TITLE OF THE INVENTION

A humanized monoclonal antibody to SFRP2 for tumor associated macrophage reduction and polarization in cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 63/568,165, filed March 21, 2024, which is hereby incorporated by reference herein in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

This application contains a Sequence Listing, which is submitted electronically via EFS-Web as an XML Document formatted sequence listing with a file name "206085-0178-00WO_Sequence_Listing.xml" having a creation date of Feubrary 26, 2025, and having a size of 26,178 bytes. The sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Triple-negative breast cancer (TNBC) is an aggressive and diverse subtype of breast cancer. It disproportionately affects African American women and is associated with high recurrence rates and distant metastases (Dent R, et al., Clin Cancer Res 2007;13(15 Pt 1):4429-34). While targeted therapies are limited, chemotherapy remains the primary treatment. Immunotherapy, particularly anti-PD-L1 and anti PD-1 therapies show promise in treating TNBC (Hossain F, et al., Cancers (Basel) 2021;13(15)). However, overall survival remains poor. Novel drugs that address chemotherapy resistance and enhance the tumor immune microenvironment offer hope for improving patient outcomes.

Thus, there is a need in the art for more effective therapeutic strategies for treating solid tumors. This invention satisfies this unmet need.

SUMMARY OF THE INVENTION

In some embodiments, the present invention provides methods of inducing macrophage polarization in a subject having a solid tumor, the method comprising administering to the subject an inhibitor of SFRP2.

In some embodiments, the inhibitor is selected from the group consisting of a chemical compound, a protein, a peptide, a peptidomimetic, an antibody, an antibody fragment, an antibody mimetic, a chimeric antibody, a bispecific antibody, a ribozyme, a small molecule chemical compound, a monobody, a short hairpin RNA, RNAi, siRNA, miRNA, or a nucleic acid encoding an antisense nucleic acid molecule.

In some embodiments, the inhibitor is an antibody. In some embodiments, the antibody is an anti-SFRP2 antibody.

In some embodiments, the anti-SFRP2 antibody comprises a complementary determining region (CDR) H1 comprising the amino acid sequence of SEQ ID NO:19, a CDR H2 comprising the amino acid sequence of SEQ ID NO:20, a CDR H3 comprising the amino acid sequence of SEQ ID NO:21, a CDR LI comprising the amino acid sequence of SEQ ID NO:22, a CDR L2 comprising the amino acid sequence of SEQ ID NO:23, and a CDR L3 comprising the amino acid sequence of SEQ ID NO:24.

In some embodiments, the solid tumor is breast cancer. In some embodiments, the breast cancer is triple negative metastatic breast cancer.

In some embodiments, the macrophage is polarized into a classically activated M1.

In some embodiments, the classically activated M1 expresses at least one M1 marker, wherein the M1 marker is selected from the group consisting of G-CSF, GM-CSF, IFNg, IL-1a, IL-1B, IL2, IL3, IL5, IL7, IL12p70, IL16, IL27, CCL2, CXCL9, TARC, TIMP1, or TNF-α.

In some embodiments, the present invention provides methods of reducing the number of tumor associated macrophages in a subject, the method comprising administering to the subject an inhibitor of SFRP2.

In some embodiments, the inhibitor is selected from the group consisting of: a chemical compound, a protein, a peptide, a peptidomimetic, an antibody, an antibody fragment, an antibody mimetic, a chimeric antibody, a bispecific antibody, a

ribozyme, a small molecule chemical compound, a monobody, a short hairpin RNA, RNAi, siRNA, miRNA, or a nucleic acid encoding an antisense nucleic acid molecule.

In some embodiments, the inhibitor is an antibody. In some embodiments, the antibody is an anti-SFRP2 antibody.

In some embodiments, the anti-SFRP2 antibody comprises a complementary determining region (CDR) H1 comprising the amino acid sequence of SEQ ID NO:19, a CDR H2 comprising the amino acid sequence of SEQ ID NO:20, a CDR H3 comprising the amino acid sequence of SEQ ID NO:21, a CDR LI comprising the amino acid sequence of SEQ ID NO:22, a CDR L2 comprising the amino acid sequence of SEQ ID NO:23, and a CDR L3 comprising the amino acid sequence of SEQ ID NO:24.

