) and the change from baseline in post-treatment for endpoints derived from tumor tissues were estimated.

Partial and Complete Responses in Patient's receiving 6 µg/kg dose of SEQ ID NO: 1

Initial data from the ongoing Phase 1/2 Study provided a partial response in a urethral melanoma patient during Part B monotherapy dose expansion cohort receiving 6 µg/kg of SEQ ID NO: 1. Prior to joining the study, the patient had been treated with surgical dissection for urethral melanoma followed by one year of adjuvant nivolumab treatment. While on the study this patient showed a reduction in serum lactate dehydrogenase (LDH) between cycle 1 and cycle 8 treatments. While on the study, the patient had the following benefits during each cycle of treatment as follows: Cycle 2- stable disease (SD); 8% increase in target lesions from baseline; Cycle 4- SD; 17% reduction in target lesions form baselines; Cycle 6 partial response (PR) 32% reduction in lesions from baseline; Cycle 8-confirmed PR as per RECIST, 35% reduction in target lesions from baseline.

Partial and Complete Responses in Patient's receiving 3 µg/kg dose of SEQ ID NO: 1 in Combination with Pembrolizumab

Initial data from the ongoing Phase 1/2 Study in patients receiving a combination of 3 µg/kg dose of SEQ ID NO: 1 in combination with pembrolizumab yielded at least one complete responses (CR) and several partial responses (PR) in a patients having the following types of cancers respectively, Ovarian (CR); Ovarian (PR); Ovarian (PR) esophageal (PR); and TNBC (iPR by iRECIST).

Data from Expansion Dose of Cohort 6 at 8 µg/kg/day

Pharmacokinetics of SEQ ID NO: 1 after IV Administration

As discussed in Example 1, RP2D was determined to be a daily dose of 6 µg/kg. As per the study design, a new Cohort 6 began enrollment in the study to be tested at a daily dose of 8 µg/kg. Two patients were enrolled with up to 4 more patients expected to be enrolled. What follows is the data received from the first two patients to be enrolled.

SEQ ID NO: 1 serum concentration vs time profiles after the first IV dose (Cycle 1 Day 1) of SEQ ID NO: 1 are depicted in Figure 11. Mean peak (C_{max}) and total serum exposure (AUC) of SEQ ID NO: 1 across the dose range evaluated in Part A described in Example 1 are shown in Figure 12.

After the first IV dose of SEQ ID NO: 1, peak serum SEQ ID NO: 1 concentrations were reached at the end of the 30 min (0.5 h) infusion, and thereafter declined in an exponential manner. Systemic exposure to SEQ ID NO: 1 (C_{max} and AUC_{last}) increased with increase in dose. The increase in C_{max} was approximately dose proportional over the dose range of 0.1 µg/kg to 8 µg/kg. The increase in AUC_{last} was greater than dose proportional over the dose range 0.1 µg/kg to 3 µg/kg but approximately dose proportional from 3 µg/kg to 8 µg/kg.

The 6 µg/kg dose was selected as the recommended Phase 2 dose (Example 1) for IV dosing of SEQ ID NO: 1. Enrollment at the 8 µg/kg is ongoing with two patients treated at this dose level.

Pharmacodynamic Effects of SEQ ID NO: 1 after IV Administration

The time course of cell populations of total NK cells, total $CD8^+$ T cells and regulatory T cells (T_{reg}) in peripheral blood after the first two cycles of treatment with IV SEQ ID NO: 1 are depicted in Figure 13. The fold changes from baseline (FCB) on Cycle 1 Day 8 (C1D8) and Cycle 2 Day 8 (C2D8) in total NK cells, total $CD8^+$ T cells and T_{reg} across the dose range evaluated in the Phase are depicted in Figure 14.

SEQ ID NO: 1 induced dose dependent increase in circulating NK and $CD8^+$ T cells with minimal, non-dose dependent effect on T_{reg} cells, and the 6 and 8 ug/kg dose levels have the most robust elevation of NK cells and $CD8^+$ T cells without a significant change in the T_{reg} profile.

In addition, in evaluating the serum cytokine concentrations in patients treated at the various dose levels of SEQ ID NO: 1, transient elevation of serum concentrations of interferon gamma (IFNγ) and IL-6 were observed in patients receiving higher doses (> 1 µg/kg) of SEQ ID NO: 1 (Figure 15). The peak IL-6 response was interestingly observed at the 3 µg/kg dose level, with reduced serum IL-6 levels at doses higher than 3 µg/kg, including 6 and 8 µg/kg.

IL-6 is a pro-inflammatory cytokine that is not thought to contribute to the antitumor response but is likely associated with general inflammation and side effects of treatment including fever. Increased levels of IL-6 in the serum and tumor site has been demonstrated in several cancers. Usually this increase is accompanied with a poor prognosis and lower survival rate. Downregulation of IL-6 has been correlated with a better response to cancer treatment.

