COMBINED TREATMENT WITH A SIRPalpha-4-1BBL FUSION PROTEIN FOR CANCER

RELATED APPLICATION/S

This application claims the benefit of priority of US Provisional Patent Application Nos. 63/559,224 filed on February 29, 2024 and 63/652,203 filed on May 28, 2024, the contents of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING STATEMENT

The XML file, entitled 102256.xml, created on February 19, 2025, comprising 64,164 bytes, submitted concurrently with the filing of this application is incorporated herein by reference.

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to combined treatment with a SIRPα-4-1BBL fusion protein for cancer.

Cancer therapy has advanced significantly in recent years, with an increasing focus on targeted approaches to improve efficacy and reduce side effects. Current treatment modalities include radiation, surgery, chemotherapy and immunotherapy (including e.g., cell-based therapy, antibody-based therapy and cytokine therapy). Non-limiting examples of recent advances in treatment include LONSURF® (trifluridine/tipiracil), which functions by inhibiting thymidine phosphorylase leading to DNA damage within cancer cells; and AVASTIN® (bevacizumab), which hinders angiogenesis thereby depriving tumors of their blood supply. On the other hand, immune checkpoint therapy has emerged as a breakthrough therapy in the treatment of cancer. Currently, there are several immune checkpoint inhibitor drugs approved for the treatment of cancer patients, and more of these classes of therapeutic agents are under development.

Despite the advancements in cancer therapy, not all patients respond to these treatments, underscoring the ongoing need for additional therapeutic options.

Dual Signaling Proteins (DSP), also known as Signal-Converting-Proteins (SCP), are bi-functional fusion proteins that link an extracellular portion of a type I membrane protein (extracellular amino-terminus), to an extracellular portion of a type II membrane protein (extracellular carboxyl-terminus), forming a fusion protein with two active sides (see e.g., US Patent Nos. 7,569,663 and 8,039,437). Several such DSPs have been described, including for example PD1-4-1BBL and SIRPα-4-1BBL (see e.g., International Patent Application Publication No. WO2018/127919 and WO2018/127917). By binding to their native corresponding ligands or

receptors, DSPs affect signaling cascades, cell growth, survival and other cellular phenotypes, depending on their composition.

SIRPα (signal-regulatory protein alpha) is a cell surface receptor of the immunoglobulin superfamily. SIRPα is expressed mainly on the surface of immune cells from the phagocyte lineage like macrophages and dendritic cells (DC). CD47, the ligand of SIRPα, functions as an inhibitor of phagocytosis through ligation of SIRPα expressed on phagocytes and serves as a "don't eat me signal". While CD47 is ubiquitously expressed at low levels on normal cells, multiple tumors express increased levels of CD47 compared to their normal cell counterparts and over-expression of CD47 enables tumors to escape innate immune system surveillance through evasion of phagocytosis.

4-1BBL is the activating ligand of the 4-1BB receptor (CD137), a member of the TNF receptor superfamily and a potent activation-induced T cell costimulatory molecule. 4-1BBL is presented on a variety of antigen presenting cells (APCs), including dendritic cells (DCs), B cells, and macrophages. While the 4-1BB receptor is not detected (<3%) on resting T cells or T cell lines, it is stably upregulated when T cells are activated. 4-1BB activation upregulates expression of survival genes, enhances cell division, induces cytokine production and prevents activation induced cell death in T-cells.

The unique composition of a SIRPα-4-1BBL fusion protein was shown to facilitate targeted activation of adaptive immunity at a tumor site and was previously suggested for cancer therapy (see e.g., International Patent Application Publication Nos. WO2018/127919 and WO2020/012486; and ClinicalTrials(dot)gov ID NCT04440735, A Study of DSP107 Alone and in Combination With Atezolizumab for Patients With Advanced Solid Tumors, www (dot)clinicaltrials(dot)gov/study/NCT04440735).

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) at least two agents selected from the group consisting of an agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1, an agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF, and Trifluridine/Tipiracil, thereby treating the cancer in the subject.

According to an aspect of some embodiments of the present invention there is provided a combination of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) at least two agents selected from the group consisting of an agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1, an agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF, and Trifluridine/Tipiracil, for use in treating cancer in a subject in need thereof.

According to some embodiments of the invention, the at least two agents comprise the agent which inhibits activity or expression of the receptor-ligand combination PD1-PD-L1 and the agent which inhibits activity or expression of the receptor-ligand combination VEGFR-VEGF.

According to some embodiments of the invention, the at least two agents comprise the agent which inhibits activity or expression of the receptor-ligand combination PD1-PD-L1, the agent which inhibits activity or expression of the receptor-ligand combination VEGFR-VEGF, and the Trifluridine/Tipiracil.

According to an aspect of some embodiments of the present invention there is provided a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) at least two agents selected from the group consisting of an antibody which inhibits binding of PD-L1 to PD1, an anti-VEGF antibody and Trifluridine/Tipiracil,

thereby treating the cancer in the subject.

According to an aspect of some embodiments of the present invention there is provided a combination of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) at least two agents selected from the group consisting of an antibody which inhibits binding of PD-L1 to PD1, an anti-VEGF antibody and Trifluridine/Tipiracil,

for use in treating cancer in a subject in need thereof.

According to some embodiments of the invention, the at least two agents comprise the antibody which inhibits binding of PD-L1 to PD1, the anti-VEGF antibody and the Trifluridine/Tipiracil.

According to an aspect of some embodiments of the present invention there is provided a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) Trifluridine/Tipiracil,

thereby treating the cancer in the subject.

According to an aspect of some embodiments of the present invention there is provided a combination of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) Trifluridine/Tipiracil,

for use in treating cancer in a subject in need thereof.

According to an aspect of some embodiments of the present invention there is provided a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) an agent which inhibits a receptor tyrosine kinase (RTK) signaling pathway, wherein the agent is not any of Bevacizumab, Ramucirumab, Cetuximab, Panitumumab, Pertuzumab, Trastuzumab, Ertumaxomab and Cixutumumab,

thereby treating the cancer in the subject.

According to an aspect of some embodiments of the present invention there is provided a combination of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) an agent which inhibits a receptor tyrosine kinase (RTK) signaling pathway, wherein the agent is not any of Bevacizumab, Ramucirumab, Cetuximab, Panitumumab, Pertuzumab, Trastuzumab, Ertumaxomab and Cixutumumab,

for use in treating cancer in a subject in need thereof.

According to some embodiments of the invention, the RTK is selected from the group consisting of FGFR, VEGFR, PDGFR, KIT, FLT3, TRKB, AXL, MET, TIE-2 and EPH.

According to some embodiments of the invention, the agent inhibits activity or expression of a receptor-ligand combination FGFR-FGF.

According to some embodiments of the invention, the FGFR is FGFR-3.

According to some embodiments of the invention, the agent inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF.

According to some embodiments of the invention, the VEGF is VEGF-C.

According to some embodiments of the invention, the VEGFR is VEGFR-3.

According to some embodiments of the invention, the agent is a tyrosine kinase inhibitor (TKI).

According to some embodiments of the invention, the agent is selected from the group consisting of Regorafenib, Pazopanib, Pemigatinib, Futibatinib, Infigratinib phosphate, Erdafitinib, Sunitinib, Sorafenib, Cabozantinib, Lenvatinib, Axitinib, Tivozanib and Vandetanib.

According to some embodiments of the invention, the agent is a polynucleotide.

According to some embodiments of the invention, the agent is a polypeptide.

According to some embodiments of the invention, the agent is Zaltrap.

According to some embodiments of the invention, the agent is an antibody.

According to some embodiments of the invention, the method or the combination for use further comprising an agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1.

According to some embodiments of the invention, cells of the cancer express CD47.

According to some embodiments of the invention, cells of the cancer express PD-L1.

According to some embodiments of the invention, the cancer is a solid tumor.

According to some embodiments of the invention, the cancer is selected from the group consisting of a GI tract cancer, liver cancer, pancreatic cancer, lung cancer, renal cancer, bladder cancer, breast cancer, and head and neck cancer.

According to some embodiments of the invention, the cancer is a colorectal cancer.

According to some embodiments of the invention, the cancer is a microsatellite stable (MSS) cancer.

According to some embodiments of the invention, the subject:

(a) has histologically confirmed, inoperable, microsatellite stable and/or proficient mismatch repair colorectal carcinoma by local testing;

(b) has progressed on or is intolerant to fluoropyrimidine, irinotecan, oxaliplatin, bevacizumab and EGFR inhibitor;

(c) wherein recurrence within 12 months of last adjuvant chemo counts as progression; and

(d) wherein when the cancer is characterized by BRAF V600E, HER2 amp or overexpression, or KRAS G12C, the subject may have also received one line of prior targeted therapy.

According to some embodiments of the invention, the SIRPα-4-1BBL fusion protein is in a form of a homo-trimer.

According to some embodiments of the invention, the SIRPα-4-1BBL fusion protein is characterized by a single amino acid linker between the SIRPα amino acid sequence and the 4-1BBL amino acid sequence.

According to some embodiments of the invention, the linker is glycine.

According to some embodiments of the invention, the SIRPα-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 1.

According to some embodiments of the invention, the SIRPα-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 1.

According to some embodiments of the invention, the SIRPα-4-1BBL fusion protein is administered intravenously.

According to some embodiments of the invention, the SIRPα-4-1BBL fusion protein is administered at a dose of 0.1 – 50 mg / kg.

According to some embodiments of the invention, the SIRPα-4-1BBL fusion protein is administered at a dose of about 10 mg / kg.

According to some embodiments of the invention, the SIRPα-4-1BBL fusion protein is administered in a 4 weeks cycle.

According to some embodiments of the invention, the 4 weeks cycle comprises 3 administrations on days 1, 8 and 15 of each cycle.

According to some embodiments of the invention, the 4 weeks cycle comprises 4 administrations on days 1, 8, 15 and 22 of each cycle.

According to some embodiments of the invention, the agent which inhibits activity or expression of the receptor-ligand combination PD1-PD-L1 is an antibody which inhibits binding of PD-L1 to PD1.

According to some embodiments of the invention, the antibody which inhibits binding of PD-L1 to PD1 is an anti-PD-L1 antibody.

According to some embodiments of the invention, the anti-PD-L1 antibody comprises Atezolizumab.

According to some embodiments of the invention, the antibody which inhibits binding of PD-L1 to PD1 is an anti-PD1 antibody.

According to some embodiments of the invention, the antibody which inhibits binding of PD-L1 to PD1 is administered intravenously.

According to some embodiments of the invention, the antibody which inhibits binding of PD-L1 to PD1 is administered at a dose of about 1680 mg.

According to some embodiments of the invention, the antibody which inhibits binding of PD-L1 to PD1 is administered once every 4 weeks.

According to some embodiments of the invention, the agent which inhibits activity or expression of the receptor-ligand combination VEGFR-VEGF is an anti-VEGF antibody.

According to some embodiments of the invention, the anti-VEGF antibody comprises Bevacizumab.

According to some embodiments of the invention, the anti-VEGF antibody is administered intravenously.

According to some embodiments of the invention, the anti-VEGF antibody is administered at a dose of about 5 mg/kg.

According to some embodiments of the invention, the anti-VEGF antibody is administered once every 2 weeks.

According to some embodiments of the invention, the Trifluridine/Tipiracil molar ratio is 1 : 0.5.

According to some embodiments of the invention, the Trifluridine/Tipiracil is administered orally.

According to some embodiments of the invention, the Trifluridine/Tipiracil is administered at a dose of 70-160 mg Trifluridine /m^2/day.

According to some embodiments of the invention, the Trifluridine/Tipiracil is administered twice a day.

According to some embodiments of the invention, the Trifluridine/Tipiracil is administered in a 4 weeks' cycle.

According to some embodiments of the invention, the 4 weeks' cycle comprises administrations for 5 consecutive days, followed by 2 days' rest, followed by administrations for 5 consecutive days.

According to some embodiments of the invention, first administration of the at least two agents starts on the same day.

According to some embodiments of the invention, first administration of the (i) and the (ii) starts on the same day.

According to some embodiments of the invention, a cycle of treatment with the SIRPα-4-1BBL fusion protein, the antibody which inhibits binding of PD-L1 to PD1 and the anti-VEGF antibody includes:

four administrations of the SIRPα-4-1BBL fusion protein having a 1 week' interval, wherein a first administration is on day 1 of the cycle;

the antibody which inhibits binding of PD-L1 to PD1 is administered once during the cycle; and

the anti-VEGF antibody is administered twice during the cycle with 2 weeks intervals.

According to some embodiments of the invention, treatment with the SIRPα-4-1BBL fusion protein, the antibody which inhibits binding of PD-L1 to PD1 and the anti-VEGF antibody is in a 4 weeks cycle, wherein:

the SIRPα-4-1BBL fusion protein is administered 4 times during the cycle, on days 1, 8, 15 and 22 of the cycle;

the antibody which inhibits binding of PD-L1 to PD1 is administered once during the cycle, on day 1 of the cycle; and

the anti-VEGF antibody is administered twice during the cycle, on days 1 and 15 of the cycle.

According to some embodiments of the invention, a cycle of treatment with the SIRPα-4-1BBL fusion protein, the antibody which inhibits binding of PD-L1 to PD1, the anti-VEGF antibody and the Trifluridine/Tipiracil includes:

three administrations of the SIRPα-4-1BBL fusion protein having a 1 week' interval, wherein a first administration is on day 1 of the cycle,

the antibody which inhibits binding of PD-L1 to PD1 is administered once during the cycle,

the anti-VEGF antibody is administered twice during the cycle with 2 weeks intervals; and

the Trifluridine/Tipiracil is administered for 10 days during the cycle.

According to some embodiments of the invention, treatment with the SIRPα-4-1BBL fusion protein, the antibody which inhibits binding of PD-L1 to PD1, the anti-VEGF antibody and the Trifluridine/Tipiracil is in a 4 weeks cycle, wherein:

the SIRPα-4-1BBL fusion protein is administered 3 times during the cycle, on days 1, 8 and 15 of the cycle;

the antibody which inhibits binding of PD-L1 to PD1 is administered once during the cycle, on day 1 of the cycle;

the anti-VEGF antibody is administered twice during the cycle, on days 1 and 15 of the cycle; and

the Trifluridine/Tipiracil is administered twice a day for a total of 10 days during the cycle, on days 1-5 and 8-12 of the cycle.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIG. 1 is graph demonstrating the increase in VEGF-C protein levels in plasma samples of CRC patients pre- and 12 weeks post- treatment with the indicated dose of DSP107 in combination with Atezolizumab.

FIGs. 2A-E are histograms demonstrating CD47, VEGFR2 and PD-L1 expression on cancer cell lines following stimulation in the presence of IFNγ or DFO as compared to unstimulated cells (non-treated) and to an isotype control. Figure 2A represents CD47 expression on colon cancer cell lines; Figure 2B represents VEGFR2 on colon cancer cell lines; Figure 2C represents PD-L1 expression on colon cancer cell lines; Figure 2D represents PD-L1 expression on SK-OV-3 ovarian carcinoma cells and Figure 2E represents PD-L1 expression on A549 lung adenocarcinoma cells. Shown are MFI values.

FIG. 3 shows bar graphs demonstrating the percentages of PBMCs expressing the activation markers PD-L1, 41BB and CD25 after partial activation with CD3/CD28 Dynabeads (1:10 bead to cell ratio) as compared to non-activated PBMCs. Shown are results of PBMCs obtained from two independent healthy donors.

FIGs. 4A-B are bar graphs demonstrating the percentages of dead cancer cell line cells following co-culturing with human PBMCs and treatment with DSP107, anti-VEGF or anti- PD-L1 antibodies as monotherapy or in combinations, as indicated on the graphs.

FIGs. 5A-B are bar graphs demonstrating the effect of treatment with DSP107, anti-PD-L1 (Atezolizumab) or combination of both compounds on M2c macrophages mediated phagocytosis of human cancer cells. Figure 5A shows % phagocytosis following 2 hours of co-culturing; and Figure 5B shows the % of CD11b-/CFSE+ cancer cells remaining in the co-culture.

FIGs. 6A-B are bar graphs demonstrating the effect of treatment with DSP107, anti-PD-L1 (Avelumab) or combination of both compounds on M2c macrophages mediated phagocytosis of human cancer cells. Figure 6A shows % phagocytosis following 2 hours of co-culturing; and Figure 6B shows the % of CD11b-/CFSE+ cancer cells remaining in the co-culture.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to combined treatment with a SIRPα-4-1BBL fusion protein for cancer.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

Cancer therapy has advanced significantly in recent years, with an increasing focus on targeted approaches to improve efficacy and reduce side effects. Current treatment modalities include radiation, surgery, chemotherapy and immunotherapy (including e.g., cell-based therapy, antibody-based therapy and cytokine therapy). Despite the advancements in cancer therapy, not all patients respond to these treatments, underscoring the ongoing need for additional therapeutic options.

While reducing specific embodiments of the present invention to practice, the present inventors have devised a clinical protocol for the treatment of advanced microsatellite stable (MSS) colorectal cancer using a SIRPα-41BBL fusion protein in combination with Atezolizumab, Bevacizumab (AVASTIN®) (Example 8 of the Examples section which follows) or in combination with Atezolizumab, Bevacizumab (AVASTIN®) and trifluridine/tipiracil (LONSURF®) (Example 1 of the Examples section which follows). In addition, the present inventors have discovered that treatment with a SIRPα-41BBL fusion protein alone or in combination with Atezolizumab results in increased plasma levels of FGFR3 and VRGF-C (Example 2 of the Examples section which follows).

Consequently, the present teachings suggest the use of a combined treatment for cancer.

Thus, according to an aspect of the present invention, there is provided a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) at least two agents selected from the group consisting of an agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1, an agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF, and Trifluridine/Tipiracil,

thereby treating the cancer in the subject.

According to an additional or an alternative aspect of the present invention, there is provided a combination of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) at least two agents selected from the group consisting of an agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1, an agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF, and Trifluridine/Tipiracil,

for use in treating cancer in a subject in need thereof.

According to an additional or an alternative aspect of the present invention, there is provided a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) at least two agents selected from the group consisting of an antibody which inhibits binding of PD-L1 to PD1, an anti-VEGF antibody and Trifluridine/Tipiracil,

thereby treating the cancer in the subject.

According to an additional or an alternative aspect of the present invention there is provided a combination of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) at least two agents selected from the group consisting of an antibody which inhibits binding of PD-L1 to PD1, an anti-VEGF antibody and Trifluridine/Tipiracil,

for use in treating cancer in a subject in need thereof.

According to an additional or an alternative aspect of the present invention there is provided a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) Trifluridine/Tipiracil,

thereby treating the cancer in the subject.

According to an additional or an alternative aspect of the present invention there is provided a combination of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) Trifluridine/Tipiracil,

for use in treating cancer in a subject in need thereof.

According to an additional or an alternative aspect of the present invention, there is provided a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) an agent which inhibits a receptor tyrosine kinase (RTK) signaling pathway, wherein said agent is not any of Bevacizumab, Ramucirumab, Cetuximab, Panitumumab, Pertuzumab, Trastuzumab, Ertumaxomab and Cixutumumab,

thereby treating the cancer in the subject.

According to an additional or an alternative aspect of the present invention, there is provided a combination of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) an agent which inhibits a receptor tyrosine kinase (RTK) signaling pathway, wherein said agent is not any of Bevacizumab, Ramucirumab, Cetuximab, Panitumumab, Pertuzumab, Trastuzumab, Ertumaxomab and Cixutumumab,

for use in treating cancer in a subject in need thereof.

As used herein the term "SIRPα (Signal Regulatory Protein Alpha, also known as CD172a)" refers to the polypeptide of the SIRPA gene (corresponding to the human Gene ID

140885) or a functional homolog e.g., functional fragment thereof. According to specific embodiments, the term "SIRPα" refers to a functional homolog of SIRPα polypeptide. According to specific embodiments, SIRPα is human SIRPα. According to a specific embodiment, the SIRPα protein refers to the human protein, such as provided in the following GenBank Number NP_001035111, NP_001035112, NP_001317657 or NP_542970.

According to specific embodiments, SIRPα amino acid sequence comprises SEQ ID NO: 6 or a functional fragment thereof.

According to specific embodiments, SIRPα amino acid sequence comprises SEQ ID NO: 6.

According to specific embodiments, SIRPα amino acid sequence consists of SEQ ID NO: 6.

According to specific embodiments, the term "SIRPα" refers to a functional homolog of SIRPα polypeptide.

As use herein, the phrase "functional homolog of the SIRPα polypeptide" or "functional fragment of the SIRPα polypeptide", refers to a portion of the polypeptide which maintains the activity of the full length SIRPα e.g., CD47 binding.

Assays for testing binding are well known in the art and include, but not limited to flow cytometry, BiaCore, bio-layer interferometry Blitz® assay, HPLC.

According to a specific embodiment, the CD47 protein refers to the human protein, such as provided in the following GenBank Numbers NP_001768 or NP_942088.

According to specific embodiments, the SIRPα binds CD47 with a Kd of 0.1 – 100 μM, 0.1 – 10 μM, 1-10 μM, 0.1-5 μM, or 1-2 μM as determined by SPR, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the SIRPα comprises an extracellular domain of SIRPα or a functional fragment thereof.

According to specific embodiments, SIRPα amino acid sequence comprises SEQ ID NO: 2 or a functional fragment thereof.

According to specific embodiments, SIRPα amino acid sequence comprises SEQ ID NO: 2.

According to specific embodiments, SIRPα amino acid sequence consists of SEQ ID NO: 2.

The term "SIRPα" also encompasses functional homologues (naturally occurring or synthetically/recombinantly produced), which exhibit the desired activity (*i.e.*, binding CD47). Such homologues can be, for example, at least 70 %, at least 75 %, at least 80 %, at least 81 %, at

least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical or homologous to the polypeptide SEQ ID NO: 6 or 2 or any other SIRPα amino acid sequence disclosed herein; or at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical to the polynucleotide sequence encoding same (as further described hereinbelow).

As used herein, "identity" or "sequence identity" refers to global identity, *i.e.*, an identity over the entire amino acid or nucleic acid sequences disclosed herein and not over portions thereof.

Sequence identity or homology can be determined using any protein or nucleic acid sequence alignment algorithm such as Blast, ClustalW, and MUSCLE.

The homolog may also refer to an ortholog, a deletion, insertion, or substitution variant, including an amino acid substitution, as further described hereinbelow.

According to specific embodiments, the SIRPα polypeptide may comprise conservative and non-conservative amino acid substitutions (also referred to herein as mutations). Such substitutions are known in the art and disclosed e.g., in Weiskopf K et al. Science. (2013); 341(6141):88-91, the contents of which are fully incorporated herein by reference.

When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences which differ by such conservative substitutions are considered to have "sequence similarity" or "similarity". Means for making this adjustment are well-known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., according to the algorithm of Henikoff S and Henikoff JG. [Amino acid substitution matrices from protein blocks. Proc. Natl. Acad. Sci. U.S.A. 1992, 89(22): 10915-

9]. Additional description on conservative amino acid and non-conservative amino acid substitutions is further provided hereinbelow.

According to specific embodiments, one or more amino acid mutations are located at an amino acid residue selected from: L4, V6, A21, A27, I31, E47, K53, E54, H56, V63, L66, K68, V92 and F96 corresponding to the SIRPα amino acid sequence set forth in SEQ ID NO: 2.

According to specific embodiments, the SIRPα amino acid sequence comprises a mutation at an amino acid residue selected from the group consisting of L4, A27, E47 and V92 corresponding to the SIRPα amino acid sequence set forth in SEQ ID NO: 2.

According to specific embodiments, one or more amino acid mutations are selected from the group consisting of: L4V or L4I, V6I or V6L, A21V, A27I or A27L, I31F or I31T, E47V or E47L, K53R, E54Q, H56P or H56R, V63I, L66T or L66G, K68R, V92I and F94L or F94V corresponding to the SIRPα amino acid sequence set forth in SEQ ID NO: 2.

According to specific embodiments, the SIRPα amino acid sequence comprises a mutation selected from the group consisting of L4I, A27I, E47V and V92I corresponding to the SIRPα amino acid sequence set forth in SEQ ID NO: 2.

As used herein, the phrase "corresponding to the SIRPα amino acid sequence set forth in SEQ ID NO: 2" or "corresponding to SEQ ID NO: 2" intends to include the homologous amino acid residue(s) in terms of composition and localization in any other SIRPα amino acid sequence. The identify of such amino acid residue(s) can be determined using any sequence alignment algorithm, such as Blast, ClustalW, and MUSCLE.

According to specific embodiments, the SIRPα amino acid sequence comprises SEQ ID NO: 4 or a functional fragment thereof.

According to specific embodiments, the SIRPα amino acid sequence comprises SEQ ID NO: 4.

According to specific embodiments, the SIRPα amino acid sequence consists of SEQ ID NO: 4.

The SIRP amino acid sequence of some embodiments of the present invention is at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical or homologous to SEQ ID NO: 2, 3, 4 or 5.

According to specific embodiments, the SIRPα amino acid sequence does not comprise the amino acid segment K117 – Y343 corresponding to SEQ ID NO: 2.

According to specific embodiments, the SIRPα amino acid sequence does not comprise any of amino acid residues K117 – Y343 corresponding to SEQ ID NO: 2.

According to specific embodiments, the SIRPα amino acid sequence does not comprise SEQ ID NO: 51.

According to specific embodiments, the SIRPα amino acid sequence comprises SEQ ID NO: 3 or 5.

According to specific embodiments, the SIRPα amino acid sequence consists of SEQ ID NO: 3 or 5.

According to specific embodiments, SIRPα amino acid sequence comprises 100-504, 100-500 amino acids, 150-450 amino acids, 200-400 amino acids, 250-400 amino acids, 300-400 amino acids, 320-420 amino acids, 340-350 amino acids, 300-400 amino acids, 340-450 amino acids, 100-200 amino acids, 100 - 150 amino acids, 100 - 125 amino acids, 100 - 120 amino acids, 100 - 119 amino acids, 105 – 119 amino acids, 110 – 119 amino acids, 115 – 119 amino acids, 105 – 118 amino acids, 110 – 118 amino acids, 115 – 118 amino acids, 105 – 117 amino acids, 110 – 117 amino acids, 115 – 117 amino acids, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, SIRPα amino acid sequence is 300-400 amino acids in length.

According to specific embodiments, SIRPα amino acid sequence is 340-450 amino acids in length.

According to specific embodiments, SIRPα amino acid sequence is 343 amino acids in length.

According to specific embodiments, SIRPα amino acid sequence is 116 amino acids in length.

As used herein the term "4-1BBL (also known as CD137L and TNFSF9)" refers to the polypeptide of the TNFSF9 gene (corresponding to human Gene ID 8744) or a functional homolog e.g., functional fragment thereof. According to specific embodiments, 4-1BBL is human 4-1BBL. According to a specific embodiment, the 4-1BBL protein refers to the human protein, such as provided in the following GenBank Number NP_003802.

According to specific embodiments, 4-1BBL amino acid sequence comprises SEQ ID NO: 17 or a functional fragment thereof.

According to specific embodiments, 4-1BBL amino acid sequence comprises SEQ ID NO: 17.

According to specific embodiments, 4-1BBL amino acid sequence consists of SEQ ID NO: 17.

According to specific embodiments, the term "4-1BBL" refers to a functional homolog of 4-1BBL polypeptide.

As use herein, the phrase "functional homolog of a polypeptide of the TNFSF9 gene" or "functional fragment of a polypeptide of the TNFSF9 gene", refers to a portion of the polypeptide which maintains the activity of the full length 4-1BBL e.g., (i) binding 4-1BB, (ii) activating 4-1BB signaling pathway, (iii) activating immune cells expressing 4-1BB, (iv) forming a homotrimer.

According to specific embodiments, the functional 4-1BBL homolog is capable of at least (i) binding to 4-1BB.

According to specific embodiments, the functional 4-1BBL homolog is capable of (i), (ii), (iii), (i)+(ii), (i)+(iii), (ii)+(iii).