In some embodiments, the subject has a solid tumor.

In some embodiments, the present invention provides methods comprising: a) administering to a subject an inhibitor of SFRP2 for the treatment or prevention of a disease or disorder, and b) measuring a response selected from the group consisting of: a change in the level of interferon-gamma (IFN-γ), a change in macrophage polarization, or a change in the number of tumor associated macrophages.

In some embodiments, the level of interferon-gamma (IFN-γ) is increased, thereby indicating that the inhibitor is effective in treating the disease or disorder in the subject.

In some embodiments, macrophage polarization to a classically activated M1 is increased, thereby indicating that the inhibitor is effective in treating the disease or disorder in the subject.

In some embodiments, the number of tumor associated macrophages is decreased, thereby indicating that the inhibitor is effective in treating the disease or disorder in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of various embodiments of the invention will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, illustrative embodiments are

shown in the drawings. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

Figure 1A through Figure 1K depict representative data demonstrating that TNBC tumor samples from patients and TNBC cell lines express SFRP2 and CD38, while hematopoietic cells express CD38 only. Figure 1A depicts representative data demonstrating that SFRP2 abundance was examined in 88 core biopsies from 44 patients using IHC. ANOVA did not reveal statistically significant differences in the mean percent staining between TNBC, endocrine receptor-positive, or Her2neu-positive breast cancer. Figure 1B depicts a negative control core without primary antibody. Figure 1C depicts representative data illustrating that cell phenotyping and segmentation analysis displays cells positively stained for SFRP2, shown in green. Figure 1D depicts representative data demonstrating a low positive core with fewer green, positively stained cells. Figure 1E depicts that eighty-eight core biopsies from a human TNBC TMA (with 44 tumors in duplicate) were stained with IHC for CD38. ANOVA did not reveal statistically significant differences in the mean percent staining between TNBC, endocrine receptor-positive, or Her2neu-positive breast cancer. Figure 1F depicts a negative control core. Figure 1G depicts representative data illustrating that cell phenotyping and segmentation analysis display cells positively stained for CD38, shown in green. Figure 1H depicts representative data demonstrating the low positive core has less green. Figure 1I depicts a representative western blot showing co-expression of SFRP2 and CD38 in three TNBC breast cancer cell lines. Gene expression in human hematopoietic cell types of CD38 (Figure 1J) and SFPR2 (Figure 1K). Expression of CD38 is high in blood and immune cells (B cells, T-cells, granulocytes, macrophages, Kupfer cells, and erythroid cells), while expression of SFRP2 is minimal across all studied cell types. Human Protein Atlas is available from http:// www.proteinatlas.org. SFRP2 and CD38 co-localize in the tumor microenvironment in human TNBC.

Figure 2A through Figure 2N depict representative colocalization of SFRP2 and CD38 in the TNBC tumor microenvironment using multiplex IHC analysis. Four human TNBC tumors underwent multiplex IHC staining with antibodies to SFRP2, CD68, CD38, CD19, cytokeratin, and CD3. Figure 2A depicts representative InForm

spatial analysis demonstrating a high degree of cells in the tumor, TAMs, TILs, and B-cells staining positively for SFRP2. Figure 2B depicts a high degree of cells in the tumor, TAMs, TILs, and B-cells stained positively for CD38. A representative human TNBC tumor stains positively for cytokeratin, shown in white (Figure 2C), SFRP2, in orange (Figure 2D) CD38, in teal (Figure 2E), and the colocalization of cytokeratin, SFRP2, and CD38, in yellow (Figure 2F). The same tumor also stains positively for CD3, shown in yellow (Figure 2G), SFRP2, in orange (Figure 2H), CD38, in teal (Figure 2I), and the colocalization of CD3, SFRP2, and CD38, in green and indicated by an arrow (Figure 2J). Lastly, the tumor stains positively for CD68 shown in green (Figure 2K), SFRP2, orange (Figure 2L), CD38, teal (Figure 2M), and colocalization of CD68, SFRP2, and CD38, in yellow and indicated by an arrow (Figure 2N).