IFNγ is a very important marker of cytotoxic effector cell function, and $CD8^+$ T cells and NK cells produce IFNγ upon activation. IFNγ is associated with the antitumor immune response, and significant elevation was observed at the 3, 6, and 8 µg/kg dose levels of SEQ ID 1. Notably, elevation of IFNγ was observed at the 6 and 8 ug/kg dose levels where the IL-6 levels were much lower than the peak level observed at 3 µg/kg. The IFNγ levels observed after treatment with 8 ug/kg SEQ ID NO: 1 were approximately 4 to 5-fold higher even than observed at 3 and 6 µg/kg. This suggests that the 6 and 8 µg/kg dose levels and potentially higher dose levels of 10, 12, 15 µg/kg or greater may have a particular advantage in terms of antitumor immune activity relative to inflammatory activity in comparison to lower dose levels of SEQ ID NO: 1.

The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents and published or unpublished United States patent applications cited herein are incorporated by reference. All published foreign patents and patent applications cited herein are hereby incorporated by reference. All other published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims. It should also be understood that the embodiments described herein are not mutually exclusive and that features from the various embodiments may be combined in whole or in part in accordance with the invention.

CLAIMS

What is claimed is:

1. Use of the fusion protein of SEQ ID NO: 1 in the manufacture of a medicament for treating cancer in a patient, wherein the fusion protein is to be administered at a dose of at least ~~about~~ 6 µg/kg/day to ~~about~~ 15 µg/kg per day.

2. The use of claim 1, wherein:

the dose is 6 µg/kg/day, 8 µg/kg/day, 10 µg/kg/day, 12 µg/kg/day, 14 µg/kg/day, or 15 µg/kg/day;

the patient has an improved safety profile as compared to a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment, optionally wherein the patient has a lower risk of capillary leak syndrome or cytokine release syndrome;

the dose is to be administered by intravenous (I.V.) injection or infusion;

the dose is a fixed dose based on a 60-70 kg human;

the dose is a fixed dose and wherein the patient is a child, optionally wherein the child weighs ~~about~~ 15 kg to ~~about~~ 50 kg;

the use results in at least a partial response in the patient;

the use further comprises repeated administration of the fusion protein if the cancer reoccurs, or a new cancer develops in the patient; or

the mean fold change from baseline (FCB) in IFNγ present in a patient's peripheral blood, serum or plasma is at least ~~about~~ 2-fold, at least ~~about~~ 3-fold, at least ~~about~~ 4-fold, or at least ~~about~~ 5-fold.

3. The use of claim 1, wherein administration results in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in T regulatory (Treg) cells, optionally wherein:

the increase in circulating NK cells and CD8+ cells is at least 2 fold over baseline;

the increase in circulating NK cells and CD8+ cells is greater relative to the increase in circulating Treg cells;

an increase in circulating NK cells and CD8+ cells is greater relative to the increase in circulating Treg cells as compared to the increase in circulating NK cells

and CD8+ cells relative to the increase in circulating Treg cells in a patient receiving high dose rhIL-2 treatment; and/or
the patient has a lower risk of capillary leak syndrome.

4. The use of claim 1, wherein the cancer being treated is a solid tumor or a blood cancer, optionally wherein:
the solid tumor is a carcinoma, sarcoma or lymphoma;
the cancer being treated is renal cell carcinoma (RCC), melanoma, breast cancer, pancreatic cancer, prostate cancer, non-small cell lung cancer, liver cancer, colon and rectal cancer, bladder cancer, cervical cancer, thyroid cancer, esophageal cancer, oral cancer, mesothelioma, and non-melanoma skin cancer;
the blood cancer is leukemia, non-Hodgkin lymphoma, Hodgkin lymphoma and multiple myeloma; and/or
the size of the solid tumor is reduced.

5. The use of claim 1, wherein the fusion protein of SEQ ID NO: 1 is to be administered by intravenous injection or infusion at a dose of at least ~~about~~ 6 µg/kg to ~~about~~ 15 µg/kg per day as a once a day administration for ~~about~~ 1 day to ~~about~~ 5 days, optionally wherein:
the fusion protein is to be administered once a day for at least ~~about~~ 2 consecutive days, at least ~~about~~ 3 consecutive days, at least ~~about~~ 4 consecutive days, or at least ~~about~~ 5 consecutive days;
the fusion protein is to be administered once a day on non-consecutive days for no more than ~~about~~ 5 total non-consecutive of days of administration; and/or
the fusion protein is to be administered followed by a rest period of at least ~~about~~ 9 consecutive days, optionally wherein the rest period is at least ~~about~~ 16 consecutive days.