According to specific embodiments, the functional 4-1BBL homolog is capable of (i)+(ii)+(iii).

According to specific embodiments, the functional 4-1BBl homolog is capable of (iv), (i)+(iv), (ii)+(iv), (iii)+(iv), (i)+(ii)+(iv), (i)+(iii)+(iv), (ii)+(iii)+(iv).

According to specific embodiments, the functional 4-1BBL homolog is capable of (i)+(ii)+(iii)+(iv).

According to a specific embodiment, the 4-1BB protein refers to the human protein, such as provided in the following GenBank Number NP_001552.

Assays for testing binding are well known in the art and are further described hereinabove

According to specific embodiments, the 4-1BBL binds 4-1BB with a Kd of about 0.1 – 1000 nM, 0.1 – 100 nM, 1-100 nM, or 55.2 nM as determined by SPR, each possibility represents a separate embodiment of the claimed invention.

Assays for testing trimerization are well known in the art and include, but not limited to NATIVE-PAGE, SEC-HPLC 2D gels, gel filtration, SEC-MALS, Analytical ultracentrifugation (AUC) Mass spectrometry (MS), capillary gel electrophoresis (CGE).

As used herein the terms "activating" or "activation" refer to the process of stimulating an immune cell (e.g., T cell, B cell, NK cell, phagocytic cell) that results in cellular proliferation, maturation, cytokine production, phagocytosis and/or induction of regulatory or effector functions.

According to specific embodiments, activating comprises co-stimulating.

As used herein the term "co-stimulating" or "co-stimulation" refers to transmitting a secondary antigen independent stimulatory signal (e.g., 4-1BB signal) resulting in activation of the immune cell.

According to specific embodiments, activating comprises suppressing an inhibitory signal (e.g., CD47 signal) resulting in activation of the immune cell.

Methods of determining signaling of a stimulatory or inhibitory signal are well known in the art and also disclosed in the Examples section which follows, and include, but are not limited to, binding assay using e.g., BiaCore, HPLC or flow cytometry, enzymatic activity assays such as kinase activity assays, and expression of molecules involved in the signaling cascade using e.g., PCR, Western blot, immunoprecipitation and immunohistochemistry. Additionally or alternatively, determining transmission of a signal (co-stimulatory or inhibitory) can be effected by evaluating immune cell activation or function. Methods of evaluating immune cell activation or function are well known in the art and include, but are not limited to, proliferation assays such as CFSE staining, MTS, Alamar blue, BRDU and thymidine incorporation, cytotoxicity assays such as CFSE staining, chromium release, Calcin AM, cytokine secretion assays such as intracellular cytokine staining, ELISPOT and ELISA, expression of activation markers such as CD25, CD69, CD137, CD107a, PD1, and CD62L using flow cytometry.

According to specific embodiments, determining the signaling activity or activation is effected *in-vitro* or *ex-vivo* e.g., in a mixed lymphocyte reaction (MLR), as further described hereinbelow.

For the same culture conditions, the signaling activity or the immune cell activation or function are generally expressed in comparison to the signaling, activation or function in a cell of the same species but not contacted with the activating agent e.g., SIRPα-4-1BBL fusion protein; or contacted with a vehicle control, also referred to as control.

According to specific embodiments, the 4-1BBL comprises an extracellular domain of 4-1BBL or a functional fragment thereof.

According to specific embodiments, 4-1BBL amino acid sequence comprises SEQ ID NO: 8 or a functional fragment thereof.

According to specific embodiments, 4-1BBL amino acid sequence comprises SEQ ID NO: 8.

According to specific embodiments, 4-1BBL amino acid sequence consists of SEQ ID NO: 8.

The term "4-1BBL" also encompasses functional homologues (naturally occurring or synthetically/recombinantly produced), which exhibit the desired activity (as defined

hereinabove). Such homologues can be, for example, at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical or homologous to the polypeptide SEQ ID NO: 17 or 8 or any other 4-1BBL amino acid sequence disclosed herein; or at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical to the polynucleotide sequence encoding same (as further described hereinbelow).

According to specific embodiments, the 4-1BBL polypeptide may comprise conservative amino acid substitutions, as further described hereinabove and below.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise the amino acid segment A1 – V6, A1 – G14 or A1-E23 corresponding to SEQ ID NO: 8.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise any of amino acid residues A1 – V6 or A1 – G14 or A1-E23 corresponding to SEQ ID NO: 8.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise SEQ ID NO: 52.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise SEQ ID NO: 53.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise the amino acid segment G198 – E205 corresponding to SEQ ID NO: 8.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise any of amino acid residues G198 – E205 corresponding to SEQ ID NO: 8.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise SEQ ID NO: 54.

As used herein, the phrase “corresponding to SEQ ID NO: 8” intends to include the homologous amino acid residue(s) in terms of composition and localization in any other 4-1BBL amino acid sequence. The identify of such amino acid residue(s) can be determined using any sequence alignment algorithm, such as Blast, ClustalW, and MUSCLE.

According to specific embodiments, 4-1BBL amino acid sequence comprises 100-254 amino acids, 150-250 amino acids, 100-250 amino acids, 150-220 amino acids, 180-220 amino acids, 180 – 210 amino acids, 185 – 205 amino acids, 185 – 200 amino acids, 185 – 199 amino

acids, 170 – 197 amino acids, 170 – 182 amino acids, 190-210 amino acids, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, 4-1BBL amino acid sequence is 191 amino acids in length.

The 4-1BBL of some embodiments of the present invention is at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical or homologous to SEQ ID NO: 7, 8, 9, 10, 11, 12, 13, 14 or 15.

According to specific embodiments, the 4-1BBL amino acid sequence comprises SEQ ID NO: 7, 8, 9, 10, 11, 12, 13, 14 or 15 or a functional fragment thereof.

According to specific embodiments, the 4-1BBL amino acid sequence comprises SEQ ID NO: 7, 8, 9, 10, 11, 12, 13, 14 or 15.

According to specific embodiments, the 4-1BBL amino acid sequence consists of SEQ ID NO: 7, 8, 9, 10, 11, 12, 13, 14 or 15.

According to specific embodiments, the 4-1BBL amino acid sequence comprises SEQ ID NO: 7.

According to specific embodiments, the 4-1BBL amino acid sequence consists of SEQ ID NO: 7.

According to specific embodiments, the 4-1BBL amino acid sequence comprised in the SIRPα-1BBL fusion protein disclosed herein comprises three repeats of a 4-1BBL amino acid sequence.

According to specific embodiments, each of the three repeats is capable of at least one of: (i) binding 4-1BB, (ii) activating 4-1BB signaling pathway, (iii) activating immune cells expressing 4-1BB, (iv) forming a homotrimer.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise a linker between each of the three repeats of said 4-1BBL amino acid sequence.

According to other specific embodiments, the 4-1BBL amino acid sequence comprises a linker between each of the three repeats of the 4-1BBL amino acid sequence. Any linker known in the art can be used with specific embodiments of the invention. Non-limiting examples of linkers that can be used are described in details hereinbelow.

A non-limiting example of such a 4-1BBL amino acid sequence is provided in SEQ ID NO: 16.

The terms “DSP”, “fusion protein”, “chimeric protein” and “chimera” are used herein interchangeably, and refer to an amino acid sequence having two or more parts which are not found together in a single amino acid sequence in nature.

As used herein, the terms “protein”, “peptide” and “polypeptide”, which are interchangeably used herein, encompass native peptides (either degradation products, synthetically synthesized peptides or recombinant peptides) and peptidomimetics (typically, synthetically synthesized peptides), as well as peptoids and semipeptoids which are peptide analogs, which may have, for example, modifications rendering the peptides more stable while in a body or more capable of penetrating into cells. Such modifications include, but are not limited to N terminus modification, C terminus modification, peptide bond modification, backbone modifications, and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein. Further details in this respect are provided hereinunder.

The polypeptides of some embodiments of the invention may be synthesized and purified by any techniques that are known to those skilled in the art of peptide synthesis, such as, but not limited to, solid phase and recombinant techniques.

The term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally in vivo, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids.

Additional description on polypeptides, amino acids, conservative and non-conservative substitutions, modifications, protecting groups, stabilizing moieties, detectable tags, cleavable moieties, methods of production and the like are described in details in International Patent Application Publication Nos. WO2018/127919 and WO2020/012486, the contents of which are fully incorporated in here by reference.

The fusion protein of some embodiments of the present invention comprises a SIRPα amino acid sequence and a 4-1BBL amino acid sequence (referred to herein as a SIRPα-4-1BBL fusion protein).

Non-limiting examples of such fusion proteins that can be used with specific embodiments of the invention are described in International Patent Application Publication Nos. WO2018/127919 and WO2020/012486, the contents of which are incorporated herein in full by reference.

According to specific embodiments, the SIRPα is N-terminal to the 4-1BBL.

According to other specific embodiments, the SIRPα is C-terminal to the 4-1BBL.

The SIRPα-4-1BBL fusion protein of some embodiments of the present invention can comprise any SIRPα amino acid sequence as defined herein; and any 4-1BBL amino acid sequence as defined herein.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is capable of least one of:

(i) binding CD47 and 4-1BB,

(ii) activating 4-1BB signaling pathway in an immune cell (e.g., T cell) expressing 4-1BB;

(iii) activating immune cells (e.g., T cells) expressing said 4-1BB; and/or

(iv) enhancing phagocytosis of pathologic cells expressing CD47 by phagocytes compared to same in the absence of SIRPα-4-1BBL fusion protein.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is capable of (i), (ii), (iii), (iv), (i)+(ii), (i)+(iii), (i)+(iv), (ii)+(iii), (ii)+(iv), (i)+(ii)+(iii), (i)+(ii)+(iv), (ii)+(iii)+(iv).

According to specific embodiments, the SIRPα-4-1BBL fusion protein is capable of (i)+(ii)+(iii)+(iv).

Methods of determining binding, activating 4-1BB signaling pathway and activating immune cells are well known in the art and are further described hereinabove and below.

According to specific embodiments, the SIRPα-4-1BBL fusion protein enhances phagocytosis of pathologic cells expressing CD47 by phagocytes.

Methods of analyzing phagocytosis are well known in the art and include for example killing assays, flow cytometry and/or microscopic evaluation (live cell imaging, fluorescent microscopy confocal microscopy, electron microscopy).

According to specific embodiments, the SIRPα-4-1BBL is soluble (i.e., not immobilized to a synthetic or a naturally occurring surface).

According to specific embodiments, the SIRPα-4-1BBL is immobilized to a synthetic or a naturally occurring surface.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is in a form of at least a homo-trimer.

According to specific embodiments, at least 10 %, at least 20 %, at least 30 %, at least 40 %, at least 50 %, at least 60 %, at least 70 %, at least 80 %, at least 90 %, at least 95 % of the SIRPα-4-1BBL fusion protein is in a form of at least a homo-trimer, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the at least homo-trimer comprises a homo-trimer.

According to specific embodiments, the at least homo-trimer is a homo-tetramer.

According to specific embodiments, the at least homo-trimer is a homo-pentamer.

According to specific embodiments, the at least homo-trimer is a homo-hexamer.

Methods of determining trimerization are well known in the art and include, but are not limited to NATIVE-PAGE, SEC-HPLC, 2D gels, gel filtration, SEC MALS, Analytical ultracentrifugation (AUC) Mass spectrometry (MS), capillary gel electrophoresis (CGE).

According to specific embodiments the at least homo-trimer is at least 100 Kd, at least 140 kD, at least 160 kD, at least 180 kD at least 200 kD, at least 220 kD, at least 240 kD, at least 250 kD in molecular weight as determined by SEC MALS.

According to specific embodiments the at least homo-trimer is at least 100 kD in molecular weight as determined by SEC MALS.

According to specific embodiments, the at least homo-trimer is at least 240 kD in molecular weight as determined by SEC MALS.

According to specific embodiments, the at least homo-trimer is about 250 – 270 kD in molecular weight as determined by SEC MALS.

According to specific embodiments, the SIRPα-4-1BBL does not comprise a linker between the SIRPα amino acid sequence and the 4-1BBL amino acid sequence.

According to specific embodiments, the SIRPα-4-1BBL fusion protein comprises a linker between the SIRPα amino acid sequence and the 4-1BBL amino acid sequence.

Any linker known in the art can be used with specific embodiments of the invention.

According to specific embodiments, the linker may be derived from naturally-occurring multi-domain proteins or is an empirical linker as described, for example, in Chichili et al., (2013), Protein Sci. 22(2): 153-167, Chen et al., (2013), Adv Drug Deliv Rev. 65(10): 1357-1369, the entire contents of which are hereby incorporated by reference. In some embodiments, the linker may be designed using linker designing databases and computer programs such as those described in Chen et al., (2013), Adv Drug Deliv Rev. 65(10): 1357-1369 and Crasto et al (2000), Protein Eng. 13(5):309-312, the entire contents of which are hereby incorporated by reference.

According to specific embodiments, the linker is a synthetic linker such as PEG.

According to specific embodiments, the linker is an Fc domain or the hinge region of an antibody (e.g., of IgG, IgA, IgD or IgE) or a fragment thereof.

According to other specific embodiments, the linker is not an Fc domain or a hinge region of an antibody or a fragment thereof.

According to specific embodiments, the linker is an Fc domain or the hinge region of human IgG4, such as provided for example in SEQ ID NO: 45, 46 or 47.

According to specific embodiments, the linker is an Fc domain or the hinge region of human IgG1, such as provided for example in SEQ ID NO: 48, 49 or 50.

According to specific embodiments, the Fc domain or the hinge region linker may comprise conservative and non-conservative amino acid substitutions (also referred to herein as mutations). Such substitutions are known in the art. According to specific embodiments, the linker may be functional. For example, without limitation, the linker may function to improve the folding and/or stability, improve the expression, improve the pharmacokinetics, and/or improve the bioactivity of the SIRPα-4-1BBL fusion protein. In another example, the linker may function to target the SIRPα-4-1BBL fusion protein to a particular cell type or location.

According to specific embodiments, the linker is a polypeptide.

Non-limiting examples of polypeptide linkers include linkers having the sequence LE, GGGGS (SEQ ID NO: 32), (GGGGS)n (n=1-4) (SEQ ID NO:32, 33, 35, 55), GGGGSGGGG (SEQ ID NO: 34), (GGGGS)x2 (SEQ ID NO: 35), (GGGGS)x2+GGGG (SEQ ID NO: 36), (Gly)8 (SEQ ID NO: 60), (Gly)6(SEQ ID NO: 61), (EAAAK)n (n=1-3) (SEQ ID NO: 37, 56, 57), A(EAAAK)nA (n = 2-5) (SEQ ID NO: 38, 39, 58, 59), AEAAAKEAAAKA (SEQ ID NO: 39), A(EAAAK)4ALEA(EAAAK)4A (SEQ ID NO: 40), PAPAP (SEQ ID NO: 41), K ESGSVSS EQ LAQ FRS LD (SEQ ID NO: 42), EGKSSGSGSESKST (SEQ ID NO: 43), GSAGSAAGSGEF (SEQ ID NO: 44), and (XP)n, with X designating any amino acid, e.g., Ala, Lys, or Glu.

In some embodiments, the SIRPα-4-1BBL comprises a linker at a length of one to six amino acids.

According to specific embodiments, the linker is substantially comprised of glycine and/or serine residues (e.g., about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 95%, or about 97% or 100 % glycines and serines).

According to specific embodiments, the linker is a single amino acid linker.

In some embodiments of the invention, the amino acid which links SIRPα and 4-1BBL is glycine, also referred to herein as SIRPα-G-4-1BBL fusion protein.

According to specific embodiments, the SIRPα-4-1BBL fusion protein amino acid sequence is at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and 18-31.

According to specific embodiments, the SIRPα-4-1BBL fusion protein comprises an amino acid sequence having at least 85 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and 18-31, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the SIRPα-4-1BBL fusion protein comprises an amino acid sequence having at least 90 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and 18-31, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the SIRPα-4-1BBL fusion protein comprises an amino acid sequence having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and 18-31, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the SIRPα-4-1BBL fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and 18-31, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the SIRPα-4-1BBL fusion protein consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and 18-31, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the SIRPα-4-1BBL fusion protein comprises SEQ ID NO: 1.

According to specific embodiments, the SIRPα-4-1BBL fusion protein consists of SEQ ID NO: 1.

The methods and uses disclosed herein relate to a combination therapy which comprises the SIRPα-4-1BBL fusion protein and at least one additional therapeutic agent.

According to specific embodiments, the additional agent is an inhibitor.

As used herein, the term "inhibitor" refers to an agent which inhibits activity or expression of a specific target protein or a protein combination (e.g., receptor-ligand combination).

According to specific embodiments, the inhibitor specifically binds the target or a polynucleotide encoding same.

As used herein, the term "specifically binds" refers to the ability of the inhibitor to bind the target protein or a polynucleotide encoding same at a higher affinity compared to other proteins or polynucleotides having different nucleic acid sequences.

Higher affinity can be, for examples, of at least 2, 5, 10, 100, 1000, 10000, or 100,000 or 1,000,000 fold or more using the same affinity assay. Methods of determining binding are well

known in the art and include e.g., BiaCore, HPLC, Surface Plasmon Resonance assay (SPR) and flow cytometry.

According to specific embodiments, the inhibitor binds the target with a Kd $\leq 10^{-3}$ M, 10^{-4} M, 10^{-5} M, 10^{-6} M, $\leq 10^{-7}$ M, $\leq 10^{-8}$ M, $\leq 10^{-9}$ M, 10^{-10} M, 10^{-11} M, 10^{-12} M, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the inhibitor binds the target protein or a polynucleotide encoding same with no cross reactivity with other proteins or polynucleotides.

According to specific embodiments, the inhibitor binds multiple members being part of the target protein family (e.g., multiple RTK family members, multiple RTK ligands etc.)

According to other specific embodiments, the inhibitor binds a single member of the target protein family (e.g., a single RTK family member e.g., VEGFR, FGFR, a single RTK ligand e.g., VEGF, FGF, a single receptor e.g., FGFR3, a single ligand VEGFC, VEGFA, etc.).

According to other specific embodiments, the inhibitor inhibits activity and/or expression of the target by affecting an upstream or downstream molecule in a signaling pathway, also referred to herein as an activator or effector in any of these pathways.

The inhibitor may be a reversible or an irreversible inhibitor.

The inhibitor may be a competitive or non-competitive inhibitor.

As used herein the phrase "inhibit activity or expression" refers to a decrease in activity or expression in the presence of the agent in comparison to same in the absence of the agent, as determined by e.g., PCR, Western-blot, ELISA, activity assay, kinase assay and the like, depending on the target. According to specific embodiments, the decrease is a statistically significant decrease. According to a specific embodiment, the change is in at least 5 %, 10 %, 20 %, 30 %, 40 % or even higher say, 50 %, 60 %, 70 %, 80 %, 90 % or more than 99 %. According to specific embodiments, the change is at least 1.5 fold, at least 2 fold, at least 3 fold, at least 5 fold, at least 10 fold, or at least 20 fold as compared to same in the absence of the agent.

Inhibiting activity or expression can be effected at the protein level (e.g., antibodies, small molecules, inhibitory peptides, enzymes that cleave the polypeptide, aptamers and the like) but may also be effected at the genomic (e.g., homologous recombination and site-specific endonucleases) and/or the transcript level using a variety of molecules which interfere with transcription and/or translation (e.g., RNA silencing agents) of a target protein.

According to specific embodiments, the inhibitor is an antibody.

The term "antibody" as used in this invention includes intact molecules as well as functional fragments thereof (that are capable of binding to an epitope of an antigen).

As used herein, the term "epitope" refers to any antigenic determinant on an antigen to which the paratope of an antibody bind. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or carbohydrate side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics.

According to specific embodiments, the antibody is a whole or intact antibody.

According to specific embodiments, the antibody comprises an Fc domain.

According to specific embodiments, the antibody is an antibody fragment.

According to a specific embodiment, the antibody fragments include, but are not limited to, single chain, Fab, Fab' and $F(ab')_2$ fragments, Fd, Fcab, Fv, dsFv, scFvs, diabodies, minibodies, nanobodies, Fab expression library or single domain molecules such as VH and VL that are capable of binding to an epitope of the antigen in an HLA restricted manner.

Suitable antibody fragments for practicing some embodiments of the invention include a complementarity-determining region (CDR) of an immunoglobulin light chain (referred to herein as "light chain"), a complementarity-determining region of an immunoglobulin heavy chain (referred to herein as "heavy chain"), a variable region of a light chain, a variable region of a heavy chain, a light chain, a heavy chain, an Fd fragment, and antibody fragments comprising essentially whole variable regions of both light and heavy chains such as an Fv, a single chain Fv Fv (scFv), a disulfide-stabilized Fv (dsFv), an Fab, an Fab', and an F(ab')2, or antibody fragments comprising the Fc region of an antibody.

According to specific embodiments, the identity of the amino acid residues in the antibody that make up the variable region and/or the CDRs is determined by the method of Kabat et al. (See, e.g., Kabat et al., 1992, Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, NIH, Washington D.C.).

Functional antibody fragments comprising whole or essentially whole variable regions of both light and heavy chains are defined as follows:

(i) Fv, defined as a genetically engineered fragment consisting of the variable region of the light chain (VL) and the variable region of the heavy chain (VH) expressed as two chains;

(ii) single chain Fv ("scFv"), a genetically engineered single chain molecule including the variable region of the light chain and the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

(iii) disulfide-stabilized Fv ("dsFv"), a genetically engineered antibody including the variable region of the light chain and the variable region of the heavy chain, linked by a genetically engineered disulfide bond.

(iv) Fab, a fragment of an antibody molecule containing a monovalent antigen-binding portion of an antibody molecule which can be obtained by treating whole antibody with the enzyme papain to yield the intact light chain and the Fd fragment of the heavy chain which consists of the variable and CH1 domains thereof;

(v) Fab', a fragment of an antibody molecule containing a monovalent antigen-binding portion of an antibody molecule which can be obtained by treating whole antibody with the enzyme pepsin, followed by reduction (two Fab' fragments are obtained per antibody molecule);

(vi) F(ab')2, a fragment of an antibody molecule containing a monovalent antigen-binding portion of an antibody molecule which can be obtained by treating whole antibody with the enzyme pepsin (i.e., a dimer of Fab' fragments held together by two disulfide bonds);

(vii) Single domain antibodies or nanobodies are composed of a single VH or VL domains which exhibit sufficient affinity to the antigen; and

(viii) Fcab, a fragment of an antibody molecule containing the Fc portion of an antibody developed as an antigen-binding domain by introducing antigen-binding ability into the Fc region of the antibody.

Methods of producing polyclonal and monoclonal antibodies as well as fragments thereof are well known in the art (See for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, 1988, incorporated herein by reference).

It will be appreciated that for human therapy, humanized or human antibodies are preferably used.

According to specific embodiments, the antibody is a humanized antibody. Humanized forms of non-human (e.g., murine) antibodies are chimeric molecules of immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab').sub.2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues form a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The

humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

According to specific embodiments, the antibody is a human antibody.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(1):86-95 (1991)]. Similarly, human antibodies can be made by introduction of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., Bio/Technology 10: 779-783 (1992); Lonberg et al., Nature 368: 856-859 (1994); Morrison, Nature 368 812-13 (1994); Fishwild et al., Nature Biotechnology 14, 845-51 (1996); Neuberger, Nature Biotechnology 14: 826 (1996); and Lonberg and Huszar, Intern. Rev. Immunol. 13, 65-93 (1995).

Selection of the therapeutic antibody used is well within the capability of those skilled in the art. Non-limiting examples of antibodies that can be used with specific embodiments of the present invention are provided infra.

Another inhibitor may be an aptamer. As used herein, the term "aptamer" refers to double stranded or single stranded RNA molecule that binds to specific molecular target, such as a protein. Various methods are known in the art which can be used to design protein specific aptamers. The skilled artisan can employ SELEX (Systematic Evolution of Ligands by Exponential Enrichment) for efficient selection as described in Stoltenburg R, Reinemann C, and Strehlitz B (Biomolecular engineering (2007) 24(4):381-403).

Other non-limiting examples of agents which can be used as an inhibitor with some embodiments of the invention include, but not limited to small molecules, inhibitory peptides, Fc-fusion polypeptides, enzymes that cleave the protein etc.

It will be appreciated that a non-functional analogue of at least a catalytic or binding portion of a target protein can be also used as an inhibitor.

Thus, according to specific embodiments, the inhibitor is a polypeptide.

Inhibition at the nucleic acid level is typically effected using a nucleic acid agent, having a nucleic acid backbone, DNA, RNA, mimetics thereof or a combination of same. The nucleic acid agent may be encoded from a DNA molecule or provided to the cell *per se*.

Thus, according to specific embodiments, the inhibitor is a polynucleotide.

The inhibitor of some embodiments of the invention can be an RNA silencing agent. As used herein, the phrase "RNA silencing" refers to a group of regulatory mechanisms [e.g., RNA interference (RNAi), transcriptional gene silencing (TGS), post-transcriptional gene silencing (PTGS), quelling, co-suppression, and translational repression] mediated by RNA molecules which result in the inhibition or "silencing" of the expression of a corresponding protein-coding gene. RNA silencing has been observed in many types of organisms, including plants, animals, and fungi.

As used herein, the term "RNA silencing agent" refers to an RNA which is capable of specifically inhibiting or "silencing" the expression of a target gene. In certain embodiments, the RNA silencing agent is capable of preventing complete processing (e.g., the full translation and/or expression) of an mRNA molecule through a post-transcriptional silencing mechanism. RNA silencing agents include non-coding RNA molecules, for example RNA duplexes comprising paired strands, as well as precursor RNAs from which such small non-coding RNAs can be generated. Exemplary RNA silencing agents that can be used with specific embodiments of the invention include dsRNAs such as siRNAs, miRNAs and shRNAs.

In one embodiment, the RNA silencing agent is capable of inducing RNA interference.

In another embodiment, the RNA silencing agent is capable of mediating translational repression.

According to an embodiment of the invention, the RNA silencing agent is specific to the target RNA and does not cross inhibit or silence other targets or a splice variant which exhibits 99% or less global homology to the target gene, e.g., less than 98 %, 97 %, 96 %, 95 %, 94 %, 93 %, 92 %, 91 %, 90 %, 89 %, 88 %, 87 %, 86 %, 85 %, 84 %, 83 %, 82 %, 81 % global homology to the target gene; as determined by PCR, Western blot, Immunohistochemistry and/or flow cytometry.

RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs).

Following is a detailed description on RNA silencing agents that can be used according to specific embodiments of the present invention.

DsRNA, siRNA and shRNA - The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes. The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex.

Accordingly, some embodiments of the invention contemplate use of dsRNA to downregulate protein expression from mRNA.

The term "siRNA" refers to small inhibitory RNA duplexes (generally between 18-30 base pairs) that induce the RNA interference (RNAi) pathway. Typically, siRNAs are chemically synthesized as 21mers with a central 19 bp duplex region and symmetric 2-base 3'-overhangs on the termini, although it has been recently described that chemically synthesized RNA duplexes of 25-30 base length can have as much as a 100-fold increase in potency compared with 21mers at the same location. The observed increased potency obtained using longer RNAs in triggering RNAi is suggested to result from providing Dicer with a substrate (27mer) instead of a product (21mer) and that this improves the rate or efficiency of entry of the siRNA duplex into RISC.

It has been found that position of the 3'-overhang influences potency of an siRNA and asymmetric duplexes having a 3'-overhang on the antisense strand are generally more potent than

those with the 3'-overhang on the sense strand (Rose et al., 2005). This can be attributed to asymmetrical strand loading into RISC, as the opposite efficacy patterns are observed when targeting the antisense transcript.

The strands of a double-stranded interfering RNA (e.g., an siRNA) may be connected to form a hairpin or stem-loop structure (e.g., an shRNA). Thus, as mentioned, the RNA silencing agent of some embodiments of the invention may also be a short hairpin RNA (shRNA).