Figure 3A through Figure 3E depict representative experimental results demonstrating that SRP2 mAb treatment is associated with an increase in IFN-Ɣ levels in TAMs and a significant boost in T-cell proliferation. Figure 3A depicts representative data demonstrating TAM enriched single cell suspension was treated in vitro for 1 hour with 10uM IgG1 control or 10uM hSFRP2 mAb. Cell lysates were collected and analyzed with western blot. IFN-Ɣ levels increase in TAMs treated for 1 and 24 hours with hSFPR2 mAb. The intensity ratio represents the protein levels from test samples compared to the vinculin loading control as described in Material and Methods. Figure 3B depicts representative qRT-PCR results showing a 2.35-fold increase in IFN-Ɣ mRNA levels in 1-hour hSFRP2 mAb-treated TAMs compared to 1-hour IgG1 control-treated TAMs (n=3 for both groups, *p<.002). Graph created with BioRender.com. Figure 3C depicts analysis of IFN-Ɣ and SFRP2 expression TCGA data from 1075 entries that shows a statistically significant inverse correlation between IFN-Ɣ and SFRP2 ($p<0.0001$). Figure 3D depicts representative flow cytometry data analyzing T-cell proliferation after co-culture of TAMs treated with IgG1 control or hSFRP2 mAb. These data show significant MFI increases in CD25 in T-cells co-cultured with hSFRP2 mAb treated TAMs when compared to T-cells co-cultured with IgG1 control treated TAMs (n=3, **p<.05). Figure 3E depicts representative data demonstrating flow cytometry analysis of T-cell proliferation after co-culture of TAMs treated with IgG1 control or hSFRP2 mAb. These data showed significant MFI increases in CD69 in T-cells co-

cultured with hSFRP2 mAb-treated TAMs when compared to T-cells co-cultured with IgG1 control-treated TAMs (n=3, ***p<.05).

Figure 4A through Figure 4G depict representative experimental results demonstrating that SFRP2 mAb treatment reduces the number of metastases and is associated with an increase in apoptosis in tumors, as well as an increase in the M1/M2 TAM ratio and in the levels of IFN-γ. Figure 4A depicts EO771.LMB tumor cells were injected via tail vein into C57/BL6 mice and treated with IgG1 control for 7 days (representative lung picture) and hSFRP2 mAb 8 mg/kg iv q for 3 days (representative lung picture). After 3 weeks, the lungs were resected, and surface metastatic lesions were counted. The bar graph indicates a higher incidence of lung metastases in IgG1 control-treated mice (gray, n=15) than in hSFPR2 mAb-treated mice (black, n=15, $p<0.05$). Figure 4B depicts a representative TUNEL assay on FFPE EO771.LMB lung sections from mice treated with IgG1 control and hSFRP2 mAb. These data show significantly more apoptotic cells in the hSFRP2 mAb treatment group (n=15, *p,<0.05) Figure 4C depicts PY8119 cells injected via tail vein into C57/BL6 mice and treated with IgG1 control (n=11) versus hSFRP2 mAb (n=11). The bar chart shows a statistically significant reduction in lung metastases in hSFRP2 mAb-treated mice compared to the control ($p<0.05$). Figure 4D depicts FFPE PY8119 lung sections stained with TUNEL assay, and the number of apoptotic cells/20 x HPF were counted. There was a significant increase in apoptotic cells in lung metastases from mice treated with hSFRP2 mAb (n=10) compared to IgG1 control (n=10, *$p<0.05$). Figure 4E depicts data demonstrating that FFPE E0771 lung sections stained with antibodies to CD86, CD163, and F4/80. M1/M2 ratios for hSFRP2 mAb treated mice were increased compared to IgG1 treated mice (*p=0.028, n=3). Figure 4F depicts data demonstrating that FFPE PY8119 lung sections stained with antibodies against CD86, CD163, and F4/80. M1/M2 ratios for hSFRP2 mAb treated mice were increased compared to IgG1 treated mice (*p= 0.036, n=3). Figure 4G depicts a representative ELISA revealing an increase of IFN-Y protein in the serum of EO771.LMB ($p<0.0001$ n=5) and PY8119 ($p<0.0001$ n=7) tumor-bearing mice following treatment with hSFRP2 mAb.