6. The use of claim 1, wherein the fusion protein of SEQ ID NO: 1 is to be administered in at least two courses of treatment, the first course of treatment comprising administration by intravenous injection or infusion at a dose of at least ~~about~~ 6 µg/kg to ~~about~~ 15 µg/kg per day for a period of ~~about~~ 1 to ~~about~~ 5 consecutive or non-consecutive days followed by a rest period of at least ~~about~~ 9 consecutive days

followed by a second course of treatment wherein the fusion protein of SEQ ID NO: 1 is to be administered by intravenous injection or infusion at a dose of at least ~~about~~ 6 µg/kg to ~~about~~ 15 µg/kg per day for a period of at least ~~about~~ 1 to at least ~~about~~ 5 consecutive or nonconsecutive days, followed by a rest period of at least ~~about~~ 9 consecutive days, optionally wherein:

administration of the fusion protein for the first course of treatment and for the second course of treatment is for a period of 5 consecutive days or 5 non-consecutive days prior to a rest period of at least ~~about~~ 9 days, optionally wherein the rest period is at least ~~about~~ 16 days or wherein the rest period during the first course of treatment is ~~about~~ 9 consecutive days and wherein the rest period for the second course of treatment is ~~about~~ 16 or more consecutive days;

the second course of treatment begins at least ~~about~~ 24 hours or more after completion of the first course of treatment; and/or

the use further comprises a third course of treatment following the second course of treatment, optionally wherein:

the third course of treatment begins within ~~about~~ 24 hours or more after completion of the second course of treatment;

the use further comprises a fourth course of treatment following the third course of treatment, optionally wherein the fourth course of treatment begins ~~about~~ 24 hours or more after completion of the third course of treatment; and/or

wherein subsequent courses of treatment begin ~~about~~ 24 hours or more after completion of the prior course of treatment until the cancer is treated or until the patient is no longer benefitting from the treatment.

7. The use of claim 1, wherein a therapeutically effective amount of a therapeutic agent is to be further co-administered to the patient, optionally wherein the therapeutic agent is a PARP inhibitor, a cytotoxic agent, a chemotherapeutic agent, or an immune checkpoint inhibitor, optionally wherein the immune checkpoint inhibitor inhibits the interaction of PD-1 and PD-L1, optionally wherein the immune checkpoint inhibitor is pembrolizumab.

8. The use of claim 7, wherein the fusion protein of SEQ ID NO: 1 is to be administered in at least two courses of treatment, the first course of treatment comprising

administration by intravenous injection or infusion at a dose of at least ~~about~~ 6 µg/kg to ~~about~~ 15 µg/kg per day once a day for 1 to 5 days followed by a rest period of at least ~~about~~ 16 consecutive days followed by a second course of treatment wherein a dose of at least ~~about~~ 6 µg/kg to ~~about~~ 15 µg/kg per day is to be administered by intravenous injection or infusion for a period 1 to 5 consecutive days, followed by a rest period of at least ~~about~~ 16 consecutive days and wherein pembrolizumab is to be co-administered once during the 1 to 5 days of administration during the first course and second course of treatment.

9. The use of claim 7, wherein:

the fusion protein is to be administered once a day for at least ~~about~~ 2 consecutive days, at least ~~about~~ 3 consecutive days, at least ~~about~~ 4 consecutive days, or at least ~~about~~ 5 consecutive days;

the fusion protein of SEQ D NO: 1 is to be administered once a day on non-consecutive days for no more than ~~about~~ 5 total non-consecutive of days of administration;

the second course of treatment begins at least ~~about~~ 24 hours or more after completion of the first course of treatment; and/or

the use further comprises a third course of treatment following the second course of treatment, optionally wherein:

the third course of treatment begins within ~~about~~ 24 hours or more after completion of the second course of treatment;

the use further comprises a fourth course of treatment following the third course of treatment, optionally wherein the fourth course of treatment begins ~~about~~ 24 hours or more after completion of the third course of treatment; and/or

wherein subsequent courses of treatment begin ~~about~~ 24 hours or more after completion of the prior course of treatment until the cancer is treated or until the patient is no longer benefitting from the treatment.

10. The use of claim 8, wherein the pembrolizumab is to be co-administered prior to, simultaneously with, or subsequent to, administration of the fusion protein of SEQ ID NO:1, optionally wherein:

the pembrolizumab is to be co-administered in a separate composition from the fusion protein of SEQ ID NO: 1;

the pembrolizumab is to be co-administered in an amount of 200 mg by I.V. injection or infusion;

the pembrolizumab is to be administered on the first day of administration of the fusion protein of SEQ ID NO: 1 during the first course of treatment;

the pembrolizumab is to be administered on the first day of administration of the fusion protein of SEQ ID NO: 1 during the second course of treatment; and/or

the pembrolizumab is to be administered on the first day of administration of the fusion protein of SEQ ID NO: 1 during subsequent courses of treatment.