The term "shRNA", as used herein, refers to an RNA agent having a stem-loop structure, comprising a first and second region of complementary sequence, the degree of complementarity and orientation of the regions being sufficient such that base pairing occurs between the regions, the first and second regions being joined by a loop region, the loop resulting from a lack of base pairing between nucleotides (or nucleotide analogs) within the loop region. The number of nucleotides in the loop is a number between and including 3 to 23, or 5 to 15, or 7 to 13, or 4 to 9, or 9 to 11. Some of the nucleotides in the loop can be involved in base-pair interactions with other nucleotides in the loop. Examples of oligonucleotide sequences that can be used to form the loop include 5'-CAAGAGA-3' and 5'-UUACAA-3' (International Patent Application Nos. WO2013126963 and WO2014107763). It will be recognized by one of skill in the art that the resulting single chain oligonucleotide forms a stem-loop or hairpin structure comprising a double-stranded region capable of interacting with the RNAi machinery.

Synthesis of RNA silencing agents suitable for use with some embodiments of the invention can be effected as follows. First, the target mRNA sequence is scanned downstream of the AUG start codon for AA dinucleotide sequences. Occurrence of each AA and the 3' adjacent 19 nucleotides is recorded as potential siRNA target sites. Preferably, siRNA target sites are selected from the open reading frame, as untranslated regions (UTRs) are richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNA endonuclease complex [Tuschl ChemBiochem. 2:239-245]. It will be appreciated though, that siRNAs directed at untranslated regions may also be effective, as demonstrated for GAPDH wherein siRNA directed at the 5' UTR mediated about 90 % decrease in cellular GAPDH mRNA and completely abolished protein level (www(dot)ambion(dot)com/techlib/tn/91/912(dot)html).

Second, potential target sites are compared to an appropriate genomic database (e.g., human, mouse, rat etc.) using any sequence alignment software, such as the BLAST software available from the NCBI server (www(dot)ncbi(dot)nlm(dot)nih(dot)gov/BLAST/). Putative target sites which exhibit significant homology to other coding sequences are filtered out.

Qualifying target sequences are selected as template for siRNA synthesis. Preferred sequences are those including low G/C content as these have proven to be more effective in mediating gene silencing as compared to those with G/C content higher than 55 %. Several target sites are preferably selected along the length of the target gene for evaluation. For better evaluation of the selected siRNAs, a negative control is preferably used in conjunction. Negative control siRNA preferably includes the same nucleotide composition as the siRNAs but lack significant homology to the genome. Thus, a scrambled nucleotide sequence of the siRNA is preferably used, provided it does not display any significant homology to any other gene.

It will be appreciated that, and as mentioned hereinabove, the RNA silencing agent of some embodiments of the invention need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides.

According to another embodiment the RNA silencing agent may be a miRNA.

The term "microRNA", "miRNA", and "miR" are synonymous and refer to a collection of non-coding single-stranded RNA molecules of about 19-28 nucleotides in length, which regulate gene expression. miRNAs are found in a wide range of organisms and have been shown to play a role in development, homeostasis, and disease etiology.

Below is a brief description of the mechanism of miRNA activity.

Genes coding for miRNAs are transcribed leading to production of an miRNA precursor known as the pri-miRNA. The pri-miRNA may form a hairpin with a stem and loop.

The hairpin structure of the pri-miRNA is recognized by Drosha, which is an RNase III endonuclease. Drosha typically recognizes terminal loops in the pri-miRNA and cleaves the pri-miRNA with a staggered cut typical of RNase III endonucleases yielding a pre-miRNA stem loop with a 5' phosphate and ~2 nucleotide 3' overhang. The pre-miRNA is then actively transported from the nucleus to the cytoplasm by Ran-GTP and the export receptor Ex-portin-5.

The double-stranded stem or the 5' phosphate and 3' overhang at the base of the stem loop of the pre-miRNA is then recognized by Dicer, which is also an RNase III endonuclease. Dicer then cleaves off the terminal loop two helical turns away from the base of the stem loop leaving an additional 5' phosphate and ~2 nucleotide 3' overhang. The resulting siRNA-like duplex, which may comprise mismatches, comprises the mature miRNA and a similar-sized fragment known as the miRNA*. miRNA* sequences may be found in libraries of cloned miRNAs but typically at lower frequency than the miRNAs.

Although initially present as a double-stranded species with miRNA*, the miRNA eventually becomes incorporated as a single-stranded RNA into a ribonucleoprotein complex

known as the RNA-induced silencing complex (RISC) while the miRNA* is removed and degraded.

The RISC identifies target nucleic acids based on high levels of complementarity between the miRNA and the mRNA, especially by nucleotides 2-7 of the miRNA.

The target sites in the mRNA may be in the 5' UTR, the 3' UTR or in the coding region.

miRNAs may direct the RISC to downregulate gene expression by either of two mechanisms: mRNA cleavage or translational repression. The miRNA may specify cleavage of the mRNA if the mRNA has a certain degree of complementarity to the miRNA. When a miRNA guides cleavage, the cut is typically between the nucleotides pairing to residues 10 and 11 of the miRNA. Alternatively, the miRNA may repress translation if the miRNA does not have the requisite degree of complementarity to the miRNA.

It will be appreciated from the description provided herein above that contacting cells with a miRNA may be effected by transfecting/loading the cells with e.g. the mature double stranded miRNA, the pre-miRNA or the pri-miRNA.

The pre-miRNA sequence may comprise from 45-90, 60-80 or 60-70 nucleotides.

The pri-miRNA sequence may comprise from 45-30,000, 50-25,000, 100-20,000, 1,000-1,500 or 80-100 nucleotides.

Antisense – Antisense is a single stranded RNA designed to prevent or inhibit expression of a gene by specifically hybridizing to its mRNA. Downregulation of a target can be effected using an antisense polynucleotide capable of specifically hybridizing with an mRNA transcript encoding the target protein.

Design of antisense molecules which can be used to efficiently downregulate a target must be effected while considering two aspects important to the antisense approach. The first aspect is delivery of the oligonucleotide into the cytoplasm of the appropriate cells, while the second aspect is design of an oligonucleotide which specifically binds the designated mRNA within cells in a way which inhibits translation thereof.

The prior art teaches of a number of delivery strategies which can be used to efficiently deliver oligonucleotides into a wide variety of cell types [see, for example, Jääskeläinen et al. Cell Mol Biol Lett. (2002) 7(2):236-7; Gait, Cell Mol Life Sci. (2003) 60(5):844-53; Martino et al. J Biomed Biotechnol. (2009) 2009:410260; Grijalvo et al. Expert Opin Ther Pat. (2014) 24(7):801-19; Falzarano et al, Nucleic Acid Ther. (2014) 24(1):87-100; Shilakari et al. Biomed Res Int. (2014) 2014: 526391; Prakash et al. Nucleic Acids Res. (2014) 42(13):8796-807 and Asseline et al. J Gene Med. (2014) 16(7-8):157-65]

In addition, algorithms for identifying those sequences with the highest predicted binding affinity for their target mRNA based on a thermodynamic cycle that accounts for the energetics of structural alterations in both the target mRNA and the oligonucleotide are also available [see, for example, Walton et al. Biotechnol Bioeng 65: 1-9 (1999)]. Such algorithms have been successfully used to implement an antisense approach in cells.

In addition, several approaches for designing and predicting efficiency of specific oligonucleotides using an in vitro system were also published (Matveeva et al., Nature Biotechnology 16: 1374 - 1375 (1998)].

Thus, the generation of highly accurate antisense design algorithms and a wide variety of oligonucleotide delivery systems, enable an ordinarily skilled artisan to design and implement antisense approaches suitable for downregulating expression of known sequences without having to resort to undue trial and error experimentation.

Nucleic acid agents can also operate at the DNA level as summarized infra.

Suppressing the biological function of a target can also be achieved by inactivating the gene via introducing targeted mutations involving loss-of function alterations (e.g., point mutations, deletions and insertions) in the gene structure.

As used herein, the phrase "loss-of-function alterations" refers to any mutation in the DNA sequence of a gene which results in downregulation of the expression level and/or activity of the expressed product, i.e., the mRNA transcript and/or the translated protein. Non-limiting examples of such loss-of-function alterations include a missense mutation, *i.e.*, a mutation which changes an amino acid residue in the protein with another amino acid residue and thereby abolishes the enzymatic activity of the protein; a nonsense mutation, *i.e.*, a mutation which introduces a stop codon in a protein, e.g., an early stop codon which results in a shorter protein devoid of the enzymatic activity; a frame-shift mutation, *i.e.*, a mutation, usually, deletion or insertion of nucleic acid(s) which changes the reading frame of the protein, and may result in an early termination by introducing a stop codon into a reading frame (e.g., a truncated protein, devoid of the enzymatic activity), or in a longer amino acid sequence (e.g., a readthrough protein) which affects the secondary or tertiary structure of the protein and results in a non-functional protein, devoid of the enzymatic activity of the non-mutated polypeptide; a readthrough mutation due to a frame-shift mutation or a modified stop codon mutation (*i.e.*, when the stop codon is mutated into an amino acid codon), with an abolished enzymatic activity; a promoter mutation, *i.e.*, a mutation in a promoter sequence, usually 5' to the transcription start site of a gene, which results in down-regulation of a specific gene product; a regulatory mutation, *i.e.*, a mutation in a region upstream or downstream, or within a gene, which affects the expression of the gene product; a deletion

mutation, *i.e.*, a mutation which deletes coding nucleic acids in a gene sequence and which may result in a frame-shift mutation or an in-frame mutation (within the coding sequence, deletion of one or more amino acid codons); an insertion mutation, *i.e.*, a mutation which inserts coding or non-coding nucleic acids into a gene sequence, and which may result in a frame-shift mutation or an in-frame insertion of one or more amino acid codons; an inversion, *i.e.*, a mutation which results in an inverted coding or non-coding sequence; a splice mutation *i.e.*, a mutation which results in abnormal splicing or poor splicing; and a duplication mutation, *i.e.*, a mutation which results in a duplicated coding or non-coding sequence, which can be in-frame or can cause a frame-shift.

Methods of introducing nucleic acid alterations to a gene of interest are well known in the art [see for example Menke D. Genesis (2013) 51: - 618; Capecchi, Science (1989) 244:1288-1292; Santiago et al. Proc Natl Acad Sci USA (2008) 105:5809-5814; International Patent Application Nos. WO 2014085593, WO 2009071334 and WO 2011146121; US Patent Nos. 8,771,945, 8,586,526, 6,774,279 and UP Patent Application Publication Nos. 20030232410, 20050026157, US20060014264; the contents of which are incorporated by reference in their entireties] and include targeted homologous recombination (e.g. "Hit and run", "double-replacement"), site specific recombinases (e.g. the Cre recombinase and the Flp recombinase), PB transposases (e.g. Sleeping Beauty, piggyBac, Tol2 or Frog Prince), genome editing by engineered nucleases (e.g. meganucleases, Zinc finger nucleases (ZFNs), transcription-activator like effector nucleases (TALENs) and CRISPR/Cas system) and genome editing using recombinant adeno-associated virus (rAAV) platform. Agents for introducing nucleic acid alterations to a gene of interest can be designed publically available sources or obtained commercially from Transposagen, Addgene and Sangamo Biosciences.

Methods for qualifying efficacy and detecting sequence alteration are well known in the art and include, but not limited to, DNA sequencing, electrophoresis, an enzyme-based mismatch detection assay and a hybridization assay such as PCR, RT-PCR, RNase protection, in-situ hybridization, primer extension, Southern blot, Northern Blot and dot blot analysis.

Sequence alterations in a specific gene can also be determined at the protein level using e.g., chromatography, electrophoretic methods, immunodetection assays such as ELISA and western blot analysis and immunohistochemistry.

Non-limiting examples of inhibitors that can be used with specific embodiments of the invention are further described in details hereinbelow.

According to specific embodiments, the agent inhibits activity or expression of a receptor-ligand combination PD1-PD-L1.

Non-limiting examples of such agents, including nucleotides, expression vectors, small molecules, peptides, non-functional PD1, soluble PD1 or fragments thereof that bind to PD1 ligand(s) and prevent binding to the endogenous PD1 receptor, non-functional PDL-1/PDL-2 or fragments thereof that bind to but do not promote signaling by PD1, fusion protein containing the binding portion of PD1 fused to a constant region such as an Fc region of an immunoglobulin molecule, antibodies etc., are known in the art and disclosed for example in International Patent Application Publication No. WO2017/009842, the contents of which are hereby incorporated by reference in their entirety. Non-limiting examples of small molecules that can be used with specific embodiments of the invention include CA-170, BMS-202, INCB086550. Non-limiting examples of decoy soluble PD1 or PD-L1 polypeptide or an Fc fusion thereof that can be used with specific embodiments of the invention include AMP-224 (described in e.g., Smothers et al. (2013) Annals of Oncology, 24 (Supplement 1): i7-i17), and the PD-L1 fusion proteins described in US Patent Application Publication No. US20170189476. In addition, suitable siRNAs directed PD1 or PD-L1 can be obtained from Thermo Fisher Scientific, Invitrogen and Cell Signaling Technologies.

According to specific embodiments, the agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1 is an antibody.

According to specific embodiments, the antibody inhibits binding of PD-L1 to PD1.

As used herein the phrase "antibody which inhibits binding of PD-L1 to PD1" refers to an antibody capable of decreasing binding of PD-L1 to PD1 as compared to same in the absence of the antibody, which may be determined by any method known in the art including e.g., flow cytometry, BiaCore, bio-layer interferometry Blitz® assay, HPLC. According to specific embodiments, the decrease is a statistically significant decrease. According to specific embodiments, the decrease is of at least 2 %, at least 5 % at least 10 %, 20 %, 30 %, 40 % or even higher say, 50 %, 60 %, 70 %, 80 %, 90 %, 99 % or even 100 %. According to specific embodiments the decrease is at least 1.5 fold, at least 2 fold, at least 3 fold, at least 5 fold, at least 10 fold, or at least 20 fold as compared to same in the absence of the antibody.

Such an antibody may be an anti-PD-L1 antibody or an anti-PD1 antibody.

As used herein, the term "anti-PD-L1 antibody" refers to any antibody capable of binding to a PD-L1 polypeptide.

As used herein the term "PD-L1 (Programmed death-ligand 1, also known as cluster of differentiation 274 (CD274) or B7 homolog 1)" refers to the polypeptide of the CD274 gene (corresponding to the human Gene ID 29126) or a functional homolog e.g., functional fragment thereof. According to specific embodiments, PD-L1 is human PD-L1. According to a specific

embodiment, the PD-L1 protein refers to the human protein, such as provided in the following GenBank Number NP_001254635 and NP_054862.

As use herein, the phrase "functional homolog of the PD-L1 polypeptide" or "functional fragment of the PD-L1 polypeptide", refers to a portion of the polypeptide which maintains the activity of the full-length PD-L1 e.g., PD-1 and B7-1 binding.

Assays for testing binding are well known in the art and are further described herein above.

The anti-PD-L1 antibody of some embodiments of the invention inhibits the interaction between PD-L1 and its receptors, PD-1 and B7-1.

Therapeutic anti-PD-L1 antibodies are known in the art and are also commercially available. Non-limiting examples include Atezolizumab, Avelumab and Durvalumab.

According to specific embodiments, the anti-PD-L1 antibody is Atezolizumab (a humanized immunoglobulin G1 (IgG1) monoclonal antibody, sold under the brand name TECENTRIQ®).

As used herein, the term "anti-PD1 antibody" refers to any antibody capable of binding to a PD1 polypeptide.

As used herein the term "PD1 (Programmed Death 1, also known as CD279)" refers to the polypeptide of the *PDCD1* gene (corresponding to the human Gene ID 5133) or a functional homolog e.g., functional fragment thereof. According to specific embodiments, PD1 is human PD1. According to a specific embodiment, the PD1 protein refers to the human protein, such as provided in the following GenBank Number NP_005009.

As use herein, the phrase "functional homolog of the PD1 polypeptide" or "functional fragment of the PD1 polypeptide", refers to a portion of the polypeptide which maintains the activity of the full length PD1 e.g., PD-L1 and PD-L2 binding.

Assays for testing binding are well known in the art and are further described herein above.

Therapeutic anti-PD1 antibodies are known in the art and are also commercially available. Non-limiting examples include Nivolumab, Pembrolizumab, Cemiplimab, Dostarlimab, Retifanlimab and Toripalimab.

According to specific embodiments, the agent inhibits a receptor tyrosine kinase (RTK) signaling pathway.

As used herein, the phrase "an agent which inhibits a receptor tyrosine kinase (RTK) signaling pathway" refers to an agent capable of decreasing activity or expression of a component of a signaling pathway that starts with binding of an RTK to its ligand as compared to same in the absence of the agent, which may be determined by any method known in the art including e.g., kinase assay, flow cytometry, BiaCore, bio-layer interferometry Blitz® assay, HPLC. The

components of the RTK signaling pathway are well known in the art and disclosed e.g., in Lemmon MA et al. (2010) Cell 141(7): 1117-34, the contents of which are fully incorporated herein by reference. Non-limiting examples of components of the RTK signaling pathway include an RTK ligand, an RTK, adaptor and scaffold proteins such as Shp2, Grb2 and SOS, Ras, MAPK pathway (e.g., RAF, MEK, ERK), PI3K-Akt pathway (e.g., PI3K, PIP2, PIP3, Akt, mTOR), Rho GTPase pathway (e.g., Rho, Rac, Cdc42), PLCγ/PKC pathway (e.g., PLCγ, PIP2, IP3, DAG, PKC), transcription factors (e.g. MYC, FOS, JUN).

According to specific embodiments, the agent effects the RTK-ligand combination or a molecule which is upstream of the RTK-ligand combination.

According to specific embodiments, the agent effects the RTK-ligand combination or an effector of RTK (i.e., a molecule which is downstream of the RTK-ligand combination) being part of the RTK signaling pathway.

According to specific embodiments, the agent inhibits activity or expression of the RTK-ligand combination

As used herein, the term "receptor tyrosine kinase (RTK)" refers to a cell surface receptor having an intracellular catalytic tyrosine kinase domain (E.C. no, 2.7.10.1, can transfer phosphate groups from ATP to tyrosine residues on the receptor or on downstream signaling proteins), which activates a signaling pathways within the cell following binding to its ligand. RTKs are well known to the skilled in the art and described for example in Robinson DR (2000) Oncogene 19: 5548-57; Hubbard SR (1999) Prog Biophys Mol Biol 71: 343-58; and Lemmon MA et al. (2010) Cell 141(7): 1117-34, the contents of which are fully incorporated herein by reference. According to specific embodiments, the RTK is human RTK. Non-limiting examples of RTKs encompassed by specific embodiments of the invention include, fibroblast Growth factor receptor (FGFR), vascular endothelial growth factor receptor (VEGFR), epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), insulin receptor (IR), KIT (also known as CD117), Fms-Like Tyrosine Kinase 3 (FLT3), Tropomyosin Receptor Kinase B (TRKB), AXL Receptor Tyrosine Kinase (AXL), Tyrosine Kinase with Immunoglobulin and EGF Homology Domains 2 (TIE-2), Ephrin Receptor (EPH), hepatocyte growth factor receptor (MET or HGFR), tropomyosin receptor kinase (IRK), leukocyte receptor tyrosine kinase, angiopoietin receptor, receptor tyrosine kinase-like orphan receptors (DOR), discoidin domain receptor (DDR), rearranged during transfection receptor (RETR).

According to specific embodiments, the RTK is selected from the group consisting of FGFR, VEGFR, PDGFR, KIT, FLT3, TRKB, AXL, MET, TIE-2 and EPH.

According to specific embodiments, the agent which inhibits RTK signaling pathway does not include the agents which are described in International Patent Application Publication Nos WO2018/127919 and WO2020/012486.

According to specific embodiments, the agent which inhibits RTK signaling pathway is not any of Bevacizumab, Ramucirumab, Cetuximab, Panitumumab, Pertuzumab, Trastuzumab, Ertumaxomab and Cixutumumab.

Non-limiting examples of such agents that can be used with specific embodiments of the present invention are described in details hereinbelow.

According to specific embodiments, the agent is a tyrosine kinase inhibitor (TKI).

As used herein the term "tyrosine kinase inhibitors (TKIs)" refers to a small molecule that binds the kinase domain of an RTK and inhibits the signaling pathway. Typically, TKIs can be categorized to four groups: (1) ATP-competitive inhibitors, which bind predominantly to the ATP-binding site of the kinase when this site is in the active conformation; (2) inhibitors that recognize and bind to the non-active conformation of the ATP-binding site of the kinase, thus making activation energetically unfavorable; (3) allosteric inhibitors, that bind outside of the ATP-binding site, modifying the tridimensional structure of the receptor and disrupting the interaction between the ATP and the kinase pocket; and (4) covalent inhibitors, that bind irreversibly by covalently bonding to the ATP-binding site of the target kinase.

The TKI can be specific to a specific RTK family member or can inhibit multiple RTK family members.

TKIs are well known to the skilled in the art. Non limiting examples of TKI that can be used with specific embodiments of the invention include Regorafenib, Pazopanib, Pemigatinib, Futibatinib, Infigratinib phosphate, Erdafitinib, Sunitinib, Sorafenib, Cabozantinib, Lenvatinib, Axitinib, Tivozanib, Vandetanib, Cabozantinib, Erlotinib, Gefitinib, Afatinib, Osimertinib, Imatinib, Dasatinib, Nilotinib, Bosutinib, Ponatinib, Lapatinib, Meratinib, Tucatinib, Vandetanib Capmatinib, Tepotinib, Savolitinib.

According to specific embodiments, the agent is an antibody.

Antibodies targeting an RTK or a ligand thereof are known in the art and include, but not limited to Olaratumab, Bemarituzumab, Vofatamab, IMC-EB10, ANA-12, YM327.6S2, Onartuzumab, Emibetuzumab, REGN910, Vanticumab, KMT203.

In addition, suitable siRNAs directed against RTKs can be obtained from Thermo Fisher Scientific, Invitrogen and Cell Signaling Technologies.

According to specific embodiments, the RTK is FGFR.

As used herein, the term "FGFR (fibroblast growth factor receptor)" refer to a receptor that binds a fibroblast growth factor and includes FGFR1 (CD331), FGFR2 (CD332), FGFR3 (CD333), FGFR4 (CD334), FGFRL1 and FGFR6.

According to specific embodiments, FGFR is FGFR3.

As used herein the term "FGFR3" (also known CD333) refers to the polypeptide of the FGFR3 gene (corresponding to the human Gene ID 2261). According to specific embodiments, FGFR3 is human FGFR3. According to a specific embodiment, the FGFR3 protein refers to the human protein, such as provided in the following GenBank Numbers NP_000133, NP_001156685, NP_075254, NP_001341738, NP_001341739.

As used herein the term "FGF" refer to a family of growth factor proteins and includes FGF1 to 10 or a functional homolog e.g., functional fragment thereof.

According to specific embodiments, the FGF is selected from the group consisting of FGF1, FGF2 and FGF9.

As used herein the term "FGF1 (fibroblast growth factor 1)" refers to the polypeptide of the FGF1 gene (corresponding to the human Gene ID 2246) or a functional homolog e.g., functional fragment thereof. According to specific embodiments, FGF1 is human FGF1. According to a specific embodiment, the FGF1 protein refers to the human protein, such as provided in the following GenBank Numbers NP_000791, NP_001138364, NP_001138406, NP_001138407, NP_001244134.

As used herein the term "FGF2 (fibroblast growth factor 2)" refers to the polypeptide of the FGF2 gene (corresponding to the human Gene ID 2247) or a functional homolog e.g., functional fragment thereof. According to specific embodiments, FGF2 is human FGF2. According to a specific embodiment, the FGF2 protein refers to the human protein, such as provided in the following GenBank Numbers NP_001997, NP_001348594.

As used herein the term "FGF9 (fibroblast growth factor 9)" refers to the polypeptide of the FGF9 gene (corresponding to the human Gene ID 2254) or a functional homolog e.g., functional fragment thereof. According to specific embodiments, FGF9 is human FGF9. According to a specific embodiment, the FGF9 protein refers to the human protein, such as provided in the following GenBank Numbers NP_002001.

As use herein, the phrase "functional homolog of the FGF polypeptide" or "functional fragment of the FGF polypeptide", refers to a portion of the polypeptide which maintains the activity of the full-length FGF e.g., binding to a FGF receptor [e.g., FGFR-3] and inducing a signaling cascade.

According to specific embodiments, the agent inhibits activity or expression of a receptor-ligand combination FGFR-FGF.

Non-limiting examples of such agents include Bemarituzumab, MGD3379, Erdafitinib, Pemigatinib, Infigratinib, Lucitanib, Dovitinib, Nintedanib, Regorafenib, Pazopanib, Futibatinib, Infigratinib, Sorafenib. In addition, suitable siRNAs directed at FGF or FGFR can be commercially obtained from e.g., Creative Biolabs.

According to specific embodiments, the RTK is VEGFR.

As used herein, the term "VEGFR (vascular endothelial growth factor receptor)" refer to a receptor that binds a vascular endothelial growth factor and includes VEGFR1 (FLT1), VEGFR2 (KDR) and VEGFR3 (FLT4).

According to specific embodiments, VEGFR is VEGFR3 (also known as Flt-4).

As used herein the term "VEGFR3" (also known FLT4) refers to the polypeptide of the FLT4 gene (corresponding to the human Gene ID 2324). According to specific embodiments, VEGFR3 is human VEGFR3. According to a specific embodiment, the VEGFR3 protein refers to the human protein, such as provided in the following GenBank Numbers NP_002011, NP_891555, NP_001341918.

According to specific embodiments, VEGFR is VEGFR2

As used herein the term "VEGFR2" (also known KDR) refers to the polypeptide of the KDR gene (corresponding to the human Gene ID 3791). According to specific embodiments, VEGFR2 is human VEGFR2. According to a specific embodiment, the VEGFR2 protein refers to the human protein, such as provided in the following GenBank Numbers NP_002244.

According to specific embodiments, VEGFR is VEGFR1.

As used herein the term "VEGFR1" refers to the polypeptide of the FLT1 gene (corresponding to the human Gene ID 2321). According to specific embodiments, VEGFR1 is human VEGFR1. According to a specific embodiment, the VEGFR1 protein refers to the human protein, such as provided in the following GenBank Numbers NP_001153392, NP_001153502, NP_001153503, NP_002010.

As used herein the term "VEGF" (Vascular Endothelial Growth Factor) refers to a family of growth factor proteins and includes VEGF-A, VEGF-B, VEGF-C and VEGF-D, or a functional homolog e.g., functional fragment thereof.

According to specific embodiments, the VEGF is VEGFA.

As used herein the term "VEGFA (Vascular Endothelial Growth Factor A)" refers to the polypeptide of the VEGFA gene (corresponding to the human Gene ID 7422) or a functional homolog e.g., functional fragment thereof. According to specific embodiments, VEGFA is human

VEGFA. According to a specific embodiment, the VEGFA protein refers to the human protein, such as provided in the following GenBank Numbers NP_001020537, NP_001020538, NP_001020539, NP_001020540, and NP_001020541.

According to specific embodiments, the VEGF is VEGFC.

As used herein the term "VEGFC (Vascular Endothelial Growth Factor C)" refers to the polypeptide of the VEGFC gene (corresponding to the human Gene ID 7424) or a functional homolog e.g., functional fragment thereof. According to specific embodiments, VEGFC is human VEGFC. According to a specific embodiment, the VEGFC protein refers to the human protein, such as provided in the following GenBank Number np_005420.

As use herein, the phrase "functional homolog of the VEGF polypeptide" or "functional fragment of the VEGF polypeptide", refers to a portion of the polypeptide which maintains the activity of the full-length VEGF e.g., binding to a VEGF receptor [e.g. VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), VEGFR-3] and inducing a signaling cascade.

According to specific embodiments, the agent inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF.

According to specific embodiments, the agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF is an antibody.

According to specific embodiments, the antibody inhibits binding of VEGF to VEGFR.

As used herein the phrase "antibody which inhibits binding of VEGF to VEGFR" refers to an antibody capable of decreasing binding of VEGFR to VEGF as compared to same in the absence of the antibody, which may be determined by any method known in the art including e.g., flow cytometry, BiaCore, bio-layer interferometry Blitz® assay, HPLC. According to specific embodiments, the decrease is a statistically significant decrease. According to specific embodiments, the decrease is of at least 2 %, at least 5 % at least 10 %, 20 %, 30 %, 40 % or even higher say, 50 %, 60 %, 70 %, 80 %, 90 %, 99 % or even 100 %. According to specific embodiments the decrease is at least 1.5 fold, at least 2 fold, at least 3 fold, at least 5 fold, at least 10 fold, or at least 20 fold as compared to same in the absence of the antibody.

Such an antibody may be an anti-VEGF antibody or an anti-VEGFR antibody.

According to specific embodiments, the antibody is an anti-VEGF antibody.

As used herein, the term "anti-VEGF antibody" refers to any antibody capable of binding to a VEGF polypeptide.

Assays for testing binding are well known in the art and are further described herein above.

The antibody of some embodiments of the invention inhibits the interaction between VEGF and its receptor(s).

Therapeutic anti-VEGF antibodies are known in the art and are also commercially available. Non-limiting examples include Bevacizumab, Aflibercept, Ranibizumab and Brolucizumab.

According to specific embodiments, the anti-VEGF antibody is Bevacizumab (a humanized IgG1 monoclonal antibody, sold under the brand name AVASTIN®).

According to other specific embodiments, the anti-VEGF antibody is not Bevacizumab.

According to specific embodiments, the antibody is an anti-VEGFR antibody

As used herein, the term "anti-VEGFR antibody" refers to any antibody capable of binding to a VEGFR polypeptide.

Assays for testing binding are well known in the art and are further described herein above.

Therapeutic anti-VEGFR antibodies are known in the art and are also commercially available. A Non-limiting example includes Ramucirumab, AK109, Olinvacimab, IMC-1121B.

According to other specific embodiments, the agent is not Bevacizumab.

Non-limiting examples of agents targeting the receptor-ligand combination VEGFR-VEGF include Zaltrap (aflibercept), Regorafenib, Pazopanib, Sunitinib, Sorafenib, Cabozantinib, Lenvatinib, Axitinib, Tivozanib, Vandetanib. In addition, suitable siRNAs directed at FGF or FGFR can be commercially obtained from e.g., Creative Biolabs and Abbexa.

As used herein the term "Trifluridine/Tipiracil (FTD–TPI)" refers to a combination drug comprising both trifluridine and tipiracil.

Trifluridine is a nucleoside analog, specifically a fluorinated thymidine analog, CAS No. 70-00-8.

Tipiracil is a thymidine phosphorylase inhibitor, CAS No. 183204-74-2. Thymidine phosphorylase is an enzyme that breaks down thymidine, a component of DNA. By inhibiting this enzyme, tipiracil helps increase the concentration of trifluridine.

According to specific embodiments, the Trifluridine/Tipiracil molar ratio is 1 : 0.5.

Such a drug is known in the art and disclosed for example in US Patent Nos. US9943537 and US10456399, the contents of which are fully incorporated herein by reference; and is also sold under the brand name LONSURF®.

As noted hereinabove, the present disclosure relates to a combined treatment comprising a SIRPα-4-1BBL fusion protein and at least one additional agent.

According to specific embodiments, the combined treatment comprises a SIRPα-4-1BBL fusion protein and at least two additional agents.

According to specific embodiments, the combined treatment comprises a SIRPα-4-1BBL fusion protein and at least three additional agents.

Thus, according to specific embodiments, the combined treatment comprises a SIRPα-4-1BBL fusion protein + Trifluridine/Tipiracil; a SIRPα-4-1BBL fusion protein + Trifluridine/Tipiracil + an agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1; a SIRPα-4-1BBL fusion protein + Trifluridine/Tipiracil + an agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF; a SIRPα-4-1BBL fusion protein + an agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1+ an agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF; or a SIRPα-4-1BBL fusion protein + Trifluridine/Tipiracil + an agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1+ an agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF.

According to specific embodiments, the combined treatment comprises a SIRPα-4-1BBL fusion protein + an agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1 + an agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF.

According to specific embodiments, the combined treatment comprises a SIRPα-4-1BBL fusion protein + Trifluridine/Tipiracil + an agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1+ an agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF.

According to specific embodiments, the combined treatment comprises a SIRPα-4-1BBL fusion protein + antibody which inhibits binding of PD-L1 to PD1+ anti-VEGF antibody.

According to specific embodiments, the combined treatment comprises a SIRPα-4-1BBL fusion protein + Trifluridine/Tipiracil; a SIRPα-4-1BBL fusion protein + Trifluridine/Tipiracil + antibody which inhibits binding of PD-L1 to PD1; a SIRPα-4-1BBL fusion protein + Trifluridine/Tipiracil + anti-VEGF antibody; a SIRPα-4-1BBL fusion protein + antibody which inhibits binding of PD-L1 to PD1+ anti-VEGF antibody; or a SIRPα-4-1BBL fusion protein + Trifluridine/Tipiracil + antibody which inhibits binding of PD-L1 to PD1+ anti-VEGF antibody.

According to specific embodiments, the combined treatment comprises a SIRPα-4-1BBL fusion protein + an anti-PD-L1 antibody + anti-VEGF antibody.

According to specific embodiments, the combined treatment comprises a SIRPα-4-1BBL fusion protein + Trifluridine/Tipiracil + an anti-PD-L1 antibody + anti-VEGF antibody.

According to specific embodiments, the combined treatment comprises a SIRPα-4-1BBL fusion protein + agent which inhibits a receptor tyrosine kinase (RTK) signaling pathway.

According to specific embodiments, each of the agents in the combined treatment is in a separate formulation.

According to other specific embodiments, some or all of the agents in the combined treatment are in a co-formulation.

According to specific embodiments, the combined treatment has a combined improved anti-cancer or anti-tumor activity (see e.g., Example 4 of the Example section which follows). As used herein the phrase "combined improved anti-cancer or anti-tumor activity" refers to at least additive but also synergistically improved anti-cancer or anti-tumor activity as compared to treatment with each of the agents when administered as a single agent, which may be determined by the effect on e.g., tumor size, tumor regression, symptoms of the disorder or subject's survival.

According to specific embodiments the combined treatment has an additive effect.

According to specific embodiments, the combined treatment has a synergistic effect.

The term "treating" or "treatment" refers to inhibiting, preventing or arresting the development of a pathology (disease, disorder or medical condition e.g., cancer) and/or causing the reduction, remission, or regression of a pathology or a symptom of a pathology (e.g., cancer). Those of skill in the art will understand that various methodologies and assays can be used to assess the development of a pathology, and similarly, various methodologies and assays may be used to assess the reduction, remission or regression of a pathology.

According to specific embodiments, the disease or pathology is cancer.

As used herein, the term cancer encompasses both malignant and pre-malignant cancers.

With regard to pre-malignant or benign forms of cancer, optionally the compositions and methods thereof may be applied for halting the progression of the pre-malignant cancer to a malignant form.

Cancers which can be treated by the methods of some embodiments of the invention can be any solid or non-solid cancer and/or cancer metastasis. One of skill in the art will appreciate that the methods and uses provided herein for treating cancer may be generally applicable to all known or to-be-discovered cancerous cell phenotypes and cancerous growths. Specific embodiments relate to any size and shape of tumors, including large, spread and amorphic cancerous outgrowths.

According to specific embodiments, the cancer comprises malignant cancer.

Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, lung cancer (including small-cell lung cancer, non-small-cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer,

colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulvar cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); Burkitt lymphoma, Diffused large B cell lymphoma (DLBCL), small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high-grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); T cell lymphoma, Hodgkin lymphoma, chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Acute myeloid leukemia (AML), Acute promyelocytic leukemia (APL), Hairy cell leukemia; chronic myeloblastic leukemia (CML); and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome. Preferably, the cancer is selected from the group consisting of breast cancer, colorectal cancer, rectal cancer, non-small cell lung cancer, non-Hodgkins lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, Kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, melanoma, ovarian cancer, mesothelioma, and multiple myeloma. The cancerous conditions amenable for treatment of the invention include metastatic cancers.

According to specific embodiments, the cancer comprises pre-malignant cancer.

Pre-malignant cancers (or pre-cancers) are well characterized and known in the art (refer, for example, to Berman JJ. and Henson DE., 2003. Classifying the precancers: a metadata approach. BMC Med Inform Decis Mak. 3:8). Classes of pre-malignant cancers amenable to treatment via the method of the invention include acquired small or microscopic pre-malignant cancers, acquired large lesions with nuclear atypia, precursor lesions occurring with inherited hyperplastic syndromes that progress to cancer, and acquired diffuse hyperplasias and diffuse metaplasias. Examples of small or microscopic pre-malignant cancers include HGSIL (High grade squamous intraepithelial lesion of uterine cervix), AIN (anal intraepithelial neoplasia), dysplasia of vocal cord, aberrant crypts (of colon), PIN (prostatic intraepithelial neoplasia). Examples of acquired large lesions with nuclear atypia include tubular adenoma, AILD (angioimmunoblastic lymphadenopathy with dysproteinemia), atypical meningioma, gastric polyp, large plaque parapsoriasis, myelodysplasia, papillary transitional cell carcinoma in-situ, refractory anemia with excess blasts, and Schneiderian papilloma. Examples of precursor lesions occurring with inherited hyperplastic syndromes that progress to cancer include atypical mole syndrome, C cell adenomatosis and MEA. Examples of acquired diffuse hyperplasias and diffuse metaplasias

include AIDS, atypical lymphoid hyperplasia, Paget's disease of bone, post-transplant lymphoproliferative disease and ulcerative colitis.

According to specific embodiments, the cancer is a solid tumor.

According to specific embodiments, the tumor is a primary tumor.

According to specific embodiments, the cancer is selected from the group consisting of a GI tract cancer, liver cancer, pancreatic cancer, lung cancer, renal cancer, bladder cancer, breast cancer, and head and neck cancer.

According to specific embodiments, the cancer is colorectal cancer (e.g., colorectal carcinoma).

According to specific embodiments, the cancer is characterized by BRAF V600E, HER2 amp or overexpression, or KRAS G12C.

According to specific embodiments, the cancer is positive for the microsatellite instability (MSI) [e.g., high microsatellite instability (MSI-H)] and/or the mismatch repair deficient (dMMR) marker.

According to other specific embodiments, the cancer is negative for the microsatellite instability (MSI) and/or the mismatch repair deficient (dMMR) marker.

According to specific embodiments, the cancer is positive for the microsatellite stable (MSS) and/or mismatch repair proficient (pMMR) marked.

Thus, according to specific embodiments, the cancer is an MSS cancer.

Methods of determining MSI / dMMR and MSS / pMMR are well known in the art and include Next-generation sequencing (NGS), Fluorescent multiplex PCR and C, immunohistochemistry, single-molecule molecular inversion probes (smMIPs).

Thus, according to specific embodiments, the method comprises determining microsatellite stability and/or mismatch repair in a biological sample obtained from the subject.

According to specific embodiments, cells of the cancer express PD-L1. In other words, according to specific embodiments, cells of the cancer present PD-L1 on their cell membrane.

According to specific embodiments, cells of the cancer express CD47. In other words, according to specific embodiments, cells of the cancer present CD47 on their cell membrane.

Methods of determining expression and/or presentation are well known in the art and include PCR, Western blot, immunostaining, flow cytometry and the like.

Thus, according to specific embodiments, the method comprises determining presentation of PD-L1 and/or CD47 in a biological sample obtained from the subject.

As used herein, the term “subject” includes mammals, e.g., human beings at any age and of any gender. According to specific embodiments, the term “subject” refers to a subject who suffers

from the pathology (e.g., cancer). According to specific embodiments, this term encompasses individuals who are at risk to develop the pathology.

According to specific embodiments, the subject has histologically confirmed, inoperable, tumor (e.g., colorectal carcinoma).

According to specific embodiments, the subject has histologically confirmed, inoperable, microsatellite stable and/or proficient mismatch repair colorectal carcinoma by local testing.

According to specific embodiments, the subject is at least 18 years of age.

According to specific embodiments, the subject does not have past or current history of autoimmune disease or immune deficiency.

According to specific embodiments, the subject does not suffer from a non-cancerous disease selected from the group consisting of liver disease, lung disease, cardiovascular disease, vascular disease, gastrointestinal disease, uncontrolled hypertension, active ulcer, untreated bone fracture and active infection,

According to specific embodiments, the subject has not been subjected to organ or stem cell transplantation.

According to specific embodiments, the subject has been treated with at least one, at least two, or at least three previous line(s) of anti-cancer therapy.

According to specific embodiments, the subject progressed on or has shown intolerance to fluoropyrimidine, irinotecan, oxaliplatin, bevacizumab and/or epidermal growth factor receptor (EGFR) inhibitor treatment.

According to specific embodiments, recurrence within 12 months of last adjuvant chemo counts as progression.

According to specific embodiments, the subject has not been treated with immunotherapy or immune checkpoint inhibitor.

According to specific embodiments, the subject has not been treated with Trifluridine/Tipiracil (LONSURF®), regorafenib and/or fruquintinib.

According to specific embodiments, the subject has not been treated with an-anti-VEGF, antibody which inhibits binding of PD-L1 to PD1, Trifluridine/Tipiracil and/or a combination thereof.

According to specific embodiments, the subject has not been treated with an-anti-VEGF (e.g., Bevacizumab), anti-PD-L1 (e.g., Atezolizumab), Trifluridine/Tipiracil (LONSURF®) and/or a combination thereof.

According to specific embodiments, the subject has not been treated with a T cell therapy such as Chimeric Antigen Receptor (CAR)-T cells.

According to specific embodiments, when the cancer is characterized by BRAF V600E, HER2 amp or overexpression, or KRAS G12C, the subject may have also received one line of prior targeted therapy.

According to specific embodiments, the subject complies with the Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 or 1.

According to specific embodiments, the subject satisfies all the inclusion criteria outlined in Table 1 hereinbelow.

According to specific embodiments, the subject does not satisfy any of the exclusion criteria outlined in Table 1 hereinbelow.

According to specific embodiments, the subject is characterized by increased levels of a TKI or a ligand thereof, such as FGFR-3 or VEGF-C.

Hence, according to specific embodiments, the method further comprises determining levels of a TKI or a ligand thereof, such as FGFR-3 or VEGF-C, in a sample obtained from the subject (e.g., blood, plasma, tumor biopsy).

According to specific embodiments, the determining is effected prior to or following treatment with the SIRPα-4-1BBL fusion protein.

According to specific embodiments, increased levels of the RTK or ligand thereof indicates suitability of the subject to the combined treatment.

According to specific embodiments, the combined treatment disclosed herein can be administered to a subject in combination with other established or experimental therapeutic regimen to treat cancer including, but not limited to analgesics, chemotherapeutic agents, radiotherapeutic agents, cytotoxic therapies (conditioning), hormonal therapy, antibodies and other treatment regimens (e.g., surgery) which are well known in the art.

Anti-cancer agents that can be use with specific embodiments of the invention include, but are not limited to the anti-cancer drugs Acivicin; Aclarubicin; Acodazole Hydrochloride; Acronine; Adriamycin; Adozelesin; Aldesleukin; Altretamine; Ambomycin; Ametantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrene Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate; Brequinar Sodium; Bropirimine; Busulfan; Cactinomycin; Calusterone; Caracemide; Carbetimer; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Chlorambucil; Cirolemycin; Cisplatin; Cladribine; Crisnatol Mesylate; Cyclophosphamide; Cytarabine; Dacarbazine; Dactinomycin; Daunorubicin Hydrochloride; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel; Doxorubicin; Doxorubicin Hydrochloride;

Droloxifene; Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate; Eflornithine Hydrochloride; Elsamitrucin; Enloplatin; Enpromate; Epipropidine; Epirubicin Hydrochloride; Erbulozole; Esorubicin Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole; Etoposide; Etoposide Phosphate; Etoprine; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Floxuridine; Fludarabine Phosphate; Fluorouracil; Flurocitabine; Fosquidone; Fostriecin Sodium; Gemcitabine; Gemcitabine Hydrochloride; Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Ilmofosine; Interferon Alfa-2a; Interferon Alfa-2b; Interferon Alfa-n1; Interferon Alfa-n3; Interferon Beta- I a; Interferon Gamma- I b; Iproplatin; Irinotecan Hydrochloride; Lanreotide Acetate; Letrozole; Leuprolide Acetate; Liarozole Hydrochloride; Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocol; Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate; Melengestrol Acetate; Melphalan; Menogaril; Mercaptopurine; Methotrexate; Methotrexate Sodium; Metoprine; Meturedepa; Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane; Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole; Nogalamycin; Ormaplatin; Oxisuran; Paclitaxel; Pegaspargase; Peliomycin; Pentamustine; Peplomycin Sulfate; Perfosfamide; Pipobroman; Piposulfan; Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium; Porfiromycin; Prednimustine; Procarbazine Hydrochloride; Puromycin; Puromycin Hydrochloride; Pyrazofurin; Riboprine; Rogletimide; Safingol; Safingol Hydrochloride; Semustine; Simtrazene; Sparfosate Sodium; Sparsomycin; Spirogermanium Hydrochloride; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Sulofenur; Talisomycin; Taxol; Tecogalan Sodium; Tegafur; Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone; Testolactone; Thiamiprine; Thioguanine; Thiotepa; Tiazofuirin; Tirapazamine; Topotecan Hydrochloride; Toremifene Citrate; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole Hydrochloride; Uracil Mustard; Uredepa; Vapreotide; Verteporfin; Vinblastine Sulfate; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate; Vinglycinate Sulfate; Vinleurosine Sulfate; Vinorelbine Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; Zorubicin Hydrochloride. Additional antineoplastic agents include those disclosed in Chapter 52, Antineoplastic Agents (Paul Calabresi and Bruce A. Chabner), and the introduction thereto, 1202-1263, of Goodman and Gilman's "The Pharmacological Basis of Therapeutics", Eighth Edition, 1990, McGraw-Hill, Inc. (Health Professions Division).

Other anti-cancer agents that can be used with specific embodiments of the invention include antibodies such as, but are not limited to, rituximab, cetuximab, trastuzumab, edrecolomab, alemtuzumab, gemtuzumab, ibritumomab, panitumumab, Belimumab, Bevacizumab,

Bivatuzumab mertansine, Blinatumomab, Blontuvetmab, Brentuximab vedotin, Catumaxomab, Cixutumumab, Daclizumab, Adalimumab, Bezlotoxumab, Certolizumab pegol, Citatuzumab bogatox, Daratumumab, Dinutuximab, Elotuzumab, Ertumaxomab, Etaracizumab, Gemtuzumab ozogamicin, Girentuximab, Necitumumab, Obinutuzumab, Ofatumumab, Pertuzumab, Ramucirumab, Siltuximab, Tositumomab, Trastuzumab, Nivolumab, Pembrolizumab, Durvalumab, Atezolizumab, Avelumab and ipilimumab, each possibility represents a separate embodiment of the invention.

Additional anti-cancer agents that can be used with specific embodiments of the invention include an IMiD (e.g., Thalidomide, Lenalidomie, Pomalidomide), an anti-infection agent (e.g., antibiotics and anti-viral agents), and/or an immune suppressor agent (e.g., GCSF and other bone marrow stimulators, steroids).

According to specific embodiments, treatment may comprise any of the permitted concomitant medications listed in Table 1 or 4 hereinbelow.

According to specific embodiments, treatment does not comprise any of the prohibited concomitant medications listed in Table 1 or 4 hereinbelow.

According to other specific embodiments, the combined treatment disclosed herein does not comprise any additional established or experimental therapeutic regimen to treat cancer.

Any of the agents [e.g. the SRIPα-4-1BBL fusion protein, the agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1 (e.g., the antibody which inhibits binding of PD-L1 to PD1), the agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF (e.g., the anti-VEGF antibody), the agent which inhibits a receptor tyrosine kinase (RTK) signaling pathway and/or Trifluridine/Tipiracil] of some embodiments of the invention can be administered to an organism *per se*, or in a pharmaceutical composition where it is mixed with suitable carriers or excipients.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Herein the term "active ingredient" refers to the agent [e.g. the SRIPα-4-1BBL fusion protein, the agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1 (e.g., the antibody which inhibits binding of PD-L1 to PD1), the agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF (e.g., the anti-VEGF antibody), the agent which inhibits a receptor tyrosine kinase (RTK) signaling pathway and/or Trifluridine/Tipiracil] accountable for the biological effect.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, intradermal, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intracardiac, e.g., into the right or left ventricular cavity, into the common coronary artery, intravenous, intraperitoneal, intranasal, or intraocular injections.

Conventional approaches for drug delivery to the central nervous system (CNS) include: neurosurgical strategies (e.g., intracerebral injection or intracerebroventricular infusion); molecular manipulation of the agent (e.g., production of a chimeric fusion protein that comprises a transport peptide that has an affinity for an endothelial cell surface molecule in combination with an agent that is itself incapable of crossing the BBB) in an attempt to exploit one of the endogenous transport pathways of the BBB; pharmacological strategies designed to increase the lipid solubility of an agent (e.g., conjugation of water-soluble agents to lipid or cholesterol carriers); and the transitory disruption of the integrity of the BBB by hyperosmotic disruption (resulting from the infusion of a mannitol solution into the carotid artery or the use of a biologically active agent such as an angiotensin peptide). However, each of these strategies has limitations, such as the inherent risks associated with an invasive surgical procedure, a size limitation imposed by a limitation inherent in the endogenous transport systems, potentially undesirable biological side effects associated with the systemic administration of a chimeric molecule comprised of a carrier motif that could be active outside of the CNS, and the possible risk of brain damage within regions of the brain where the BBB is disrupted, which renders it a suboptimal delivery method.

Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient.

Pharmaceutical compositions of some embodiments of the invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with some embodiments of the invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose,

binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to some embodiments of the invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water-based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water-based solution, before use.

The pharmaceutical composition of some embodiments of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Alternative embodiments include depots providing sustained release or prolonged duration of activity of the active ingredient in the subject, as are well known in the art.

According to specific embodiments, the SIRPα-4-1BBL fusion protein (e.g., SEQ ID NO: 1) is administered intravenously.

According to specific embodiments, the agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1 is administered intravenously (e.g., by infusion).

According to specific embodiments, the antibody which inhibits binding of PD-L1 to PD1 [e.g., anti-PD-L1 antibody (e.g., Atezolizumab)] is administered intravenously.

According to specific embodiments, the agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF is administered intravenously (e.g., by infusion).

According to specific embodiments, the anti-VEGF antibody (e.g., Bevacizumab) is administered intravenously.

According to specific embodiments, the SIRPα-4-1BBL fusion protein, antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) and/or anti-VEGF antibody is administered by infusion.

According to specific embodiments, the infusion is over 10 – 240 minutes.

According to specific embodiments, the infusion is over 120 ± 15 minutes, 90 ± 15 minutes, 60 ± 15 minutes, 30 ± 15 minutes.

According to specific embodiments, the Trifluridine/Tipiracil (e.g., LONSURF®) is administered orally.

Pharmaceutical compositions suitable for use in context of some embodiments of the invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of a disorder (e.g., cancer) or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from in vitro and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. The data obtained from these in vitro and cell culture assays and animal studies can be

used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide levels of the active ingredient are sufficient to induce or suppress the biological effect (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is administered at a dose of 0.01 – 100 mg / kg, 0.01 – 50 mg / kg, 1 – 25 mg / kg, 5-25 mg / kg, 5 – 20 mg / kg, 10 – 20 mg / kg, or 5 – 10 mg / kg.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is administered at a dose of at least 5 mg / kg.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is administered at a dose of about 5 mg / kg.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is administered at a dose of about 10 mg / kg.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is administered at a dose of about 15 mg / kg.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is administered at a dose of about 20 mg / kg.

According to specific embodiments, the antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) is administered at a dose of 500 – 2000 mg.

According to specific embodiments, the antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) is administered at a dose of 840-1680 mg.

According to specific embodiments, the antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) is administered at a dose of about 1680 mg.

According to specific embodiments, the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) is administered at a dose of 5-15 mg /kg.

According to specific embodiments, the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) is administered at a dose of about 5 mg / kg.

According to specific embodiments, the Trifluridine/Tipiracil is administered at a dose of 70-160 mg Trifluridine /m^2/day.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

According to specific embodiments, the combined treatment comprises multiple cycles of treatment.

According to specific embodiments, treatment comprises at least 2, at lease 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 15, at least 20, at least 21, at least 22, at least 23, at least 24 cycles.

According to specific embodiments, each cycle is 3 weeks long.

According to specific embodiments, each cycle is 4 weeks long.

According to specific embodiments, first administration of the agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1, the agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF and/or the Trifluridine/Tipiracil starts on the same day.

According to specific embodiments, first administration of the antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody), the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) and/or the Trifluridine/Tipiracil starts on the same day.

According to specific embodiments, first administration of the antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody), the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) and/or the Trifluridine/Tipiracil starts on the same day of each cycle.

According to specific embodiments, first administration of all the agents in the combined treatment starts on the same day.

According to specific embodiments, first administration of all the agents in the combined treatment starts on the same day of each cycle.

According to specific embodiments, when administered on the same day, administration of Trifluridine/Tipiracil and/or the antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) will precede the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody).

According to specific embodiments, when administered on the same day, administration of Trifluridine/Tipiracil and/or the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) will precede the antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody).

According to specific embodiments, when administered on the same day, administration of Trifluridine/Tipiracil and/or the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) and/or the antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) will precede the SIRPα-4-1BBL fusion protein.

According to specific embodiments, when administered on the same day, administration of the antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) will precede the SIRPα-4-1BBL fusion protein.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is administered in 5-14 days or 5-10 days intervals.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is administered once a week.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is administered at least twice in a 4 weeks' cycle, wherein first administration is on day 1 of the cycle.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is administered at least 3 times in a 4 weeks' cycle, wherein first administration is on day 1 of the cycle.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is administered 3 or 4 times in a treatment cycle, wherein first administration is on day 1 of the cycle.

According to specific embodiments, each treatment cycle includes 3 administrations of the SIRPα-4-1BBL fusion protein having 5-10 days intervals, wherein first administration is on day 1 of the cycle.

According to specific embodiments, each treatment cycle includes 3 administrations of the SIRPα-4-1BBL fusion protein having a 1 week' interval, wherein first administration is on day 1 of the cycle.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is administered 3 times in a 4 weeks' cycle, wherein first administration is on day 1 of the cycle.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is administered 3 times in a 4 weeks' cycle, on days 1, 8 and 15 of the cycle.

According to specific embodiments, each treatment cycle includes 4 administrations of the SIRPα-4-1BBL fusion protein having a 1 week' interval, wherein first administration is on day 1 of the cycle.

According to specific embodiments, the SIRPα-4-1BBL fusion protein is administered 4 times in a 4 weeks' cycle, on days 1, 8, 15 and 22 of the cycle.

According to specific embodiments, the antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) is administered once every at least 2, at least 3 or at least 4 weeks.

According to specific embodiments, the antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) is administered at a dose of about 840 mg every two weeks, at a dose of about 1200 mg every 3 weeks or at a dose of about 1680 mg every 4 weeks.

According to specific embodiments, the antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) is administered once every 4 weeks.

According to specific embodiments, the antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) is administered once during each cycle.

According to specific embodiments, the antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) is administered on day 1 of each cycle.

According to specific embodiments, the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) is administered every at least 1, at least 2 or at least 3 weeks.

According to specific embodiments, the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) is administered at a dose of about 5-10 mg / kg every 2 weeks, or at a dose of 15 mg / kg every 3-4 weeks.

According to specific embodiments, the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) is administered every two weeks.

According to specific embodiments, the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) is administered twice during each cycle.

According to specific embodiments, first administration of the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) is on day 1 of each cycle.

According to specific embodiments, the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) is administered on days 1 and 15 of each cycle.

According to specific embodiments, the Trifluridine/Tipiracil is administered once or twice a day.