11. The use of claim 7 resulting in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in T regulatory (Treg) cells, optionally wherein:

the dose dependent increase in circulating NK cells and CD8+ cells is greater than the non-dose dependent increase in Treg cells;

the patient has an improved safety profile as compared to a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment;

the patient has a lower risk of cytokine release syndrome as compared to a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment; and/or

the patient has a lower risk of capillary leak syndrome as compared to a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment.

12. Use of the fusion protein of SEQ ID NO: 1 in the manufacture of a medicament for treating cancer in a patient, wherein the fusion protein is to be administered to the patient at a dose of at least ~~about~~ 40 µg/kg to ~~about~~ 70 µg/kg per day once weekly.

13. The use of claim 12, wherein the daily dose is ~~about~~ 50 µg/kg to ~~about~~ 60 µg/kg.

14. Use of the fusion protein of SEQ ID NO: 1 in the manufacture of a medicament for treating cancer in a patient, wherein the fusion protein is to be administered to the patient at a dose of at least ~~about~~ 50 µg/kg to ~~about~~ 60 µg/kg per day once weekly, resulting in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in circulating T regulatory (Treg)

cells, and wherein the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) is greater compared to the increase in circulating NK cells and CD8+ cells relative to the increase in circulating T regulatory (Treg) in a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment, and wherein the patient has a lower risk of capillary leak syndrome as compared to a patient receiving high dose recombinant human IL-2 (rhIL-2) treatment, optionally wherein the dose is to be administered by intravenous injection or infusion.

15. A pharmaceutical composition comprising ~~about~~ 0.4 to ~~about~~ 1 mg of the fusion protein of SEQ ID NO: 1.

16. Use of the fusion protein of SEQ ID NO: 1 in the manufacture of a medicament for treating cancer in a patient, wherein the fusion protein is to be administered to the patient at a daily dose of at least ~~about~~ 3 µg/kg to ~~about~~ 5.5 µg/kg, wherein administration results in a dose dependent increase in circulating NK cells and CD8+ cells in a patient in the absence of a dose dependent increase in circulating T regulatory (Treg) cells and wherein the increase in circulating NK cells and CD8+ cells is greater relative to the increase in circulating T regulatory cells (Treg).

17. Use of the fusion protein of SEQ ID NO: 1 in the manufacture of a medicament for treating cancer in a patient, wherein a dose of at ~~about~~ 16 µg/kg/day to ~~about~~ 70 µg/kg/day or a corresponding fixed dose based on an ~~about~~ 60-70 kg adult or ~~about~~ 12 kg to 50 kg or more child is to be intravenously administered to the patient.

18. The use of claim 17, wherein the dose is ~~about~~ 16 µg/kg/day to ~~about~~ 50 µg/kg/day of the fusion protein of SEQ ID NO: 1 or a corresponding fixed dose based on an ~~about~~ 60-70 kg adult or ~~about~~ 12 kg to 50 kg or more child, optionally wherein the corresponding fixed dose is ~~about~~ 5 µg to ~~about~~ 3 mg;

the mean fold change from baseline (FCB) in IFNγ present in a patient's peripheral blood, serum or plasma is at least ~~about~~ 2-fold, at least ~~about~~ 3-fold, at least ~~about~~ 4-fold, or at least ~~about~~ 5-fold; and/or

the fusion protein of SEQ ID NO: 1 is to be administered on non-consecutive days, optionally wherein:

the fusion protein of SEQ ID NO: 1 is to be administered once every 7 days, once every 14 days or once every 21 days of a treatment cycle;

the fusion protein of SEQ ID NO: 1 is to be administered on days 1, 7, 14 and 21 of a treatment cycle;

the fusion protein of SEQ ID NO: 1 is to be administered on days 1 and 14 of a treatment cycle; or

the fusion protein of SEQ ID NO: 1 is to be administered on days 1 and 21 of a treatment cycle.

19. The use of claim 2, wherein: the mean fold change from baseline in IL-6 is less than 4-fold; and/or the dose of the fusion protein of SEQ ID NO: 1 is ~~about~~ 6 µg/kg/day or ~~about~~ 8 µg/kg/day.

20. The use of claim 17 wherein: the mean fold change from baseline in IL-6 is less than 4-fold; and/or the dose of the fusion protein of SEQ ID NO: 1 is ~~about~~ 30 µg/kg/day or ~~about~~ 50 µg/kg/day.