According to specific embodiments, the Trifluridine/Tipiracil is administered twice a day. Under this scenario the daily dose is divided in half in each administration.

According to specific embodiments, first administration of Trifluridine/Tipiracil is on day 1 of each cycle.

According to specific embodiments, the Trifluridine/Tipiracil is administered for 5 consecutive days following by a 2 days' rest.

According to specific embodiments, the Trifluridine/Tipiracil is administered for a total of 10 days during each cycle.

According to specific embodiments, the Trifluridine/Tipiracil is administered for 5 consecutive days, followed by 2 days' rest, followed by administration for 5 consecutive days.

According to specific embodiments, the Trifluridine/Tipiracil is administered for a total of 10 days during each cycle, on days 1-5 and 8-12 of the cycle.

According to specific embodiments, the Trifluridine/Tipiracil is administered twice a day for a total of 10 days during said cycle, on days 1-5 and 8-12 of the cycle.

According to specific embodiments, a cycle of treatment with the SIRPα-4-1BBL fusion protein, antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody), antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) and Trifluridine/Tipiracil includes:

3 administrations of the SIRPα-4-1BBL fusion protein having 1 week' intervals, wherein a first administration is on day 1 of the cycle,

the antibody which inhibits binding of PD-L1 to PD1 is administered once during the cycle,

the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) is administered twice during the cycle with 2 weeks intervals; and

the Trifluridine/Tipiracil is administered for 10 days during the cycle.

According to specific embodiments, treatment with the SIRPα-4-1BBL fusion protein, antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody), antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) and Trifluridine/Tipiracil is in a 4 weeks' cycle, wherein:

said SIRPα-4-1BBL fusion protein is administered 3 times during the cycle, on days 1, 8 and 15 of the cycle;

said antibody which inhibits binding of PD-L1 to PD1 is administered once during the cycle, on day 1 of the cycle;

said antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF) antibody is administered twice during the cycle, on days 1 and 15 of the cycle; and

said Trifluridine/Tipiracil is administered twice a day for a total of 10 days during said cycle, on days 1-5 and 8-12 of the cycle.

According to specific embodiments, treatment with the SIRPα-4-1BBL fusion protein, antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody), antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) and Trifluridine/Tipiracil is in a 4 weeks' cycle, wherein:

said SIRPα-4-1BBL fusion protein (e.g., SEQ ID NO: 1) is administered 3 times during the cycle, on days 1, 8 and 15 of the cycle, in a dose of about 10 mg/kg/dose;

said antibody which inhibits binding of PD-L1 to PD1 [e.g., anti-PD-L1 antibody (e.g., Atezolizumab)] is administered once during the cycle, on day 1 of the cycle, in a dose of 1680 mg;

said antibody which inhibits binding of VEGF to VEGFR [e.g., anti-VEGF antibody (e.g., Bevacizumab)] is administered twice during the cycle, on days 1 and 15 of the cycle, in a dose of about 5 mg/kg/dose; and

said Trifluridine/Tipiracil is administered twice a day for a total of 10 days during the cycle, on days 1-5 and 8-12 of the cycle, in a dose of about 70-160 mg/m^2/day.

According to specific embodiments, a cycle of treatment with the SIRPα-4-1BBL fusion protein, antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) and antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) includes:

4 administrations of the SIRPα-4-1BBL fusion protein having 1 week' intervals, wherein a first administration is on day 1 of the cycle;

the antibody which inhibits binding of PD-L1 to PD1 is administered once during the cycle; and

the antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) is administered twice during the cycle with 2 weeks intervals.

According to specific embodiments, treatment with the SIRPα-4-1BBL fusion protein, antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) and antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) is in a 4 weeks' cycle, wherein:

said SIRPα-4-1BBL fusion protein is administered 4 times during the cycle, on days 1, 8, 15 and 22 of the cycle;

said antibody which inhibits binding of PD-L1 to PD1 is administered once during the cycle, on day 1 of the cycle; and

said antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF) antibody is administered twice during the cycle, on days 1 and 15 of the cycle.

According to specific embodiments, treatment with the SIRPα-4-1BBL fusion protein, antibody which inhibits binding of PD-L1 to PD1 (e.g., anti-PD-L1 antibody) and antibody which inhibits binding of VEGF to VEGFR (e.g., anti-VEGF antibody) is in a 4 weeks' cycle, wherein:

said SIRPα-4-1BBL fusion protein (e.g., SEQ ID NO: 1) is administered 4 times during the cycle, on days 1, 8, 15 and 22 of the cycle, in a dose of about 10 mg/kg/dose;

said antibody which inhibits binding of PD-L1 to PD1 [e.g., anti-PD-L1 antibody (e.g., Atezolizumab)] is administered once during the cycle, on day 1 of the cycle, in a dose of 1680 mg; and

said antibody which inhibits binding of VEGF to VEGFR [e.g., anti-VEGF antibody (e.g., Bevacizumab)] is administered twice during the cycle, on days 1 and 15 of the cycle, in a dose of about 5 mg/kg/dose. A non-limiting dosing regimen that can be used with specific embodiments

of the invention is provided in the "Investigational Product Route and Dosage Form" of Table 1 or 4 hereinbelow.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of some embodiments of the invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labelling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labelled for treatment of an indicated condition, as is further detailed above.

As used herein the term "about" refers to ± 10 %.

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of" means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically

disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

When reference is made to particular sequence listings, such reference is to be understood to also encompass sequences that substantially correspond to its complementary sequence as including minor sequence variations, resulting from, e.g., sequencing errors, cloning errors, or other alterations resulting in base substitution, base deletion or base addition, provided that the frequency of such variations is less than 1 in 50 nucleotides, alternatively, less than 1 in 100 nucleotides, alternatively, less than 1 in 200 nucleotides, alternatively, less than 1 in 500 nucleotides, alternatively, less than 1 in 1000 nucleotides, alternatively, less than 1 in 5,000 nucleotides, alternatively, less than 1 in 10,000 nucleotides.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques.

EXAMPLE 1

TREATMENT OF COLORECTAL CANCER WITH A SIRPα-4-1BBL FUSION PROTEIN, ATEZOLIZUMAB, BEVACIZUMAB AND LUNSURF®

Clinical Protocol – A SIRPα-4-1BBL fusion protein having an amino acid sequence as set forth in SEQ ID NO: 1 (herein referred to as "DSP107") is administered to colorectal cancer patients in combination with the anti-PD-L1 antibody Atezolizumab, the anti-VEGF antibody Bevacizumab and Trifluridine/Tipiracil (LONSURF®), according to the protocol synopsis shown in Table 1 hereinbelow.

Table 1: Protocol Synopsis

Study Title	A Phase II Study to Evaluate the Efficacy of the Combination of DSP107, Atezolizumab, Trifluridine/Tipiracil and Bevacizumab in Patients with Advanced Microsatellite Stable Colorectal Cancer
Protocol No.	DSP107_003
Clinical Sites	TBD
Study Phase	II
Planned Sample Size	Up to 50 patients will be enrolled
Therapeutic Indication	Patients ≥ 18 years of age with microsatellite stable (MSS) and/or mismatch repair proficient (pMMR) colorectal cancer (CRC) who have progressed following up to three previous lines of therapy.
Study Objectives	**Primary** • Evaluate the efficacy of the combination of DSP107, atezolizumab, trifluridine/Tipiracil and bevacizumab in patients with microsatellite stable colorectal cancer.

	• Evaluate the safety and tolerability of the combination of DSP107, atezolizumab, trifluridine/Tipiracil and bevacizumab. **Secondary** • Evaluate the pharmacokinetics of DSP107 when administered in combination with atezolizumab, trifluridine/Tipiracil and bevacizumab. • Evaluate the pharmacodynamic effect of the combination of DSP107, atezolizumab, trifluridine/Tipiracil and bevacizumab. • Evaluate DSP107 immunogenicity by measurement of anti-drug antibodies. **Exploratory** • Evaluate predictive biomarkers for association with efficacy, safety and pharmacokinetics. • Evaluate exploratory biomarkers for future drug development.
Study Design	This study will evaluate the efficacy, safety, pharmacokinetics (PK) and pharmacodynamics (PD) of DSP107 in combination with atezolizumab, trifluridine/Tipiracil (LONSURF®) and bevacizumab in patients with advanced MSS CRC. Specifically, the study will enroll CRC patients that are MSS and/or pMMR who have progressed on or shown to intolerance to fluoropyrimidine, irinotecan, oxaliplatin, bevacizumab and epidermal growth factor receptor (EGFR) inhibitor treatment. Patients with BRAF (Proto-oncogene B-Raf) V600E, HER2 (Human Epidermal Growth Factor Receptor 2) amplification or overexpression and KRAS (Kirsten Rat Sarcoma Virus) G12C may also have received one line of prior targeted therapy. Prior treatment with LONSURF® in combination with bevacizumab will not be allowed. The study population will be stratified so that no more than 50% of the patients enrolled in the study may have liver metastases at the time of screening or within 6 months of Cycle 1 Day 1. Patients with previously treated liver metastases more than 6 months prior to Cycle 1 Day 1 are not considered to have liver metastases and may enrol into any open slot. The dose of DSP107 used in this study will be 10 mg/kg/dose. The study will incorporate a “safety run-in” to allow evaluation of the safety of this dose in combination with atezolizumab, bevacizumab and LONSURF® in the first 10 patients enrolled who are DLT-evaluable. In the event of toxicity (defined

	below) in this initial cohort of 10 patients, a reduction of DSP107 dose may be recommended. Up to 50 patients evaluable for the primary endpoints will be enrolled in this study. Enrolled patients will receive DSP107 in combination with atezolizumab, bevacizumab and LONSURF® in an open-label fashion. DSP107 will be administered by intravenous (IV) infusion on Days 1, 8 and 15 of each 28-day cycle. The first DSP107 infusion will be administered over 120 (± 15) mins with premedication and IV fluids at the Investigator's discretion, to minimize the occurrence of infusion related reactions (IRRs). If well tolerated, subsequent infusions may be administered over 60 (± 15) mins with premedication and IV fluids at the Investigator's discretion. Atezolizumab will be administered at a dose of 1680 mg IV on Day 1 of every treatment cycle. DSP107 infusion will commence 30 (± 10) mins following completion of atezolizumab infusion. The first atezolizumab infusion should be administered over 60 mins. If well tolerated, subsequent infusions may be performed over 30 mins. Bevacizumab will be administered by IV infusion at a dose of 5 mg/kg/dose on Days 1 and 15 of each 28-day treatment cycle. Bevacizumab infusion will commence 30 (± 10) mins following completion of Atezolizumab infusion (and LONSURF® dosing). The first bevacizumab infusion should be administered over 90 mins. If well tolerated, the next infusion can be administered over 60 mins, and if infusion over 60 mins is well tolerated then subsequent infusion can be administered over 30 mins. Patients will receive a LONSURF® dose of 35 mg/m^2/dose up to a maximum of 80 mg/dose (based on trifluridine content). Doses will be administered orally, twice daily with food on Days 1-5 and Days 8-12 of each 28-day treatment cycle. LONSURF® dosing (together with bevacizumab infusion) will precede atezolizumab infusion. Doses taken by the patient at home will be recorded on a dosing diary. If deemed appropriate by the Investigator, subjects may hold or discontinue one or more components of the treatment regimen (or in the case of LONSURF®, have dose reduction(s)) and may continue treatment with the

	other agent(s). Subjects will only be considered off treatment when all protocol-specified anti-cancer therapies have been discontinued. The study will include up to 12 treatment cycles. Subjects still on study at the end of 12 treatment cycles may continue to receive DSP107 alone or in combination with atezolizumab ± bevacizumab ± LONSURF® until the occurrence of unacceptable toxicity, disease progression, withdrawal of consent or study closure. Patients may continue treatment beyond progression in certain circumstances. All study endpoints will be evaluated at time of initial progressive disease (PD) evaluation by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. Patients who receive treatment beyond PD will be analyzed descriptively. Patients with evidence of PD by RECIST v1.1 will be allowed to continue study treatment beyond progression provided: o The patient is tolerating treatment and is felt to be obtaining clinical benefit from study treatment, at the discretion of the treating physician. o No signs/symptoms of clinical disease progression, at the discretion of the treating physician. o No decline in ECOG performance status attributable to underlying cancer. o No rapid disease progression or disease progression at critical sites (such as spinal cord compression) requiring urgent intervention. o Patient signs a treatment beyond progression ICF. New lesions are considered measurable at the time of initial progression if they meet RECIST v1.1 criteria (i.e., longest diameter ≥ 10 mm, or ≥ 15 mm short axis for a lymph node). If subsequent imaging confirms PD, study treatments will end. Subsequent PD is defined as: o Worsening progression in target disease with an increase of at least 5 mm in the absolute value of the sum o Continued unequivocal progression in non-target disease with an increase in tumor burden. o Increase in size of previously identified new lesion(s) (an increase of at least 5 mm in the absolute value of the sum of those considered to be target new lesions) or additional new lesions.

	All subjects will be evaluated for efficacy, safety and tolerability of the regimen. Treatment response assessment will be based on Computed Tomography (CT) scans obtained after four treatment cycles (16 weeks). Treatment futility, toxicity (defined as any Grade 3 or 4 adverse event [AE] considered related to study drug) and 4-week mortality will be monitored so that enrollment can be stopped if the defined stopping boundaries are met during patient accrual. In the event of toxicity, the safety monitoring committee (SMC) may recommend a reduced DSP107 dose level. Sparse DSP107 PK profiles and DSP107 anti-drug antibody (ADA) titers will be evaluated in all patients. PD samples including paired biopsies for assessment of tumor expression of relevant targets and blood samples for measurement of carcinoembryonic antigen (CEA), ctDNA and other correlative studies will be evaluated in all patients. **Dose Limiting Toxicities and Ongoing Safety Assessment** AEs will be recorded from the time of informed consent until 30 days after the last dose of study drug. The period considered for DLT assessment will be limited to the first cycle of therapy defined as the time from the first dose of DSP107 up to and including the day when the first cycle of monotherapy or combination therapy ends (Day 28). Beyond the DLT assessment period, AE data will be collected on an ongoing basis and recorded within the CRF. However, these events will not be considered for DLT evaluation. DLT is defined as a clinically significant AE or abnormal laboratory value assessed as related to DSP107 and/or atezolizumab and unrelated to disease progression or intercurrent illness and occurring during the first treatment cycle that meets any of the following criteria: • Increased Aspartate Aminotransferase (AST) (Serum Glutamic Oxaloacetic Transaminase (SGOT)) or Alanine Transaminase (ALT) (Serum Glutamic Pyruvic Transaminase (SGPT)) > 5 x upper limit of normal (ULN) but ≤ 8 x ULN lasting for ≥ 7 days • Increased AST (SGOT) or ALT (SGPT) > 8 or total bilirubin > 3 times ULN x ULN of any duration • Laboratory abnormalities that satisfy Hy's Law i.e., ALT or AST elevation > 3 x ULN, total bilirubin elevation > 2 x ULN, absence of

	initial findings of cholestasis (i.e., absence of elevation of alkaline phosphatase to > 2 x ULN), and no other reason can be found to explain the combination of increased ALT/AST and total bilirubin • CTCAE grade 3 reduced hemoglobin (HGB) for ≥ 7 days • CTCAE grade 4 reduction in HGB of any duration • CTCAE grade 4 neutropenia or thrombocytopenia of any duration • CTCAE grade 3 neutropenia lasting for ≥ 7 days • CTCAE grade 3 thrombocytopenia with clinically significant bleeding, e.g., requiring intervention or transfusion • Neutropenic fever • Any other CTCAE grade 4 laboratory abnormality • Any other CTCAE grade 3 laboratory abnormalities that are clinically significant or require intervention • CTCAE grade ≥ 3 non-hematologic toxicity excluding: o Grade 3 nausea/vomiting or diarrhea lasting > 72 h with adequate antiemetic and other supportive care. o Grade 3 fatigue lasting > 1 week • Pericardial disorders (pericarditis, pericardial effusion, cardiac tamponade) of any grade • Grade 3 or 4 pneumonitis • Grade 4 colitis or diarrhea • Grade 4 hypophysitis • Grade 4 immune-mediated adverse reaction involving a major organ including, but not limited to the following: o Cardiac: Grade 2, 3 or 4 myocarditis o Dermatologic: Grade 4 dermatological events. Any grade Stevens Johnson Syndrome or toxic epidermal necrolysis) o Gastrointestinal: Grade 4 immune-mediated pancreatitis o General: systemic inflammatory response syndrome, histiocytic necrotizing lymphadenitis o Musculoskeletal: Grade 4 immune-mediated myositis, rhabdomyolysis o Neurological: Any grade Guillain-Barre syndrome, myasthenia gravis and immune-mediated

	meningoencephalitis. Grade 3 or 4 immune-mediated neuropathy, including facial paresis. Grade 2, 3 or 4 immune-mediated myelitis o Ophthalmological: Grade 3 or 4 ocular events, e.g., uveitis, retinal events o Renal: Grade 3 or 4 immune-mediated nephritis o Vascular: vasculitis. • Grade 3 or 4 infusion related reactions (IRRs) • Persistent grade 2 or 3 immune-mediated adverse reaction that does not recover to grade 0 or 1 within 12 weeks after last DSP107 and/or atezolizumab dose • Inability to taper corticosteroid dose to ≤ 10 mg/day prednisone (or equivalent) within 12 weeks after last DSP107 and/or atezolizumab dose • Recurrent grade 3 or 4 adverse reaction. • Any death not clearly due to the underlying disease or extraneous cause. An AE must be clinically significant to define DLT e.g., nausea and vomiting, rash, alopecia, electrolyte abnormalities (including potassium (K^+), sodium (Na^+), chloride (Cl^-), bicarbonate ($HCO3^-$), magnesium (Mg^{++}), calcium (Ca^{++}), bilirubin) that are < grade 3 will not constitute DLT. Patients experiencing ≥ grade 3 DSP107-related toxicity will be withdrawn from the study but followed for toxicity. If a patient experiencing DSP107-related toxicity is deriving clinical benefit from treatment, they may be allowed to interrupt dosing and restart treatment once toxicity has resolved at the discretion of the Investigator. Patients who experience toxicity, which is not related to DSP107, will be allowed to continue DSP107 treatment at the Investigator's discretion. To be evaluable for the DLT assessment period, a patient must complete the first cycle of treatment. If a patient is discontinued before completion of the first cycle (unless due to a DLT), an additional patient will be recruited and included in the initial safety cohort to ensure a total of ten evaluable patients. The patients that are discontinued early will be included in all the other

	relevant analyses according to the population described in the statistical analysis section.
Study Procedures	Study visits and evaluations will be performed at Screening and at scheduled timepoints following the first and subsequent doses of DSP107. Safety assessments including physical examination, vital sign measurement (pulse, blood pressure, oral temperature, oxygen (O_2) saturation and respiratory rate) and collection of blood samples for hematology, coagulation, blood chemistry and systemic cytokine and complement levels will be performed at scheduled timepoints during the study and at the Final Visit. A single electrocardiogram (ECG) will be collected at the Screening visit and at scheduled timepoints during the study. AEs and concomitant medications will be assessed and recorded throughout the study. PK samples will be collected prior to the first administration of study drug and at scheduled timepoints throughout the study to measure DSP107 PK levels. Additional samples will be collected prior to the first administration of study drug and at scheduled timepoints throughout the study to measure DSP107 ADA titers. Blood samples will be collected at scheduled timepoints for immunophenotyping of immune cells by flow cytometry. Paired fresh tumor tissue (or metastatic tumor) biopsies will be collected from at least 25 patients (one prior to study drug administration and one while on treatment) provided it is deemed safe and accessible by the Investigator. The pre-treatment tumor biopsy (within 28 days prior to the first dose of DSP107) and on-treatment (after approximately 6-8 weeks of treatment) should be collected, provided the Investigator deems the procedure to be safe. The biopsy material will include at least 2 and no more than 4 cores. Patients who do not agree to undergo this procedure may be enrolled or remain on study provided there are sufficient patients enrolled to ensure paired biopsies are collected from at least 25 patients. Tumor tissue will be analyzed for changes in the tumor microenvironment (TME), markers of immune cell activation, PD-L1 and CD47 expression and other biomarkers to facilitate retrospective correlation with clinical response. Archival biopsies may be used to determine baseline expression if fresh biopsies are not collected. PD samples will be collected at scheduled timepoints throughout the study for measurement of CEA, ctDNA and other correlative studies.

	CT scans must be performed within 28 days prior to the first dose of study drug. Imaging will be repeated at the end of treatment cycles 2 and 4 and then every 2 months until disease progression or study termination. For patients who complete 12 treatment cycles and continue DSP107 treatment ± atezolizumab ± bevacizumab ± LONSURF® beyond 1 year, the frequency of imaging may be reduced to 4 months at the discretion of the Investigator. Alternative imaging modalities may be used for these patients if clinically indicated, e.g., PET-CT. Response evaluation will be performed based on RECIST version 1.1. Scans will be read locally at the clinical site; scans may also be read centrally by an independent reader at the discretion of the Investigator. A safety follow-up visit will be performed 30 (± 7) days after the last dose of study drug. The safety follow-up period may be extended up to 90 days after the last dose of study drug for any Serious Adverse Events (SAEs) considered related to DSP107 and/or atezolizumab. All patients will be followed for up to 5 years after completion of the follow-up period. Sites will contact subjects by telephone at approximately 3-month intervals (± 1 month) after the end of the follow-up period to determine survival.
Study Duration	Patients may continue to receive DSP107 ± atezolizumab ± bevacizumab ± LONSURF® provided they continue to tolerate the drug(s), have no evidence of disease progression, and as long as they do not meet any of the criteria for subject discontinuation, the Sponsor terminates or suspends the trial or until marketing approval, whichever is earlier. The study will include up to 12 treatment cycles. Patients still on study at the end of 12 treatment cycles may continue to receive DSP107 alone or in combination with atezolizumab ± bevacizumab ± LONSURF® until the occurrence of unacceptable toxicity, disease progression, withdrawal of consent or study closure. Patients may also continue to receive study treatment(s) beyond progression under certain circumstances (see above). DSP107 treatment may also be stopped in the event of a confirmed complete response (CR) in patients who have completed at least 6 treatment cycles on study including at least one treatment cycle beyond the date the initial CR was declared. Patients who subsequently experience radiologic disease progression will be eligible for re-treatment with DSP107 at the discretion of

<table>
<tr><td></td><td>the Investigator, if no cancer treatment was administered since the last dose of DSP107, the patient meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is still open.
When a patient discontinues study drug treatment, assessments will be performed at a safety follow-up visit conducted 30 (± 7) days after the last dose of DSP107. Patients with grade 3 or 4 drug-related toxicities at this visit will be followed for up to 90 days after the last dose of study drug, until the toxicities resolve to an acceptable level or until they start another anticancer therapy, whichever occurs earlier.
All patients will be followed for up to 5 years after completion of the follow-up period. Sites will contact patients by telephone at approximately 3-month intervals (± 1 month) after the end of the follow-up period to determine survival.

<table><tr><th>Study Period</th><th>Duration</th></tr><tr><td>Screening period</td><td>28 days</td></tr><tr><td>Treatment Period</td><td>Twelve, 28-day treatment cycles</td></tr><tr><td>Follow-up safety period</td><td>Up to 90 days from the last dose of study drug</td></tr><tr><td>Survival follow-up</td><td>Up to 5 years</td></tr></table></td></tr>
<tr><td>Inclusion Criteria</td><td>Each patient must meet all the following inclusion criteria to be enrolled in the study:
1. Patient must be ≥ 18 years of age.
2. Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 or 1.
3. Capable of understanding and complying with parameters as outlined in the protocol and able to sign informed consent, approved by an Institutional Review Board (IRB) / Independent Ethics Committee (IEC) prior to the initiation of any screening or study-specific procedures.
4. Patient has:
▪ Histologically confirmed, inoperable, microsatellite stable and/or proficient mismatch repair colorectal carcinoma by local testing (only one required).</td></tr>
</table>

	▪ Progressed on or is intolerant to (as long as eligible and no contraindication per Investigator) fluoropyrimidine, irinotecan, oxaliplatin, bevacizumab and EGFR inhibitor. ▪ Recurrence within 12 months of last adjuvant chemo counts as progression. ▪ Patients with BRAF V600E, HER2 amp or overexpression, and KRAS G12C may have also received one line of prior targeted therapy. 5. Measurable disease per RECIST version 1.1: • A previously irradiated lesion can be considered a target lesion if the lesion is well defined, measurable per RECIST, and has clearly progressed. Tumor biopsies will be collected from non-target lesions that can be biopsied at acceptable risk as judged by the Investigator or if no non-target lesion is suitable for biopsy, then a RECIST target lesion used for biopsy must be ≥ 2 cm in longest diameter. 6. Male patients and female patients of childbearing potential must agree to use a highly effective method of birth control during the study and for 4 weeks after the final dose of DSP107. Additionally, females of childbearing potential who receive atezolizumab must agree to use a highly effective method of birth control during and for 5 months after the final dose of atezolizumab. • Women are considered to be of childbearing potential unless it is documented that they are over the age of 60 **OR** postmenopausal by history with no menses for 1 year and confirmed by Follicle Stimulating Hormone (FSH) (using local reference ranges) **OR** have a history of hysterectomy and/or bilateral oophorectomy **OR** have a history of bilateral tubal ligation. Highly effective methods of birth control include hormonal birth control (oral, intravaginal, transdermal, implantable, or intrauterine), intrauterine devices (IUDs), vasectomy, or any double-barrier methods (combination of male condom and spermicide with either cap, diaphragm, or sponge).

Exclusion criteria	Each patient must meet none of the following exclusion criteria to be enrolled in the study: 1. Life expectancy of ≤ 3 months. 2. Colorectal cancer that is microsatellite instability high and/or mismatch repair deficient. 3. Central nervous system (CNS) metastases, unless stable 1 month post definitive therapy with steroids discontinued 14 days prior to Cycle 1 Day 1. 4. Previously treated with any immunotherapy or immune checkpoint inhibitor. 5. Previously treated with LONSURF®, regorafenib and/or fruquintinib. 6. Unresolved adverse events ≥ grade 2 from prior anticancer therapy (except for alopecia). 7. Unable to swallow pills or has any condition expected to impair the absorption of LONSURF®. 8. History of primary immunodeficiency. 9. Past or current history of autoimmune disease or immune deficiency, including, but not limited to, myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, antiphospholipid antibody syndrome, Wegener granulomatosis, Sjogren's syndrome, Guillain-Barré syndrome, or multiple sclerosis, with the following exceptions: • History of autoimmune-related hypothyroidism who are on thyroid replacement hormone are eligible for the study. • Controlled Type 1 diabetes mellitus who are on a stable insulin regimen are eligible for the study. • Eczema, psoriasis, lichen simplex chronicus, or vitiligo with dermatologic manifestations only (e.g., patients with psoriatic arthritis are excluded) are eligible for the study provided all the following conditions are met: • Rash must cover <10% of body surface area. • Disease is well controlled at baseline and requires only low-potency topical corticosteroids.

	• No occurrence of acute exacerbations of the underlying condition requiring psoralen plus ultraviolet A radiation, methotrexate, retinoids, biologic agents, oral calcineurin inhibitors, or high potency or oral corticosteroids within the previous 12 months. 10. History of autoimmune hemolytic anemia or autoimmune thrombocytopenia. 11. History of hematological malignancy. 12. History of organ or stem cell transplantation. 13. Previously treated with Chimeric Antigen Receptor (CAR)-T cells. 14. Diagnosed with clinically significant liver disease, including alcoholic hepatitis, cirrhosis, fatty liver disease and inherited liver disease. 15. History of idiopathic pulmonary fibrosis or organizing pneumonia (e.g., bronchiolitis obliterans). 16. History of, or active, clinically significant cardiovascular dysfunction including the following: • History of stroke or transient ischemic attack within 6 months prior to Cycle 1 Day 1 • History of myocardial infarction within 6 months prior to Cycle 1 Day 1 • New York Heart Association Class III or IV cardiac disease • Uncontrolled arrhythmias or history of, or active, ventricular arrhythmia requiring medication • Coronary heart disease that is symptomatic or unstable angina. 17. History of significant vascular disease (e.g., aortic aneurysm requiring surgical repair or recent arterial thrombosis) within 6 months prior to Cycle 1 Day 1. 18. History of Grade 4 venous thromboembolism. 19. History of Grade $\geq$ 2 hemoptysis (defined as $\geq$ 2.5 mL of bright red blood per episode) or any other serious hemorrhage within 28 days prior to Cycle 1 Day 1.

	20. History or evidence of inherited bleeding diathesis or significant coagulopathy at risk of bleeding (in the absence of therapeutic anticoagulation). 21. Current or recent (within 10 days prior to Cycle 1 Day 1) use of aspirin (>325 mg/day) of clopidogrel (> 75 mg/day). The use of full-dose oral or parenteral anticoagulants for therapeutic purpose is permitted as long as the INR and aPTT is within expected therapeutic range (according to Investigator discretion) on screening labs and the patient has been on a stable dose of anticoagulants for ≥ 2 weeks prior to Cycle 1 Day 1. Prophylactic use of anticoagulants is allowed. 22. Inadequately controlled hypertension (defined as systolic blood pressure > 150 mmHg and/or diastolic blood pressure > 90 mmHg and based on an average of ≥ 3 blood pressure readings on ≥ 2 occasions. • Anti-hypertensive therapy to achieve these parameters is allowed. 23. History of hypertensive crisis or hypertensive encephalopathy. 24. Patient has had major surgery within 28 days prior to Cycle 1 Day 1 and/or the surgical wound is not fully healed or the anticipation of the need for major surgery during study treatment. 25. Patient has had minor surgical procedure, excluding placement of a vascular access device, within 7 days prior to Cycle 1 Day 1 and/or the surgical wound is not fully healed. 26. Serious non-healing wound, active ulcer or untreated bone fracture. 27. History of abdominal fistula, gastrointestinal perforation, intra-abdominal abscess, or active gastrointestinal bleeding within 6 months prior to Cycle 1 Day 1. 28. Partial or complete bowel obstruction within 3 months of Cycle 1 Day 1, current signs or symptoms of bowel obstruction, or current radiologic evidence of impending obstruction. 29. Evidence of abdominal free air not explained by paracentesis or recent surgical procedure.

30. Uncontrolled pleural effusion, pericardial effusion or ascites requiring recurrent drainage procedures (once monthly or more frequently). Patients with indwelling catheters are allowed.
31. Active tuberculosis or history of serious bacterial, viral, parasitic or systemic fungal infections requiring hospitalization and administration of intravenous antibiotics, antifungals or antivirals within 28 days prior to Cycle 1 Day 1.
32. Currently receiving other anticancer therapies or has received anticancer therapy, including chemotherapy, radiation therapy, biologic or any investigational therapy within a period of 21 days prior to Cycle 1 Day 1.
    - Palliative radiation therapy for painful bony or skin metastasis is not subject to a washout period.
33. Treated with systemic immunosuppressive medication (including, but not limited to, corticosteroids, cyclophosphamide, azathioprine, methotrexate, thalidomide and anti-Tumor Necrosis Factor alpha [TNF-α] agents) within 2 weeks prior to Cycle 1 Day 1, or anticipation of need for systemic immunosuppressive medication during the course of the study, with the following exceptions:
    - Patients who received acute, low-dose systemic immunosuppressant medication or a one-time pulse dose of systemic immunosuppressant medication (e.g., 48 h of corticosteroids for a contact allergy) at the discretion of the Investigator.
    - Subjects who received mineralocorticoids (e.g., fludrocortisone), corticosteroids for chronic obstructive pulmonary disease or asthma, or low-dose corticosteroids for orthostatic hypotension or adrenal insufficiency are eligible for the study.
34. Received a live, attenuated vaccine within 28 days prior to Cycle 1 Day 1 or anticipation of need for such a vaccine during treatment or within 5 months after the last dose of DSP107 / atezolizumab.
35. Participated in study of an investigational device or drug within 14 days or five half-lives, whichever is longer, prior to Cycle 1 Day 1.

	36. Known allergy or hypersensitivity to any of the test compounds, materials or contraindication to test product, including: • History of severe allergic anaphylactic reactions to chimeric, human or humanized antibodies or fusion proteins • Known hypersensitivity to Chinese Hamster Ovary (CHO) cell products or any component of the atezolizumab formulation. 37. Clinically significant abnormal laboratory safety tests including, but not limited to: • Platelet count $< 100 \times 10^9$/L. • Hemoglobin level < 9 g/dL. • Absolute neutrophil count $< 1.0 \times 10^9$/L. • AST and/or ALT at screening > 1.5 x ULN for subjects without known liver involvement by tumor; or > 3 x ULN for subjects with known liver involvement by tumor. • Bilirubin ≥ 1.5 x ULN (unless prior diagnosis and documentation of ongoing hemolysis or Gilbert's syndrome). • Creatinine > 1.5 x ULN or creatinine clearance ≤ 40 mL/min as calculated using Cockcroft-Gault formula. • Uncontrolled or symptomatic hypercalcemia (ionized calcium > 1.5 mmol/L, calcium > 12 mg/dL, or corrected calcium greater than ULN). • Grade ≥ 2 proteinuria, as demonstrated by ≥ 2+ protein on dipstick urinalysis and ≥ 1.0 g of protein in a 24-hour urine collection. All patients with ≥ 2+ protein on dipstick urinalysis at screening must undergo a 24-hour urine collection for protein. Patients with < 2+ protein on dipstick urinalysis are eligible for the study. 38. History of Human Immunodeficiency Virus (HIV) infection. Patients on triple therapy, regardless of HIV titer, will not be included. 39. Active hepatitis B virus (HBV) infection (chronic or acute), defined as having a positive hepatitis B surface antigen (HBsAg) test at

	screening. Patients with a past or resolved HBV infection, defined as having a negative HBsAg test and a positive total hepatitis B core antibody (HBcAb) test at screening, are eligible for the study. 40. Active hepatitis C virus (HCV) infection, defined as having a positive HCV antibody test followed by a positive HCV ribonucleic acid (RNA) test at screening. The HCV RNA test will be performed only for patients who have a positive HCV antibody test. 41. Pregnancy (positive urine pregnancy test in females of childbearing potential) or breast feeding or planning to become pregnant while enrolled in the study. 42. History or evidence of any other clinically unstable/uncontrolled disorder, condition, or disease (including, but not limited to, cardiopulmonary, renal, metabolic, hematologic or psychiatric disorders) other than their primary malignancy, that in the opinion of the Investigator would pose a risk to patient safety or interfere with study evaluations, procedures or study completion. Unable to comply with study requirements in the opinion of the Investigator.

Investigational Product Route and Dosage Form	**DSP107** The DSP107 drug product will be supplied by the Sponsor in a single-use vial intended for IV administration. Subjects will receive IV administration of DSP107 on Days 1, 8 and 15 of each treatment cycle. The dose to be administered will be 10 mg/kg/dose. The first DSP107 dose will be administered over 120 (± 15) mins with premedication and IV fluids at the Investigator's discretion, to minimize the occurrence of IRRs. If well tolerated, subsequent infusions may be administered over 60 (± 15) mins with premedication and IV fluids at the Investigator's discretion. Patients will be administered DSP107 30 (± 10) mins following completion of atezolizumab infusion. **Atezolizumab** The atezolizumab drug product will be supplied by the Sponsor in a single-use, 20 mL United State Pharmacopeia (USP)/Ph. Eur. Type 1 glass vial as a colorless to slightly yellow, sterile, preservative-free, clear liquid solution intended for IV administration. Patients will receive atezolizumab 1680 mg IV on Day 1 of every treatment cycle. Atezolizumab infusion will precede administration of DSP107 and will take place 30 (± 10) mins following completion of bevacizumab infusion The first atezolizumab infusion should be administered over 60 mins. If well tolerated, subsequent infusions may be performed over 30 mins. **Bevacizumab** The bevacizumab will be supplied by the clinical site. Patients will receive bevacizumab 5 mg/kg on Days 1 and 15 of every treatment cycle. Bevacizumab infusion (and administration of LONSURF®) will precede administration of atezolizumab. The first bevacizumab infusion should be administered over 90 mins. If the first infusion is tolerated, the next infusion can be administered over 60 mins. If infusion over 60 mins is tolerated, subsequent infusions can be administered over 30 mins.

Trifluridine/Tipiracil

LONSURF® tablets (15 mg and 20 mg formulation) will be supplied by the clinical site. Patients will receive a dose of 35 mg/m^2/dose up to a maximum of 80 mg/dose (based on trifluridine content). Doses will be administered orally, twice daily with food on Days 1-5 and Days 8-12 of each 28-day treatment cycle. LONSURF® dosing (together with bevacizumab infusion) will precede atezolizumab infusion. Please see Table 1a for recommended dosage according to body surface area (BSA):

Table 1a: Recommended LONSURF® dosage according to body surface area

BSA (m^2)	Total daily dose (mg)	Dose (mg) administered twice daily	Tablets per dose	
			15 mg	20 mg
< 1.07	70	35	1	1
1.07 - 1.22	80	40	0	2
1.23 - 1.37	90	45	3	0
1.38 - 1.52	100	50	2	1
1.53 - 1.68	110	55	1	2
1.69 - 1.83	120	60	0	3
1.84 - 1.98	130	65	3	1
1.99 - 2.14	140	70	2	2
2.15 - 2.29	150	75	1	3
≥ 2.30	160	80	0	4

Concomitant Medications and Dose Adjustment	All treatments that the Investigator considers necessary for a subject's welfare may be administered at the discretion of the Investigator in keeping with the community standards of medical care. All concomitant medications received within 30 days prior to the screening visit and up to 30 days after the last dose of study treatment should be recorded. Concomitant medications administered more than 30 days after the last dose of study treatment will be recorded if they are associated with a SAE. All concomitant medication will be recorded on the CRF including all prescription, over the counter (OTC), herbal supplements and IV medications, blood products and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date should also be included in the CRF. Pre-medication and permitted medication for DSP107 AE management: • *Premedication* with antihistamines (e.g., diphenhydramine, promethazine, etc.), antipyretics and/or analgesics is permitted prior to DSP107 IV administration to minimize the occurrence of grade 1 or 2 infusion-related reactions (IRRs). • *Blood transfusion* will be allowed in the event of significant, symptomatic anemia at the discretion of the Investigator. **Permitted Concomitant Medications** • Antiemetic drugs (e.g., ondansetron) as required clinically based on local guidelines for subjects experiencing nausea. • Prophylactic antibiotics (e.g., quinolone or cephalosporin), anti-fungals (e.g., voriconazole) and antivirals (e.g., valacyclovir) when appropriate. • Blood products commonly required in oncology subjects. • Steroids (together with interruption of atezolizumab dosing) until the adverse reaction has reduced to grade $\leq$ 1 and the steroid dose has been tapered to $\leq$ 10 mg/day prednisone (or equivalent). **Prohibited Concomitant Medications** Subjects are prohibited from receiving the following therapies during the screening period and treatment phase of this study: • Antineoplastic systemic chemotherapy or biological therapy. • Immunotherapy or other therapy not specified in this protocol.

	• Investigational agents other than DSP107, atezolizumab, bevacizumab and LONSURF®. • Live vaccines within 4 weeks prior to the first dose of study treatment and while participating in the trial and for 5 months after the last dose of DSP107 / atezolizumab. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chickenpox, yellow fever, Bacillus Calmette–Guérin (BCG) and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed. However, intranasal influenza vaccines (e.g., Flu - Mist®) are live attenuated vaccines and are not allowed. • Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of suspected immunologic etiology or for systemic IRRs secondary to DSP107 and/or atezolizumab treatment. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor. *Note: Inhaled steroids are allowed for management of asthma, and dermatological formulations are allowed to reduce the intensity of injection site reactions.* • Medications or vaccinations specifically prohibited in the Exclusion Criteria are not allowed during the ongoing study. If there is a clinical indication for any medication or vaccination specifically prohibited during the study, discontinuation of study treatment may be required. The Investigator should discuss any questions regarding this with the Sponsor's Medical Monitor. The final decision on any supportive therapy or vaccination rests with the Investigator and/or the subject's primary physician. However, the decision to continue the subject's study treatment requires the mutual agreement of the Investigator, the Sponsor and the subject. Patients who, in the assessment of the Investigator, require the use of any of the aforementioned treatments for clinical management should be withdrawn from the study. Patients may receive other medications that the Investigator deems to be medically necessary.

	Once a patient completes or discontinues study treatment, there are no prohibitions on concomitant therapy (unless specifically mentioned above, e.g., live attenuated vaccines). **Atezolizumab Adverse Reaction Management:** *Immune-Mediated Pneumonitis* Atezolizumab can cause immune-mediated pneumonitis or interstitial lung disease, defined as requiring use of systemic corticosteroids. Fatal cases have been reported. Subjects should be monitored for signs and symptoms of pneumonitis. Suspected pneumonitis should be evaluated with radiographic imaging. In subjects with grade 2 or higher pneumonitis, administer corticosteroids, prednisone 1–2 mg/kg (kilogram)/day (or equivalent), followed by a taper. Subjects with grade 2 pneumonitis should have atezolizumab withheld until the severity has reduced to grade 1 or resolved and the corticosteroid dose is ≤ prednisone 10 mg/day (or equivalent). Subjects with grade 3 or 4 pneumonitis should permanently discontinue atezolizumab. *Immune-Mediated Hepatitis* Atezolizumab can cause liver tests abnormalities and immune-mediated hepatitis, defined as requiring use of systemic corticosteroids. Fatal cases have been reported. Subjects should be monitored for signs and symptoms of hepatitis during and after discontinuation of atezolizumab, including clinical chemistry monitoring. In subjects with grade 2 or higher elevations of ALT, AST and/or total bilirubin, administer corticosteroids, prednisone 1–2 mg/kg/day (or equivalent), followed by a taper. In subjects with AST or ALT > 3 and up to 8 times the ULN or total bilirubin > 1.5 and up to 3 times the ULN, atezolizumab should be withheld until the severity has reduced to grade 1 or resolved and the corticosteroid dose is ≤ prednisone 10 mg/day (or equivalent). Subjects with AST or ALT > 8 times ULN or total bilirubin > 3 times ULN should permanently discontinue atezolizumab. *Immune-Mediated Colitis* Atezolizumab can cause immune-mediated colitis or diarrhea, defined as requiring use of systemic corticosteroids. Subjects should be monitored for signs and symptoms of diarrhea or colitis. Atezolizumab should be withheld

	for grade 2 or 3 diarrhea or colitis. If symptoms persist for longer than 5 days or recur, administer corticosteroids, prednisone 1–2 mg/kg/day (or equivalent), followed by a taper. If the event does not improve within 48 h after initiating corticosteroids, consider adding an immunosuppressive agent. In subjects with grade 2 or 3 colitis or diarrhea, atezolizumab should be withheld until the severity has reduced to grade 1 or resolved and the corticosteroid dose is ≤ prednisone 10 mg/day (or equivalent). Subjects with grade 4 colitis or diarrhea should permanently discontinue atezolizumab. *Immune-Mediated Endocrinopathies* Atezolizumab can cause immune-mediated endocrinopathies, including thyroid disorders, adrenal insufficiency and type I diabetes mellitus, including diabetic ketoacidosis and hypophysitis/hypopituitarism. Thyroid function should be monitored prior to and periodically during treatment with atezolizumab. Hormone replacement therapy or medical management of hyperthyroidism should be initiated as clinically indicated. In the event of asymptomatic hypothyroidism, atezolizumab treatment can continue. Subjects should also be monitored for clinical signs and symptoms of adrenal insufficiency. For grade 2 or higher adrenal insufficiency, initiate prednisone 1–2 mg/kg/day (or equivalent), followed by a taper and hormone replacement as clinically indicated. Subjects should also be monitored for hyperglycemia or other signs and symptoms of diabetes. Treatment with insulin should be initiated as clinically indicated. Atezolizumab should be interrupted based on severity. In subjects with grade 2 or higher hypophysitis, initiate prednisone 1–2 mg/kg/day (or equivalent), followed by a taper and hormone replacement as clinically indicated. Atezolizumab should be interrupted based on severity. In subjects with grade 2, 3 or 4 endocrinopathies (including but not limited to hypophysitis, adrenal insufficiency, hyperthyroidism and type I diabetes mellitus) atezolizumab should be withheld until the severity has reduced to grade 1 or resolved and the subject is clinically stable on hormone replacement therapy. *Severe Cutaneous Adverse Reactions*

	Severe Cutaneous Adverse Reactions (SCARs) are an identified risk for patients receiving atezolizumab. SCARs are a heterogeneous group of immunologically mediated drug eruptions. Although rare, these events are potentially fatal, and mainly constituted by erythema multiforme, acute generalized exanthematous pustulosis, Stevens-Johnson syndrome (SJS), Toxic Epidermal Necrolysis (TEN) and drug rash with eosinophilia and systemic symptoms (DRESS). For suspected SCARs the patients should be referred to a dermatologist for further diagnosis and management. Atezolizumab should be withheld for patients with suspected SJS or TEN and atezolizumab should be permanently withdrawn for any grade confirmed SJS or TEN. *Immune-Mediated Pericardial Disorders* Atezolizumab can cause immune-mediated pericardial disorders including pericarditis, pericardial effusion and cardiac tamponade. Fatal cases have been reported. Immune-mediated pericarditis should be considered in all patients presenting with chest pain. Immune-mediated pericardial effusion and cardiac tamponade should be considered in all patients presenting with chest pain associated with dyspnea or hemodynamic instability. Cardiac tamponade should be treated as a medical emergency and consultation with a cardiologist should be sought for further management. In subjects with pericardial disorders of any grade, initiate corticosteroid treatment equivalent to methylprednisolone 1-2 mg/kg/day IV and convert to 1-2 mg/kg/day oral prednisone (or equivalent) upon improvement. Consider adding an immunosuppressive agent if the event does not improve within 48 hours. Subjects with suspected pericardial disorders should have atezolizumab withheld. Subjects with pericardial disorders of any grade should permanently discontinue atezolizumab. *Immune-Mediated Neuropathy Including Facial Paresis* Immune-mediated facial paresis is considered a rare event (< 0.1%) in patients receiving atezolizumab monotherapy. Patients should be monitored for symptoms of motor and sensory neuropathy. No fatal events have been

	reported in the monotherapy population (n = 3178) or observed across the atezolizumab clinical development program. Subjects with grade 1 immune-mediated neuropathy may continue atezolizumab treatment. Any cranial nerve disorder including facial paresis should be managed according to the guidelines for grade 2 immune-mediated neuropathy. In these subjects, atezolizumab should be withheld, and treatment should be initiated according to institutional guidelines. For general immune-mediated neuropathy, if the event resolves to grade 1 or better, atezolizumab may be resumed. For subjects with facial paresis, atezolizumab treatment may be resumed only if the event resolves fully. Subjects with grade 3 or 4 immune-mediated neuropathy including facial paresis should permanently discontinue treatment with atezolizumab. *Immune-Mediated Myelitis* Immune-mediated myelitis is considered a rare event (< 0.1%) in patients receiving atezolizumab monotherapy. No fatal events have been reported in the monotherapy population (n = 3178) or observed across the atezolizumab clinical development program. Subjects with grade 1 immune-mediated myelitis may continue atezolizumab unless symptoms worsen or do not improve. The subject must be referred to a neurologist. Subjects with grade 2, 3 or 4 immune-mediated myelitis should permanently discontinue atezolizumab. The subject must be referred to a neurologist and initiate prednisone 1–2 mg/kg/day (or equivalent) or according to institutional guidelines. *Other Immune-Mediated Adverse Reactions* Atezolizumab can cause severe and fatal immune-mediated adverse reactions. These reactions may involve any organ system and usually manifest during treatment but can also appear after treatment discontinuation. In subjects with suspected grade 2 immune-mediated adverse reactions, other causes should be excluded, and corticosteroids initiated as clinically indicated. In subjects with severe (grade 3 or 4) adverse reactions, administer corticosteroids, prednisone 1–2 mg/kg/day (or equivalent), followed by a taper. In subjects with grade 3 adverse reactions, atezolizumab should be withheld until the severity has reduced to grade 1 or resolved and the corticosteroid dose is ≤ prednisone 10 mg/day (or equivalent).

	Subjects with grade 4 adverse reactions should permanently discontinue atezolizumab. *Infusion-Related Reactions* Atezolizumab can cause severe or life-threatening IRRs. Subjects should be monitored for signs and symptoms of IRRs. In subjects with grade 1 or 2 IRRs, either interrupt or slow the rate of atezolizumab infusion. Subjects with grade 3 or 4 IRRs should permanently discontinue atezolizumab. **Bevacizumab Adverse Reaction Management:** There are no recommended dose reductions for bevacizumab. Bevacizumab should be discontinued in the event of: • Gastrointestinal perforations (including fistula formation in the gastrointestinal tract and intra-abdominal abscess formation) or fistula formation involving an internal organ • Wound dehiscence and wound healing complications requiring medical intervention • Serious hemorrhage (i.e., requiring medical intervention) • Severe arterial thromboembolic events • Hypertensive crisis or hypertensive encephalopathy • Reversible posterior leukoencephalopathy syndrome (RPLS) • Nephrotic syndrome Avastin treatment should be held in the event of: • Severe hypertension not controlled with medical management • Moderate to severe proteinuria pending further evaluation • Severe infusion reactions **LONSURF® Adverse Reaction Management:** The most common adverse reactions or laboratory abnormalities for LONSURF® in combination with bevacizumab (≥ 20%) are neutropenia, anemia, thrombocytopenia, fatigue, nausea, increased liver transaminases, increased alkaline phosphatase, decreased sodium, diarrhea, abdominal pain and decreased appetite. Due to the potential for hematological toxicities, complete blood cell counts will be obtained pre-dose and on Day 15 of each treatment cycle. LONSURF® treatment will not be initiated until:

	• Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/mL or febrile neutropenia is resolved. • Platelet count ≥ 75 x 10^9/mL • Grade 3 or 4 non-hematological adverse reactions are resolved to grade 0 or 1. Once a treatment cycle has started, LONSURF® treatment will be held for any of the following: • ANC < 0.5 x 10^9/mL or febrile neutropenia • Platelet count < 50 x 10^9/mL • Grade 3 or 4 non-hematological adverse reaction After recovery, resume LONSURF® after reducing the dose by 5 mg/m^2/dose from the previous dose, if any of the following occurred: • Febrile neutropenia • Uncomplicated grade 4 neutropenia (which has recovered to ≥ 1.5 x 10^9/mL) or thrombocytopenia (which has recovered to ≥ 75 x 10^9/mL) that results in more than 1 week delay in start of cycle. • Non-hematological grade 3 or 4 adverse reaction except for grade 3 nausea and/or vomiting controlled by anti-emetic therapy or grade 3 diarrhea responsive to anti-diarrheal medication. A maximum of 3 dose reductions are permitted. Permanently discontinue LONSURF® in patients who are unable to tolerate a dose of 20 mg/m^2 orally twice daily. The LONSURF® dose must not be escalated after it has been reduced. If deemed appropriate by the Investigator, subjects may hold or discontinue one or more components of the treatment regimen (or in the case of LONSURF®, have dose reduction(s)) and may continue treatment with the other agent(s). Subjects will only be considered off treatment when all protocol-specified anti-cancer therapies have been discontinued.
Study Endpoints	**Efficacy:** The proportion of patients who achieve progression-free survival (PFS) at 6 months will be the primary efficacy endpoint for the study. Secondary efficacy measures will include median PFS, objective response rate (ORR; determined using RECIST version 1.1), duration of objective response (DOR), disease control rate (DCR) and median overall survival (OS).

	Safety: Safety and tolerability will be co-primary endpoints of this study and will be evaluated on the basis of the following parameters: • General safety: Vital signs (oral temperature, blood pressure, pulse rate, respiratory rate and O_2 saturation), 12-lead ECG and physical examination • Toxicity according to the latest version of NCI-CTCAE for AEs and laboratory safety tests • Systemic complement levels following DSP107 administration (in the event of IRRs) **Pharmacokinetics:** DSP107 PK parameters will be secondary endpoints of this study. Sparse serum samples to measure circulating levels of DSP107 may be pooled with data from other studies using an established population PK model to derive PK parameters such as clearance, volume of distribution and AUC, as warranted by the data and to compare exposure in this study with that observed in previous studies. **Pharmacodynamics:** Pharmacodynamic (PD) markers will be secondary endpoints in this study. Evaluation of the PD effect of the study treatments will include: • Measurement of CEA • Measurement of ctDNA • Evaluation of paired, fresh tumor biopsies collected from at least 25 patients at pre-treatment (within 28 days prior to the first dose of DSP107) and on-treatment (after approximately 6-8 weeks of treatment) provided collection of samples is deemed safe and accessible by the Investigator. The biopsy material will include at least 2 and no more than 4 cores. Tumor tissue will be analyzed for changes in the TME, markers of immune cell activation, PD-L1 and CD47 expression and other biomarkers to facilitate retrospective correlation with clinical response. Archival biopsies may be used to determine baseline expression if fresh biopsies are not collected. • Evaluation of DSP107 ADA formation and correlation with efficacy, PK, PD and safety outcomes

	• Evaluation of correlative samples and correlation with efficacy, PK, PD and safety outcomes. **Exploratory Endpoints:** Blood and tumor biopsy samples (where available) will be collected for exploratory research to study protein and RNA biomarkers and/or circulating tumor DNA (ctDNA) that may be associated with PK, safety and efficacy.

EXAMPLE 2

FGFR3 AND VEGF-C LEVELS ARE UPREGULATED FOLLOWING TREATMENT WITH A SIRPα-4-1BBL FUSION PROTEIN

Materials and Methods:

Sample Preparation – MSS CRC patients were treated with DSP107 monotherapy (10 mg/kg) or DSP107 (10 mg/kg) combined with Atezolizumab (1200 mg) (treatment protocol is described in details in ClinicalTrials(dot)gov ID NCT04440735, A Study of DSP107 Alone and in Combination With Atezolizumab for Patients With Advanced Solid Tumors, www(dot)clinicaltrials(dot)gov/study/NCT04440735. Plasma samples were collected from the patients at baseline (i.e., pre-treatment) and compared with samples collected at week 12, four hours after treatment with DSP107 (-/+ Atezolizumab). Following, the samples were depleted of highly abundant proteins of plasma by using HighSelect™ Top14 abundant protein depletion resin. After depletion, samples were reduced and alkylated using Biognosys' Reduction and Alkylation Solution. Samples were digested overnight with sequencing grade trypsin (Promega) at a protein:protease ratio of 100:1 using the KingFisher Flex instrument. C18 clean-up for mass spectrometry was carried out using Oasis HLB µElution Plate 30µm plate (WATERS) according to the manufacturer's instructions. Peptides were dried down to complete dryness using a SpeedVac system and dissolved in LC solvent A (1 % acetonitrile in water with 0.1 % formic acid (FA)) containing Biognosys' iRT-peptide mix for retention time calibration. Peptide concentrations in mass spectrometry-ready samples were measured using the mBCA assay (Thermo Scientific™ Pierce™). Samples were transferred in MS Vials and analyzed.

HRM Mass Spectrometry - For DIA LC-MS/MS measurements, 2.0 µg of peptides per sample were injected on an in-house packed reversed phase column on a Thermo Scientific™ NeoVanquish UHPLC nano-liquid chromatography system connected to a Thermo Scientific™ Orbitrap™ Exploris 480™ mass spectrometer equipped with a Nanospray Flex™ ion source and a FAIMS Pro™ ion mobility device. LC solvents were A: water with 0.1 % FA; B: 80 % acetonitrile, 0.1 % FA in water. The nonlinear LC gradient was 1 – 50 % solvent B in 172 minutes followed

by a column washing step in 90 % B for 5 minutes, and a final equilibration step of 1 % B for 1 column volume with a flow rate set to a ramp between 500 to 250 nl / min (min 0: 500 nL / min, min 172: 250 nL / min, washing at 500 nL / min). The FAIMS-DIA method consisted per applied compensation voltage of one full range MS1 scan and 34 DIA segments as adopted from Bruderer et al. & Tognetti et al.

Database Search of DIA LC-MS/MS Data - DIA mass spectrometric data were analyzed using directDIA+ in Spectronaut software (version 18.5) using the default settings, including a 1 % false discovery rate control at PSM, peptide and protein level, allowing for 2 missed cleavages and variable modifications (N-term acetylation, deamidation, ammonia-loss and methionine and proline oxidation). The human UniProt.fasta database (Homo sapiens, 2023-07-01) was used and for the library generation, the default settings were used.

HRM Data Analysis - HRM mass spectrometric data were analyzed using Spectronaut software (version 18.5). The false discovery rate on peptide and protein level was set to 1 %, data was filtered using row-based extraction. The sample-specific library generated in this project was used for the analysis. The HRM measurements analyzed with Spectronaut were normalized using local normalization.

Data Analysis - For testing of differential protein abundance, protein intensities for each protein were analyzed using a two-sample Student's t-test. The following thresholds were applied for candidate identification: p-value < 0.05; absolute average log2 ratio > 0.58 (fold-change > 1.5). Distance in heat maps was calculated using the "manhattan" method, the clustering using "ward.D" for both axes. Principal component analysis was conducted in R using *prcomp* and a modified *ggbiplot* function for plotting, and partial least squares discriminant analysis was performed using *mixOMICS* package. Functional analysis was performed using String-db (string-db.org, version 11.5) [C]. Gene Ontology (GO) enrichment analysis was performed in Spectronaut software (Biognosys) using a human gene associations file obtained from EBI (2016-10-03). Only terms with a minimum of 2 members were considered. Topology of candidate proteins was visualized using Protter [D]. General plotting was done in R using *ggplot2* package.

Results:

An increase in FGFR3 and VEGF-C protein levels was detected in plasma samples of CRC patients following treatment with DSP107. Specifically, as shown in Table 2, an increase of between 5 to 3000-fold was detected in patients receiving 10 mg/kg DSP107 alone or in combination with Atezolizumab. In addition, as shown in Table 3 and Figure 1, an increase of

between 1.3 to 21-fold was detected in patients receiving 1-10 mg/kg DSP107 in combination with Atezolizumab.

Table 2: FGFR3 protein expression

	Monotherapy 10mg/kg		DSP107+Atezo 10mg/kg		
Patient ID	11-011	11-009	13-019	13-018	11-019
Best response*	SD 20W	PD 12W	SD 27W (-16%)	PR 75W (-86%)	PR 90W (-80%)
FGFR3**	5.78	3624.69	28.05	287.43	1.11

*Best response -determined by CT scan assessing the target lesions and following RECIST V1.1 criteria (PD=progressive disease, SD = stable disease, PR=partial response). The number next to the PD/SD/PR represents how many weeks (W)the patient stayed in this status. The number in brackets represents the precent by which the target lesion shrank.
**fold change compared to baseline.

Table 3: VEGF-C protein expression

Sample	Cohort Group	Best response*	Weeks*	Reduction in Target lesions	tumor site	VEGF-C**	
13-012	1 mg/kg combo	PD	12		Sigmoid colon	stable	x0.8
11-015	1 mg/kg combo	PD	12		Rectal	stable	x1.3
11-013	1 mg/kg combo	SD	45		Epithelioid sarcoma	stable	x0.9
13-010	1 mg/kg combo	PD	12		Rectal	increase	x21
12-007	3 mg/kg combo	SD	21		Rectal	increase	x3.6
13-013	3 mg/kg combo	SD	>95	-4%	Parotid gland	stable	x1.3
13-018	10 mg/kg combo	PR	73	-86%	Colon	increase	x3.3
11-021	10 mg/kg combo	SD	>89		Adernal	increase	x2.2
11-019	10 mg/kg combo	PR	>85	-80%	Colon	increase	x2.7
13-019	10 mg/kg combo	SD	27	-16%	Rectal	stable	x0.85

*Best response -determined by CT scan assessing the target lesions and following RECIST V1.1 criteria (PD=progressive disease, SD = stable disease, PR=partial response). The number in the Weeks column represents how long the patient stayed in this status.
**fold change compared to baseline.

Taken together, the results indicate an increase in plasma levels of FGFR3 and VEGF-C (which may indicate e.g., increased expression, increased secretion and/or reduced degradation)

following treatment with DSP107. Hence, specific embodiments suggest combining DSP107 treatment with an agent(s) inhibiting any of these signaling pathways.

EXAMPLE 3

EXPRESSION OF DIFFERENT TARGETS ON CANCER CELL LINES

In vitro human cell line models have been widely used for cancer pharmacogenomic studies to predict clinical response, to help generate pharmacogenomic hypothesis for further testing, and to help identify novel mechanisms associated with variation in drug response. To mimic the tumor cells' conditions in the tumor microenvironment (TME), different treatments are employed. Hypoxia, for example, promotes malignant behavior of cancer cells, including proliferation, migration, infestation and epithelial-mesenchymal transition (EMT), and enhances immunotherapy, chemotherapy, and radiotherapy tolerance (Chen, Z. et al. (2023) Sig Transduct Target Ther 8, 70).

The PD-1/PD-L1 checkpoint is a central mediator of immunosuppression in the TME and is primarily associated with IFNγ signaling.

In this Example, the expression of the relevant ligands for DSP107, anti-PD-L1 antibody and anti-VEGF antibody, on colon cancer cell lines was evaluated with and without Deferoxamine (DFO; a hypoxia-mimetic agent) and IFNγ treatments, as follows:

Materials:

CD47 antibody (Cat#323124, BioLegend), VEGFR2 antibody (mIgG1,Cat# 393006, BioLegend), Isotype control antibody for CD47 and VEGFR2 (mIgG1 Cat# 400122, BioLegend), PD-L1 antibody (Cat# 329708, BioLegend), Isotype control antibody for PDL-1 (mIgG2b Cat#400320, BioLegend) and; DLD-1 overexpressing PD-L1 (DLD-1 from ATCC # CCL-221), HT-29 (ATCC # HTB-38) and COLO-205 (ATCC # CCL-222) colon cancer cell lines, A549 (ATCC # CCL-185) Lung Carcinoma cell line, SK-OV-3 (ATCC # HTB-77) ovary adenocarcinoma cell line, DFO (Cat#D9533 Merk); IFN-ɣ (Cat#285-IF-100/CF, R&D), RPMI 1640 (Cat#01-100-1A, Sartorius), FBS (Cat# 10270-106, Gibco), Glutamax (Cat# 35050-038, Gibco), Pen/Strep (Cat# 15140-122, Gibco), PBS (Cat#02-023-1A, Sartotius), TrypLE (Cat# 12604-013, Gibco).

Methods:

Cells were grown in RPMI supplemented with 10 % FBS, Glutamax and Pen/Strep and left untreated or treated overnight with 100 μM DFO or 20 ng / ml IFNγ at 37 ^{0}C, 5 % CO_2. Membrane expression of CD47, PD-L1 or VEGFR2 was evaluated by flow cytometry using allophycocyanin (APC)-conjugated anti-CD47, PDL-1 or anti-VEGFR2 antibodies, respectively, and the

corresponding isotype controls. Cells were incubated with the antibodies for 30 minutes at 4 °C. Following incubation, cells were washed and analyzed by flow cytometry.

Results:

High membrane expression of CD47 was observed on the colon cancer cell lines and a slight increase in CD47 membranal expression was observed after treatment with IFNγ and DFO in all three cell lines (Figure 2A).

VEGFR2 was also expressed on all cell lines and was slightly upregulated on all 3 cell lines following DFO treatment (Figure 2B).

PD-L1 was expressed only on DLD-1 PD-L1 overexpressing cells but not on HT-29 and COLO-205 cell lines and was upregulated on these cell lines following DFO and IFNγ treatments (Figure 2C). PD-L1 was shown to be upregulated also on A549 Lung Carcinoma cell line and SK-OV-3 ovarian Adenocarcinoma cell line following treatment with IFNγ (Figures 2D-E).

EXAMPLE 4

ACTIVATION OF PBMCS AND T-CELLS FOLLOWING TREATMENT WITH SIRPα-4-1BBL FUSION PROTEIN, ANTI-VEGF AND ANTI-PDL-1

The activation of a T cell requires two signals: the first signal derives from the ligation of the T-Cell Receptor (TCR) with the Major Histocompatibility Complex (MHC)/peptide complex on the APC. The second signal is generated by cognate interactions through adhesion molecules of T cells and antigen-presenting cells and/or by cytokines produced by antigen-presenting cells. The delivery of the two signals results in gene transcription, cytokine secretion, expression of new cell surface molecules including cytokine receptors, and cellular proliferation.

Numerous methods are known in the art to determine activation of T cells, including but not limited to:

- Expression of activation markers on the surface of the T cells (for example: CD25, CD69, CD62L, CD137, CD107a, PD-1). Can be determined by e.g., staining the cells with specific antibodies and flow cytometry (FACS) analysis.

- Secretion of inflammatory cytokines (for example: IL2, IL6, IL8, INF gamma etc.). Can be determined by e.g., ELISA or Cytometric Bead Array (CBA) in FACS.

- Proliferation of T cells, measured by pre-staining of T cells with CFSE (carboxyfluorescein succinimidyl ester) or CPD (cell proliferation dye) and deviation of T-cells by CFSE/CPD dilution that is determined by FACS.

- Killing of a target cell e.g., cancer cells that is measured by pre-labeling the cancer cells using e.g., Calcine-AM reagent and measuring Calcine release into the culture medium using

luminescence plate reader or by XTT Cell Proliferation Assay, or by viability dye using flow cytometry.

Expression of activation markers

The effect of the combination of DSP107 with VEGF inhibitor and/or PDL-1 inhibitor on expression of T cells activation markers is evaluated by isolating human PBMCs from peripheral blood of healthy donors using standard Ficoll-Paque method (Grienvic et al. 2016, *Biopreserv Biobank*. 14(5):410-415), according to manufacture instructions. T cells are further separated from the PBMCs using CD3 negative selection magnetic beads. 96-wells plates are pre-coated with anti-human CD3 antibody (0.5 µg/mL), by incubation for 3 hours at 37°C. PBMCs from healthy donors or cancer patients are cultured in the anti-CD3 pre-coated plates with different concentrations of DSP107 alone or in combination with avelumab and/or anti-VEGF-A for 24-48 hours at 37 °C, 5 % CO2. Following incubation, gated CD3+ T cells are analyzed by flow cytometry for expression of the activation markers PD-1, CD25 and 4-1BB.

Proliferation

The effect of the combination of DSP107 with a VEGF inhibitor and/or a PDL-1 inhibitor, on PBMCs or purified T cells proliferation is evaluated by isolating human PBMCs from healthy donor peripheral blood using Ficoll-Paque method (Grienvic et al. 2016, *Biopreserv Biobank*. 14(5):410-415), according to manufacture instructions. The T-cells are further separated from the PBMCs by CD3 negative selection magnetic beads. Following isolation, PBMCs or T cells are labeled by CPD and the labeled cells are cultured for 3-5 days on a CD47 coated plate with addition of different concentrations of the various compounds, in the presence of sub-optimal concentrations of anti-CD3 (0.5 µg / ml) and anti CD28 (2 µg / ml) or CD3/CD28 Dynabeads (1:10 Bead: PBMCs/T-cells), and in the presence of IL-2 (20 IU/mL). Proliferation of PBMCs/T cells is determined by FACS according to the CPD level. In addition, cells are tested for 4-1BB, PD-1, CD25 and VEGFR2 surface expression at day 0 and days 3 or 5 of the experiment by flow cytometry.

Killing of target cancer cells

Materials:

DSP107 (SEQ ID NO: 1); PD-L1 antibody (Avelumab, Cat#SIM002, InVivoSIM; Atezolizumab, Cat#A2004, Selleckchem) and Bevacizumab, VEGF antibody (Cat#A20006, Selleckchem); Ficoll-Paque (Cat# 17-1440-03, GE Healthcare), X-VIVO Serum Free Media (Cat#04-448Q, Lonza), RPMI 1640 (Cat# 01-100-1A, Sartorius), FBS (Cat# 10270-106, Gibco), Glutamax (Cat# 35050-038, Gibco), Pen/ Strep (Cat# 15140-122, Gibco), TrypLE (Cat# 12604-013, Gibco), PBS (Cat#02-023-1A, Sartotius), T-cell isolation kit (Cat# 19661, Stem Cells);

CD3/CD28 dynabeads (Cat#11131D, Thermo Fischer); anti-CD3 (Cat#317315, BioLegend), soluble anti-CD28 (Cat#302978, BioLegend), 7-AAD (Cat# 00-6993-50, eBioScience), CPD (Cat# 65-0842-85, ThermoFischer), Fixable viability dye 450 (Cat# 562247, BD), CellTrace™ CFSE Cell Proliferation Dye (Cat#C34554, Thermo), CD47 antibody (Cat# 323124, BioLegend), 4-1BB antibody (mIgG1, Cat#309810, BioLegend), VEGFR2 antibody (mIgG1,Cat# 393006, BioLegend), Isotype control antibody for CD47, 4-1BB and VEGFR2 (mIgG1 Cat# 400122, BioLegend), PD-L1 antibody (Cat# 329708, BioLegend), Isotype control antibody for PD-L1 (mIgG2b Cat#400320, BioLegend) and, CD25 antibody (mIgG1, Cat# 302634, BioLegend), Isotype control antibody for CD25 (mIgG1, 400164, BioLegend), CD3 antibody (317330, BioLegend), Human TruStain (Cat#422302, BioLegend).

Methods:

T-cells mediated cytotoxicity: Human T-cells are isolated from peripheral blood samples of healthy donors or cancer patient donors using Ficoll gradient, followed by CD3 negative selection magnetic beads. Isolated T-cells, inactivated or activated with a sub-optimal concentration of anti-CD3/CD28 dynabeads (1:10 beads per cells) or by plate bound anti-CD3 (0.5 μg/mL) and soluble anti-CD28 (2 μg/mL), are co-cultured with the different colon cancer cell lines, stained with CPD at different Effector to Tumor (E:T) ratios in the presence of different concentrations and combinations of the tested compounds. Live CFSE/CPD labeled cancer cells and T-cells activation markers are analyzed by flow cytometry following detachment with cell dissociation buffer.

PBMCs mediated cytotoxicity: PBMCs were isolated from peripheral blood samples of healthy donors. Isolated PBMCs were partially activated with CD3/CD28 Dynabeads (bead: cell of 1:10), and co-cultured with CFSE labeled DLD-1 overexpressing PD-L1 cells in 8:1 E:T ratio. DLD-1 PD-L1 cells were labelled with CFSE according to the manufacturer protocol, then cells were seeded in flat 96 wells plates in serum free medium. Following a 1-hour incubation at 37 ^{0}C, different treatment combinations were added and following an additional 0.5-hour incubation at 37 ^{0}C, hPBMCs were added. Following 24 hours of co-culture, cells were detached, stained with viability dye and analyzed by flow cytometry for viability. Expression of 4-1BB and CD25 were tested on non-activated as well as activated PBMCs. CD47, PDL-1 and VEGFR2 expression was tested on the cancer cells. Cytotoxicity results are presented as the percentage of dead cancer cells from total cancer cell number, and significance was analyzed using a T-test, and p-values are presented to indicate the statistical significance of the observed differences as follows: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

Results:

PBMCs demonstrated higher percentages of cells expressing 4-1BB, CD25 and PD-L1 following activation (Figure 3). The percentages of the dead cancer cells are presented in Figure 4, using PBMCs derived from two independent donors. Co-culturing DLD-1 PD-L1 OX cells with human PBMCs, increased the percentages of dead cancer cells, compared to cancer cells cultured alone.

The use of DSP107 in conjunction with both anti-VEGF (Bevacizumab) and anti-PDL-1 (Avelumab) therapies demonstrated greater efficacy on cancer cell cytotoxicity than using any of these treatments alone or in pairs, as illustrated in Figures 4A-B.

EXAMPLE 5

THE EFFECT OF THE SIRPα-4-1BBL FUSION PROTEIN AND ANTI-PDL-1 ON MACROPHAGES

Materials:

DSP107 (SEQ ID NO: 1); anti-PD-L1 IgG1 (Avelumab, Cat#SIM002, InVivoSIM), anti PD-L1- A-glycosylated IgG1 (Atezolizumab; Cat#A2004, Selleckchem), Human cancer cell line DLD-1 (colon carcinoma) overexpressing PD-L1; Ficoll-Paque (Cat# 17-1440-03, GE Healthcare), 1X RBC Lysis Buffer (Cat# 00-4333-57, eBioscience™) RPMI 1640 (Cat# 01-100-1A, Biological industries), FBS (Cat# 12657-029, Gibco), Glutamax (Cat# 35050-038, Gibco), Pen/Strep (Cat# 15140-122, Gibco); human M-CSF (Cat# 216-MC, R&D systems); human IL-10 (Cat# 217-1L, R&D systems), CellTrace™ CFSE Cell Proliferation Dye (Cat#C34554, Thermo), Human TruStain FcX (Cat# 422302, BioLegend), CD11b antibody (Cat# 301346 BioLegend), CD47 antibody (Cat# 323124, BioLegend), Isotype control antibody for CD47 (mIgG1 Cat# 400122, BioLegend) PD-L1 antibody (Cat# 329708 BioLegend), Isotype control antibody for PD-L1 (mIgG2b Cat#400320, BioLegend).

Methods:

PBMCs were isolated from blood samples of healthy donors by using Ficoll gradient, followed by lysis of erythrocytes. Monocytes were differentiated into macrophages (M0) by culturing in RPMI 1640 culture medium supplemented with 10 % FBS and 50 ng / ml M-CSF for 6 days. For polarization into M2c macrophages (M2), M0 cells were incubated with 50 ng / mL IL-10 for 24 hours. DLD1 PD-L1 cells were labelled with CFSE dye and were pre-incubated with 5 µg / ml DSP107, 1 µg/ml Atezolizumab or 0.25 µg/ml Avelumab, or in combination of DSP107 and PD-L1 antibody, for 30 minutes at 37 ^{0}C. Following pre-incubation, DLD-1 PD-L1 cells were

mixed with M2c macrophages at effector-to-target ratio of 1 : 2. Mixed cultures were incubated in the presence of the respective compounds for 3 hours at 37 ^{0}C. Following incubation, cells were washed and stained with CD11b antibody as a macrophage marker. Phagocytosis of cancer cells by macrophages was analyzed by flow cytometry. The expression of CD47, and PD-L1 on cancer cells was also analysed by flow cytometry. Phagocytosis results are presented as the percentage of double-positive CD11b+CFSE+ cells (macrophages that engulfed cancer cells). Additionally, the absolute counts of CFSE+ cells that remained unengulfed by macrophages (CD11b-CFSE+) are also presented. The combination of DSP107 with Atezolizumab was studied in 8 independent donors and the combination with Avelumab in 3 independent donors. Statistical significance was analyzed using a T-test, and p-values are presented to indicate the statistical significance of the observed differences as follows: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

Results:

The use of DSP107 in conjunction with anti-PDL-1 (Atezolizumab or Avelumab) therapy demonstrated greater efficacy on macrophages-mediated phagocytosis than using any of these treatments alone, as illustrated in Figures 5A-6B.

EXAMPLE 6

THE EFFECT OF SIRPα-4-1BBL FUSION PROTEIN, ANTI-VEGF AND ANTI-PDL-1 ON MIGRATION AND INVASION OF CANCER CELLS

Methods:

Migration and invasion assays are performed using the Transwell system. For migration assay, medium supplemented with FBS or other chemoattractant is added to the lower well of each chamber, and cancer cells, resuspended in serum-free medium are added to the upper inserts. After 24-48 hours of incubation, cells on the upper well are removed and the cells that have migrated through the filter into the lower wells are fixed, stained and counted using microscopy or quantified by colorimetric analysis.

The invasion assay is performed similarly to the migration assay, except that the membrane is coated with an extracellular matrix (e.g., Matrigel). Treatments, including monotherapy or combination of DSP107, anti VEGF anti PD-L1 at different concentrations are added to the top and/or bottom of the Transwell.

EXAMPLE 7

THE *IN-VIVO* ANTI-TUMOR EFFECT OF SIRPα-4-1BBL FUSION PROTEIN, ANTI-VEGF AND ANTI-PDL-1/PD-1

Two different *in-vivo* mouse models are used:

1. NSG mice are inoculated with human stem cells or human PBMCs and human tumor cells.
2. C57BL/6 – human-4-1BB, human-VEGF and human-PD-1 knock-in mice inoculated with human colon cancer cell line (e.g., MC38) overexpressing the human-CD47, human-VEGFR2 and humanPD-L1.

In the two models, mice are inoculated with tumor cells intravenously (IV), intraperitoneally (IP), subcutaneously (SC) or orthotopically. Once the tumor is palpable (~80 mm^3), for SC tumors or visualized by In Vivo Imaging Systems (IVIS) for IP or orthotopic tumor models, mice are treated IV, IP, SC or orthotopically, with different doses and different regimens of the DSP107, the anti PD(L)1 and the anti-VEGF antibodies.

Mice are followed for weights and clinical signs. SC tumors are measured few times a week by micro caliper; and tumor volume is calculated according to the following equation: V = length X $width^2$ / 2. Mice Weight is measured routinely. Mice with orthotopic or peritoneal tumors are followed using cell lines expressing luciferase. Tumor growth is measured for average radiance level after administered an intraperitoneal D-luciferin 10 min prior to each in vivo system (IVIS) spectrum imaging. Tumor growth and survival are monitored through the whole experiment.

Infiltration of immune cells into the tumor is tested by resecting the tumor or draining lymph nodes, or the spleen, digestion and immune phenotyping using specific antibodies staining and flow cytometry analysis. Additionally, or alternatively, infiltration of immune cells or necrotic grade of tumors is determined by resecting the tumors, paraffin embedding and sectioning for immunohistochemistry staining with specific antibodies.

At sacrificing, mice organs and tumors are harvested and embedded into paraffin blocks for H&E and IHC staining.

Blood samples are taken from mice at different time points, according to common procedures, for the following tests: PK analysis, cytokines measurements in plasma, FACS profiling of blood cells sub-populations in circulation, hematology testing, serum chemistry testing, anti-drug-antibody (ADA) analysis and neutralizing antibodies analysis (NAB).

EXAMPLE 8

TREATMENT OF COLORECTAL CANCER WITH A SIRPα-4-1BBL FUSION PROTEIN, ATEZOLIZUMAB, AND BEVACIZUMAB

Clinical Protocol – A SIRPα-4-1BBL fusion protein having an amino acid sequence as set forth in SEQ ID NO: 1 (herein referred to as "DSP107") is administered to colorectal cancer

patients in combination with the anti-PD-L1 antibody Atezolizumab and the anti-VEGF antibody Bevacizumab, according to the protocol synopsis shown in Table 4 hereinbelow.

Table 4: Protocol Synopsis

Study Title	A Randomized, Open-Label, Phase II Study to Compare the Efficacy of DSP107, Atezolizumab and Bevacizumab versus Trifluridine/Tipiracil and Bevacizumab in Patients with Advanced Microsatellite Stable Colorectal Cancer
Protocol No.	DSP107_003
Clinical Sites	Approx 10 sites in US ± EU Additional sites may be added in these territories or elsewhere in the event of slow enrolment.
Study Phase	II
Planned Sample Size	Up to 118 patients will be enrolled
Therapeutic Indication	Patients ≥ 18 years of age with microsatellite stable (MSS) and/or mismatch repair proficient (pMMR) colorectal cancer (CRC) who have progressed following up to three previous lines of therapy.
Study Objectives	**Primary** • Determine the recommended dose (RD) of DSP107 in combination with atezolizumab and bevacizumab in patients with microsatellite stable colorectal cancer • Determine overall survival (OS) in patients with microsatellite stable colorectal cancer treated with the combination of DSP107, atezolizumab and bevacizumab compared to patients treated with trifluridine/tipiracil and bevacizumab. **Secondary** • Compare the safety and tolerability of the combination of DSP107, atezolizumab and bevacizumab versus trifluridine/tipiracil and bevacizumab as assessed by the current version of the Common Terminology Criteria for Adverse Events (CTCAE), dose-limiting toxicities (DLT), vital signs, 12-lead ECG, physical examination and

	systemic cytokine and complement levels (in the event of infusion related reactions [IRRs]). • Compare efficacy of the combination of DSP107, atezolizumab and bevacizumab versus trifluridine/tipiracil and bevacizumab including 12-month overall survival rate (12M OS), progression-free survival (PFS), objective response rate (ORR; determined by RECIST v1.1), disease control rate (DCR) and duration of response (DoR). • Evaluate efficacy of the combination of DSP107, atezolizumab and bevacizumab by iRECIST including ORR, DoR, PFS and DCR. • Evaluate the pharmacokinetics (PK) of DSP107 when administered in combination with atezolizumab and bevacizumab • Evaluate DSP107 immunogenicity by measurement of anti-drug antibodies (ADA). • Evaluate the PK of atezolizumab when administered in combination with DSP107 and bevacizumab (Stage 1 only). • Evaluate atezolizumab immunogenicity by measurement of ADA (Stage 1 only) • Evaluate the effect of treatment on quality of life (QoL) using the Functional Assessment of Cancer Therapy – Colorectal (FACT-C; Stage 2 only). **<u>Exploratory</u>** • Compare the pharmacodynamic (PD) effect of the combination of DSP107, atezolizumab and bevacizumab versus trifluridine/tipiracil and bevacizumab • Evaluate predictive biomarkers for association with efficacy, safety and pharmacokinetics • Evaluate exploratory biomarkers for future drug development • Evaluate efficacy in patients with and without liver metastases • Evaluate subsequent response in patients who treat beyond disease progression.
Study Design	This study is designed to compare the efficacy and safety of DSP107 in combination with atezolizumab and bevacizumab versus trifluridine/tipiracil (LONSURF®) and bevacizumab in patients with advanced MSS CRC.

	Specifically, the study will enroll CRC patients that are MSS and/or pMMR who have progressed on or shown to intolerance to (as long as eligible and no contraindication per Investigator) fluoropyrimidine, irinotecan, oxaliplatin, bevacizumab and epidermal growth factor receptor (EGFR) inhibitor treatment. Patients with BRAF (Proto-oncogene B-Raf) V600E, HER2 (Human Epidermal Growth Factor Receptor 2) amplification or overexpression or KRAS (Kirsten Rat Sarcoma Virus) G12C may also have received one line of prior targeted therapy. Prior treatment with LONSURF®, regorafenib or fruquintinib will not be allowed. The dose of DSP107 used in this study is planned to be 10 mg/kg/dose (dose level [DL] 1). The study will include two stages. Stage 1 ("safety run-in") will evaluate the safety of this dose in combination with atezolizumab and bevacizumab in the first 9 patients who are DLT-evaluable. If < 33% of patients experience a first-cycle DLT, the study will proceed to Stage 2 (DSP107 RD = 10 mg/kg/dose). If ≥ 33% of patients experience a first-cycle DLT, N=9 patients will be enrolled at DSP107 DL -1 (5 mg/kg/dose) with standard doses of atezolizumab and bevacizumab. If < 33% of patients experience a first-cycle DLT at DSP107 DL -1, the study will proceed to Stage 2 (DSP107 RD = 5 mg/kg/dose). If ≥ 33% of patients experience a first-cycle DLT, the study will close for futility. In Stage 2, eligible patients will be randomized 1:1 to receive either DSP107, atezolizumab and bevacizumab **or** LONSURF® and bevacizumab in an open-label fashion. Patients will be treated with either the DSP107 RD (from Stage 1) plus standard doses of atezolizumab and bevacizumab **OR** LONSURF® and bevacizumab to achieve at least 45 evaluable patients in each treatment arm. Up to 118 patients evaluable for the primary endpoints will be enrolled in this study (assuming dose de-escalation to DL -1 is required in Stage 1). For patients enrolled to the DSP107, atezolizumab and bevacizumab combination treatment arm: DSP107 will be administered by intravenous (IV) infusion on Days 1, 8, 15 and 22 of each 28-day cycle. The first DSP107 infusion will be administered over 120 (± 15) mins with premedication and IV fluids at the Investigator's discretion, to minimize the occurrence of IRRs. If well tolerated, subsequent

	infusions may be administered over 60 (± 15) mins with premedication and IV fluids at the Investigator's discretion. Atezolizumab will be administered at a dose of 1680 mg IV on Day 1 of every treatment cycle. DSP107 infusion will commence 30 (± 10) mins following completion of atezolizumab infusion. The first atezolizumab infusion should be administered over 60 mins. If well tolerated, subsequent infusions may be performed over 30 mins. Bevacizumab will be administered by IV infusion at a dose of 5 mg/kg/dose on Days 1 and 15 of each 28-day treatment cycle. In patients allocated to treatment with DSP107, atezolizumab and bevacizumab, atezolizumab infusion will commence 30 (± 10) mins following completion of bevacizumab infusion. The first bevacizumab infusion should be administered over 90 mins. If well tolerated, the next infusion can be administered over 60 mins, and if infusion over 60 mins is well tolerated then subsequent infusion can be administered over 30 mins. For patients enrolled to the LONSURF® and bevacizumab combination treatment arm: Bevacizumab will be administered as described above. Patients will receive a LONSURF® dose of 35 mg/m^2/dose up to a maximum of 80 mg/dose (based on trifluridine content). Doses will be administered orally, twice daily with food on Days 1-5 and Days 8-12 of each 28-day treatment cycle. Doses taken by the patient at home will be recorded on a dosing diary. If deemed appropriate by the Investigator, subjects may hold or discontinue one or more components of their allocated treatment regimen (or in the case of LONSURF®, have dose reduction(s)) and may continue treatment with the other agent(s). Subjects will only be considered off treatment when all protocol-specified anti-cancer therapies have been discontinued. The study will include up to 24 treatment cycles until the occurrence of unacceptable toxicity, disease progression, withdrawal of consent or study closure. Subjects still on study at the end of 24 treatment cycles will stop study treatments, enter into the survival follow-up portion of the study, and may continue to receive LONSURF® ± bevacizumab off-study at the discretion of the Investigator.

	Patients may continue treatment beyond progression in certain circumstances. All study endpoints will be evaluated at time of initial progressive disease (PD) evaluation by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. Patients who receive treatment beyond PD will be analysed descriptively. Patients with evidence of PD by RECIST v1.1 will be allowed to continue study treatment beyond progression provided: o The patient is tolerating treatment and is felt to be obtaining clinical benefit from study treatment, at the discretion of the treating physician. o No signs/symptoms of clinical disease progression, at the discretion of the treating physician. o No decline in ECOG performance status attributable to underlying cancer. o No rapid disease progression or disease progression at critical sites (such as spinal cord compression) requiring urgent intervention. o Patient signs a treatment beyond progression ICF. New lesions are considered measurable at the time of initial progression if they meet RECIST v1.1 criteria (i.e., longest diameter ≥ 10 mm, or ≥ 15 mm short axis for a lymph node). If subsequent imaging confirms PD, study treatments will end. Subsequent PD is defined as: o Worsening progression in target disease with an increase of at least 5 mm in the absolute value of the sum o Continued unequivocal progression in non-target disease with an increase in tumor burden. o Increase in size of previously identified new lesion(s) (an increase of at least 5 mm in the absolute value of the sum of those considered to be target new lesions) or additional new lesions. All subjects will be evaluated for efficacy, safety and tolerability. Treatment response assessment will be based on Computed Tomography (CT) scans obtained every 8 weeks (every two treatment cycles).
Study Procedures	Study visits and evaluations will be performed at Screening and at scheduled timepoints following the first and subsequent doses of study drugs. Safety assessments including physical examination, vital sign measurement (pulse, blood pressure, temperature, oxygen (O_2) saturation and respiratory rate), collection of urine for urinalysis, and collection of blood samples for

	hematology, coagulation, blood chemistry and systemic cytokine and complement levels will be performed at scheduled timepoints during the study and at the Final Visit. A single electrocardiogram (ECG) will be collected at the Screening visit and at scheduled timepoints during the study. AEs and concomitant medications will be assessed and recorded throughout the study. PK samples will be collected from patients enrolled to the DSP107, atezolizumab and bevacizumab combination treatment arm prior to the first administration of study drug and at scheduled timepoints throughout the study to measure DSP107 PK levels. Additional samples will be collected from these patients prior to the first administration of study drug and at scheduled timepoints throughout the study to measure DSP107 ADA titers. Blood samples will be collected at scheduled timepoints for PD assessments, including immunophenotyping of immune cells, circulating tumor DNA (ctDNA) and CEA. Paired fresh tumor tissue biopsies will be collected from at least 30 patients (15 patients from each treatment arm; one biopsy collected prior to study drug administration and one while on treatment) provided it is deemed safe and accessible by the Investigator. The pre-treatment tumor biopsy (within 28 days prior to the first dose of DSP107) and on-treatment (after approximately 6-8 weeks of treatment) should be collected, provided the Investigator deems the procedure to be safe. The biopsy material will optimally include 4 cores (at least 2 and no more than 4 cores). Patients who do not agree to undergo this procedure may be enrolled or remain on study provided there are sufficient patients enrolled to ensure paired biopsies are collected from at least 30 patients. Tumor tissue will be analyzed for changes in the tumor microenvironment (TME), markers of immune cell activation, PD-L1 and CD47 expression, and other biomarkers to facilitate retrospective correlation with clinical response. Archival biopsies may be used to determine baseline expression if fresh biopsies are not collected. CT scans must be performed within 28 days prior to the first dose of study drug. Imaging will be repeated every 8 weeks until disease progression or study termination. For patients who complete 12 treatment cycles and continue their allocated treatment beyond 1 year, the frequency of imaging may be reduced to 4 months at the discretion of the Investigator. Alternative imaging

	modalities may be used for these patients if clinically indicated, e.g., MRI and/or PET-CT. Response evaluation will be performed based on RECIST version 1.1. Scans will be read locally at the clinical site; scans may also be read centrally by an independent reader at the discretion of the Sponsor. A safety follow-up visit will be performed 30 (± 7) days after the last dose of study drug. The safety follow-up period may be extended up to 90 days after the last dose of study drug for any Serious Adverse Events (SAEs) considered related to DSP107 and/or atezolizumab and/or bevacizumab and/or LONSURF®. All patients will be followed for up to 5 years after completion of the follow-up period. Sites will contact subjects by telephone at approximately 3-month intervals (± 1 month) after the end of the follow-up period to determine survival. Alternatively, sites may contact next of kin or primary care physician or search public or hospital records to determine survival status.
Study Duration	Patients may continue to receive their allocated treatment combination provided they continue to tolerate the drug(s), have no evidence of disease progression, and they do not meet any of the criteria for subject discontinuation. The study will also end if the Sponsor terminates or suspends the trial or marketing approval is achieved, whichever is earlier. The study will include up to 24 treatment cycles until the occurrence of unacceptable toxicity, disease progression, withdrawal of consent or study closure. Patients still on study at the end of 24 treatment cycles will stop study treatments, enter into the survival follow-up portion of the study, and may continue to receive LONSURF® ± bevacizumab off-study at the discretion of the Investigator. Patients may also continue to receive study treatment(s) beyond progression under certain circumstances (see above). The allocated treatment may also be stopped in the event of a confirmed complete response (CR) in patients who have completed at least 6 treatment cycles on study including at least one treatment cycle beyond the date the initial CR was declared. Patients who subsequently experience radiologic disease progression will be eligible for re-treatment with the previously allocated treatment regimen at the discretion of the Investigator, if no cancer treatment

<table>
<tr><td></td><td>was administered since the last dose of study drug, the patient meets the safety parameters listed in the Inclusion/Exclusion criteria and the trial is still open.
When a patient discontinues all study drug treatments, assessments will be performed at a safety follow-up visit conducted 30 (± 7) days after the last dose of study drug. Patients with Grade 3 or 4 drug-related toxicities at this visit will be followed for up to 90 days after the last dose of study drug, until the toxicities resolve to an acceptable level or until they start another anticancer therapy, whichever occurs earlier.
All patients will be followed for up to 5 years after completion of the follow-up period. Sites will contact patients by telephone at approximately 3-month intervals (± 1 month) after the end of the follow-up period to determine survival.

<table><tr><th>Study Period</th><th>Duration</th></tr><tr><td>Screening period</td><td>28 days</td></tr><tr><td>Treatment Period</td><td>Up to twenty-four, 28-day treatment cycles</td></tr><tr><td>Follow-up safety period</td><td>Up to 90 days from the last dose of study drug</td></tr><tr><td>Survival follow-up</td><td>Up to 5 years</td></tr></table></td></tr>
<tr><td>Inclusion Criteria</td><td>Same as in the protocol provided in Table 1 hereinabove</td></tr>
<tr><td>Exclusion criteria</td><td>Same as in the protocol provided in Table 1 hereinabove</td></tr>
<tr><td>Investigational Product Route and Dosage Form</td><td><u>DSP107</u>
The DSP107 drug product will be supplied by the Sponsor in a single-use vial intended for IV administration. For information on the formulation and handling of DSP107, see the Pharmacy Manual and the DSP107 Investigator's Brochure (IB).
Patients allocated to treatment with DSP107, atezolizumab and bevacizumab will receive IV administration of DSP107 on Days 1, 8, 15 and 22 of each treatment cycle. The dose to be administered will be 10 mg/kg/dose. The first DSP107 dose will be administered over 120 (± 15) mins with premedication</td></tr>
</table>

	and IV fluids at the Investigator's discretion, to minimize the occurrence of IRRs. If well tolerated, subsequent infusions may be administered over 60 (± 15) mins with premedication and IV fluids at the Investigator's discretion. Patients will be administered DSP107 30 (± 10) mins following completion of atezolizumab infusion. **Atezolizumab** The atezolizumab drug product will be supplied by the Sponsor in a single-use, 20 mL United State Pharmacopeia (USP)/Ph. Eur. Type 1 glass vial as a colorless to slightly yellow, sterile, preservative-free, clear liquid solution intended for IV administration. For information on the formulation and handling of atezolizumab, see the Pharmacy Manual and the atezolizumab IB. Patients allocated to treatment with DSP107, atezolizumab and bevacizumab will receive atezolizumab 1680 mg IV on Day 1 of every treatment cycle. Atezolizumab infusion will precede administration of DSP107 and will take place 30 (± 10) mins following completion of bevacizumab infusion. The first atezolizumab infusion should be administered over 60 mins. If well tolerated, subsequent infusions may be performed over 30 mins. **Bevacizumab** The bevacizumab will be supplied by the clinical site. Patients will receive bevacizumab 5 mg/kg on Days 1 and 15 of every treatment cycle. For patients allocated to treatment with DSP107, atezolizumab and bevacizumab, atezolizumab infusion will commence 30 (± 10) mins following completion of bevacizumab infusion. The first bevacizumab infusion should be administered over 90 mins. If the first infusion is tolerated, the next infusion can be administered over 60 mins. If infusion over 60 mins is tolerated, subsequent infusions can be administered over 30 mins. **Trifluridine/Tipiracil** LONSURF® tablets (15 mg and 20 mg formulation) will be supplied by the clinical site. Patients allocated to treatment with LONSURF® and bevacizumab will receive a dose of 35 mg/m^2/dose up to a maximum of 80 mg/dose (based on trifluridine content). Doses will be administered orally, twice daily with food on Days 1-5 and Days 8-12 of each 28-day treatment cycle. Please see 4.2 for recommended dosage according to body surface area (BSA).

<table>
<tr><td></td><td>

Table 4a: Recommended LONSURF® dosage according to body surface area

<table>
<tr><th rowspan="2">BSA (m^2)</th><th rowspan="2">Total daily dose (mg)</th><th rowspan="2">Dose (mg) administered twice daily</th><th colspan="2">Tablets per dose</th></tr>
<tr><th>15 mg</th><th>20 mg</th></tr>
<tr><td>< 1.07</td><td>70</td><td>35</td><td>1</td><td>1</td></tr>
<tr><td>1.07 - 1.22</td><td>80</td><td>40</td><td>0</td><td>2</td></tr>
<tr><td>1.23 - 1.37</td><td>90</td><td>45</td><td>3</td><td>0</td></tr>
<tr><td>1.38 - 1.52</td><td>100</td><td>50</td><td>2</td><td>1</td></tr>
<tr><td>1.53 - 1.68</td><td>110</td><td>55</td><td>1</td><td>2</td></tr>
<tr><td>1.69 - 1.83</td><td>120</td><td>60</td><td>0</td><td>3</td></tr>
<tr><td>1.84 - 1.98</td><td>130</td><td>65</td><td>3</td><td>1</td></tr>
<tr><td>1.99 - 2.14</td><td>140</td><td>70</td><td>2</td><td>2</td></tr>
<tr><td>2.15 - 2.29</td><td>150</td><td>75</td><td>1</td><td>3</td></tr>
<tr><td>≥ 2.30</td><td>160</td><td>80</td><td>0</td><td>4</td></tr>
</table>

</td></tr>
<tr><td>Concomitant Medications and Dose Adjustment</td><td>Same as in the protocol provided in Table 1 hereinabove</td></tr>
<tr><td>Study Endpoints</td><td>

Primary Endpoints:

- Recommended dose (RD) of DSP107 in combination with atezolizumab and bevacizumab in patients with MSS CRC
- Overall survival in patients treated with the combination of DSP107, atezolizumab and bevacizumab versus trifluridine/tipiracil and bevacizumab in patients with MSS CRC.

Secondary Endpoints:

- Safety and tolerability in patients treated with the combination of DSP107, atezolizumab and bevacizumab versus trifluridine/tipiracil and bevacizumab as assessed by CTCAE v5.0, DLTs, vital signs, 12-

</td></tr>
</table>

	lead ECG, physical examination and systemic cytokine and complement levels (in the event of IRRs) • Efficacy of treatment with the combination of DSP107, atezolizumab and bevacizumab versus trifluridine/tipiracil and bevacizumab, including 12M OS, PFS, ORR (determined by RECIST v1.1), DCR and DoR • Efficacy of treatment with the combination of DSP107, atezolizumab and bevacizumab (determined by iRECIST) including ORR, DoR, PFS and DC • DSP107 PK when administered in combination with atezolizumab and bevacizumab • DSP107 immunogenicity by measurement of ADA • QoL as measured by FACT-C (Stage 2 only). **Exploratory Endpoints:** • PD effect of treatment with the combination of DSP107, atezolizumab and bevacizumab versus trifluridine/tipiracil and bevacizumab • Predictive biomarkers for association with efficacy, safety and PK • Exploratory biomarkers for future drug development • Efficacy, safety and PD effect in patients with and without liver metastases • Subsequent response in patients who treat beyond disease progression. **Pharmacokinetics:** Sparse serum samples to measure circulating levels of DSP107 may be pooled with data from other studies using an established population PK model to derive PK parameters such as clearance, volume of distribution and AUC, as warranted by the data and to compare exposure in this study with that observed in previous studies. **Pharmacodynamics:** Evaluation of the PD effect of the study treatments will include: • Measurement of CEA • Measurement of ctDNA • Measurement of peripheral blood immune profile • Evaluation of paired, fresh tumor biopsies collected from at least 30

	patients (15 patients from each treatment arm) at pre-treatment (within 28 days prior to the first dose of DSP107) and on-treatment (after approximately 6-8 weeks of treatment) provided collection of samples is deemed safe and accessible by the Investigator. The biopsy material will optimally include 4 cores (at least 2 and no more than 4 cores). Tumor tissue will be analysed for changes in the TME, markers of immune cell activation, PD-L1 and CD47 expression and other biomarkers to facilitate retrospective correlation with clinical response. Archival biopsies may be used to determine baseline expression if fresh biopsies are not collected. • Evaluation of DSP107 ADA formation and correlation with efficacy, PK, PD and safety outcomes • Evaluation of correlative samples and correlation with efficacy, PK, PD and safety outcomes.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

It is the intent of the applicant(s) that all publications, patents and patent applications referred to in this specification are to be incorporated in their entirety by reference into the specification, as if each individual publication, patent or patent application was specifically and individually noted when referenced that it is to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting. In addition, any priority document(s) of this application is/are hereby incorporated herein by reference in its/their entirety.

WHAT IS CLAIMED IS:

1. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) at least two agents selected from the group consisting of an agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1, an agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF, and Trifluridine/Tipiracil, thereby treating the cancer in the subject.

2. A combination of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) at least two agents selected from the group consisting of an agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1, an agent which inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF, and Trifluridine/Tipiracil, for use in treating cancer in a subject in need thereof.

3. The method of claim 1 or the combination for use of claim 2, wherein said at least two agents comprise said agent which inhibits activity or expression of said receptor-ligand combination PD1-PD-L1 and said agent which inhibits activity or expression of said receptor-ligand combination VEGFR-VEGF.

4. The method of claim 1 or the combination for use of claim 2, wherein said at least two agents comprise said agent which inhibits activity or expression of said receptor-ligand combination PD1-PD-L1, said agent which inhibits activity or expression of said receptor-ligand combination VEGFR-VEGF, and said Trifluridine/Tipiracil.

5. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) Trifluridine/Tipiracil,

thereby treating the cancer in the subject.

6. A combination of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) Trifluridine/Tipiracil,

for use in treating cancer in a subject in need thereof.

7. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) an agent which inhibits a receptor tyrosine kinase (RTK) signaling pathway, wherein said agent is not any of Bevacizumab, Ramucirumab, Cetuximab, Panitumumab, Pertuzumab, Trastuzumab, Ertumaxomab and Cixutumumab,

thereby treating the cancer in the subject.

8. A combination of:

(i) a SIRPα-4-1BBL fusion protein comprising a SIRPα amino acid sequence capable of binding CD47 and a 4-1BBL amino acid sequence capable of binding 4-1BB; and

(ii) an agent which inhibits a receptor tyrosine kinase (RTK) signaling pathway, wherein said agent is not any of Bevacizumab, Ramucirumab, Cetuximab, Panitumumab, Pertuzumab, Trastuzumab, Ertumaxomab and Cixutumumab,

for use in treating cancer in a subject in need thereof.

9. The method of claim 7 or the combination for use of claim 8, wherein said RTK is selected from the group consisting of FGFR, VEGFR, PDGFR, KIT, FLT3, TRKB, AXL, MET, TIE-2 and EPH.

10. The method of claim 7 or the combination for use of claim 8, wherein said agent inhibits activity or expression of a receptor-ligand combination FGFR-FGF.

11. The method or the combination of any one of claims 9-10, wherein said FGFR is FGFR-3.

12. The method of claim 7 or the combination for use of claim 8, wherein said agent inhibits activity or expression of a receptor-ligand combination VEGFR-VEGF.

13. The method or the combination for use of claim 12, wherein said VEGF is VEGF-C.

14. The method or the combination for use of any one of claims 9 and 12-13, wherein said VEGFR is VEGFR-3.

15. The method or the combination for use of any one of claims 7-14, wherein said agent is a tyrosine kinase inhibitor (TKI).

16. The method of claim 7 or the combination for use of claim 8, wherein said agent is selected from the group consisting of Regorafenib, Pazopanib, Pemigatinib, Futibatinib, Infigratinib phosphate, Erdafitinib, Sunitinib, Sorafenib, Cabozantinib, Lenvatinib, Axitinib, Tivozanib and Vandetanib.

17. The method or the combination for use of any one of claims 7-14, wherein said agent is a polynucleotide.

18. The method or the combination for use of any one of claims 7-14, wherein said agent is a polypeptide.

19. The method or the combination for use of any one of claims 7-14 and 18, wherein said agent is Zaltrap.

20. The method or the combination for use of any one of claims 7-14 and 18, wherein said agent is an antibody.

21. The method or the combination for use of any one of claims 7-20, further comprising an agent which inhibits activity or expression of a receptor-ligand combination PD1-PD-L1.

22. The method or the combination for use of any one of claims 1-21, wherein cells of said cancer express CD47.

23. The method or the combination for use of any one of claims 1-22, wherein cells of said cancer express PD-L1.

24. The method or the combination for use of any one of claims 1-23, wherein said cancer is a solid tumor.

25. The method or the combination for use of claim 24, wherein said cancer is selected from the group consisting of a GI tract cancer, liver cancer, pancreatic cancer, lung cancer, renal cancer, bladder cancer, breast cancer, and head and neck cancer.

26. The method or the combination for use of claim 24, wherein said cancer is a colorectal cancer.

27. The method or the combination for use of any one of claims 1-26, wherein said cancer is a microsatellite stable (MSS) cancer.

28. The method or the combination for use of any one of claims 1-6 and 22-27, wherein said subject:

(a) has histologically confirmed, inoperable, microsatellite stable and/or proficient mismatch repair colorectal carcinoma by local testing;

(b) has progressed on or is intolerant to fluoropyrimidine, irinotecan, oxaliplatin, bevacizumab and EGFR inhibitor;

(c) wherein recurrence within 12 months of last adjuvant chemo counts as progression; and

(d) wherein when said cancer is characterized by BRAF V600E, HER2 amp or overexpression, or KRAS G12C, said subject may have also received one line of prior targeted therapy.

29. The method or the combination for use of any one of claims 1-28, wherein said SIRPα-4-1BBL fusion protein is in a form of a homo-trimer.

30. The method or the combination for use of any one of claims 1-29, wherein the SIRPα-4-1BBL fusion protein is characterized by a single amino acid linker between said SIRPα amino acid sequence and said 4-1BBL amino acid sequence.

31. The method or the combination for use of claim 30, wherein the linker is glycine.

32. The method or the combination for use of any one of claims 1-31, wherein said SIRPα-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 1.

33. The method or the combination for use of any one of claims 1-31, wherein said SIRPα-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 1.

34. The method or the combination for use of any one of claims 1-33, wherein said SIRPα-4-1BBL fusion protein is administered intravenously.

35. The method or the combination for use of any one of claims 1-34, wherein said SIRPα-4-1BBL fusion protein is administered at a dose of 0.1 – 50 mg / kg.

36. The method or the combination for use of any one of claims 1-34, wherein said SIRPα-4-1BBL fusion protein is administered at a dose of about 10 mg / kg.

37. The method or the combination for use of any one of claims 1-36, wherein said SIRPα-4-1BBL fusion protein is administered in a 4 weeks cycle.

38. The method or the composition for use of claim 37, wherein said 4 weeks cycle comprises 3 administrations on days 1, 8 and 15 of each cycle.

39. The method or the composition for use of claim 37, wherein said 4 weeks cycle comprises 4 administrations on days 1, 8, 15 and 22 of each cycle.

40. The method or the combination for use of any one of claims 1-4 and 21-39, wherein said agent which inhibits activity or expression of said receptor-ligand combination PD1-PD-L1 is an antibody which inhibits binding of PD-L1 to PD1.

41. The method or the combination for use of claim 40, wherein said antibody which inhibits binding of PD-L1 to PD1 is an anti-PD-L1 antibody.

42. The method or the combination for use of claim 41, wherein said anti-PD-L1 antibody comprises Atezolizumab.

43. The method or the combination for use of claim 40, wherein said antibody which inhibits binding of PD-L1 to PD1 is an anti-PD1 antibody.

44. The method or the combination for use of any one of claims 40-43, wherein said antibody which inhibits binding of PD-L1 to PD1 is administered intravenously.

45. The method or the combination for use of any one of claims 40-44, wherein said antibody which inhibits binding of PD-L1 to PD1 is administered at a dose of about 1680 mg.

46. The method or the combination for use of any one of claims 40-45, wherein said antibody which inhibits binding of PD-L1 to PD1 is administered once every 4 weeks.

47. The method or the combination for use of any one of claims 1-4, 12 and 21-46, wherein said agent which inhibits activity or expression of said receptor-ligand combination VEGFR-VEGF is an anti-VEGF antibody.

48. The method or the combination for use of claim 47, wherein said anti-VEGF antibody comprises Bevacizumab.

49. The method or the combination for use of any one of claims 47-48, wherein said anti-VEGF antibody is administered intravenously.

50. The method or the combination for use of any one of claims 47-49, wherein said anti-VEGF antibody is administered at a dose of about 5 mg/kg.

51. The method or the combination for use of any one of claims 47-50, wherein said anti-VEGF antibody is administered once every 2 weeks.

52. The method or the combination for use of any one of claims 1-2, 4-6 and 22-51, wherein said Trifluridine/Tipiracil molar ratio is 1 : 0.5.

53. The method or the combination for use of any one of claims 1-2, 4-6 and 22-52, wherein said Trifluridine/Tipiracil is administered orally.

54. The method or the combination for use of any one of claims 1-2, 4-6 and 22-53, wherein said Trifluridine/Tipiracil is administered at a dose of 70-160 mg Trifluridine /m^2/day.

55. The method or the combination for use of claim 54, wherein said Trifluridine/Tipiracil is administered twice a day.

56. The method or the combination for use of any one of claims 1-2, 4-6 and 22-55, wherein said Trifluridine/Tipiracil is administered in a 4 weeks' cycle.

57. The method or the combination for use of claim 56, wherein said 4 weeks' cycle comprises administrations for 5 consecutive days, followed by 2 days' rest, followed by administrations for 5 consecutive days.

58. The method or the combination for use of any one of claims 1-4 and 22-57, wherein first administration of said at least two agents starts on the same day.

59. The method or the combination for use of any one of claims 1-58, wherein first administration of said (i) and said (ii) starts on the same day.

60. The method or the combination for use of any one of claims 47-50 and 58-59, wherein a cycle of treatment with said SIRPα-4-1BBL fusion protein, said antibody which inhibits binding of PD-L1 to PD1 and said anti-VEGF antibody includes:

four administrations of the SIRPα-4-1BBL fusion protein having a 1 week' interval, wherein a first administration is on day 1 of said cycle;

the antibody which inhibits binding of PD-L1 to PD1 is administered once during said cycle; and

the anti-VEGF antibody is administered twice during said cycle with 2 weeks intervals.

61. The method or the combination for use of any one of claims 47-50, wherein treatment with said SIRPα-4-1BBL fusion protein, said antibody which inhibits binding of PD-L1 to PD1 and said anti-VEGF antibody is in a 4 weeks cycle, wherein:

said SIRPα-4-1BBL fusion protein is administered 4 times during said cycle, on days 1, 8, 15 and 22 of said cycle;

said antibody which inhibits binding of PD-L1 to PD1 is administered once during said cycle, on day 1 of said cycle; and

said anti-VEGF antibody is administered twice during said cycle, on days 1 and 15 of said cycle.

62. The method or the combination for use of any one of claims 47-50, 52-55 and 58-59, wherein a cycle of treatment with said SIRPα-4-1BBL fusion protein, said antibody which inhibits binding of PD-L1 to PD1, said anti-VEGF antibody and said Trifluridine/Tipiracil includes:

three administrations of the SIRPα-4-1BBL fusion protein having a 1 week' interval, wherein a first administration is on day 1 of said cycle;

the antibody which inhibits binding of PD-L1 to PD1 is administered once during said cycle;

the anti-VEGF antibody is administered twice during said cycle with 2 weeks intervals; and

the Trifluridine/Tipiracil is administered for 10 days during said cycle.

63. The method or the combination for use of any one of claims 47-50 and 52-55, wherein treatment with said SIRPα-4-1BBL fusion protein, said antibody which inhibits binding of PD-L1 to PD1, said anti-VEGF antibody and said Trifluridine/Tipiracil is in a 4 weeks cycle, wherein:

said SIRPα-4-1BBL fusion protein is administered 3 times during said cycle, on days 1, 8 and 15 of said cycle;

said antibody which inhibits binding of PD-L1 to PD1 is administered once during said cycle, on day 1 of said cycle;

said anti-VEGF antibody is administered twice during said cycle, on days 1 and 15 of said cycle; and

said Trifluridine/Tipiracil is administered twice a day for a total of 10 days during said cycle, on days 1-5 and 8-12 of said cycle.