DETAILED DESCRIPTION

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

As used herein, each of the following terms has the meaning associated with it in this section.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

"About" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20%, ±10%, ±5%, ±1%, or ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

The term "antibody" means an immunoglobulin molecule that recognizes and specifically binds to a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing (e.g., a glycoprotein), through at least one antigen recognition site within the variable region of the immunoglobulin molecule. As used herein, the term "antibody" encompasses polyclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, human antibodies, and any other immunoglobulin molecule so long as the antibodies exhibit the desired biological activity. An antibody can be of any the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), based on the identity of their heavy-chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. The different classes of immunoglobulins have different and well known subunit structures and three-dimensional configurations. Antibodies can be naked, part of a fusion protein, or conjugated to other molecules such as toxins, radioisotopes, etc.

The term "antibody fragment" refers to a portion of an antibody. An "antigen-binding fragment," "antigen-binding domain," or "antigen-binding region," refers to a portion of an antibody that binds to an antigen. An antigen-binding fragment can contain the antigenic determining regions of an antibody (e.g., the complementarity

determining regions (CDR)). An antigen-binding fragment can contain some or all of the VH and/or VL chain polypeptides of an antibody. Examples of antigen-binding fragments of antibodies include, but are not limited to Fab, Fab′, F(ab′)2, and Fv fragments, linear antibodies, and single chain antibodies. An antigen-binding fragment of an antibody can be derived from any animal species, such as rodents (e.g., mouse, rat, or hamster) and humans or can be artificially produced.

The terms "anti-SFRP2 antibody," "SFRP2 antibody" and "antibody that binds to SFRP2" refer to an antibody that is capable of binding SFRP2 with sufficient affinity such that the antibody is useful as a diagnostic, a therapeutic, and/or as a modulator of SFRP2 activity.

A antibody that is "blocking" or that "blocks" or that is "inhibitory" or that "inhibits" is an antibody that reduces or inhibits (partially or completely) binding of its target protein to one or more ligands when the antibody is bound to the target protein, and/or that reduces or inhibits (partially or completely) one or more activities or functions of the target protein when the antibody is bound to the target protein.

The term "anti-tumor effect" as used herein, refers to a biological effect which can be manifested by various means, including but not limited to, e.g., a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, decrease in tumor cell proliferation, decrease in tumor cell survival, or amelioration of various physiological symptoms associated with the cancerous condition. An "anti-tumor effect" can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies of the invention in prevention of the occurrence of tumor in the first place.

The term "humanized" antibody or antigen-binding fragment thereof refers to forms of non-human (e.g. murine) antibodies or antigen-binding fragments that are specific immunoglobulin chains, chimeric immunoglobulins, or fragments thereof that contain minimal non-human (e.g., murine) sequences. Typically, humanized antibodies or antigen-binding fragments thereof are human immunoglobulins in which residues from the complementarity determining regions (CDRs) are replaced by residues from the CDRs of a non-human species (e.g. mouse, rat, rabbit, hamster) that have the desired specificity, affinity, and capability ("CDR grafted") (Jones et al., Nature 321:522-

525 (1986); Riechmann et al., Nature 332:323-327 (1988); Verhoeyen et al., Science 239:1534-1536 (1988)). The humanized antibody or antigen-binding fragment thereof can be further modified by the substitution of additional residues either in the Fv framework region and/or within the replaced non-human residues to refine and optimize the specificity, affinity, and/or capability of the antibody or antigen-binding fragment thereof. In general, the humanized antibody or antigen-binding fragment thereof will comprise VH and VL that comprise substantially all of at least one, and typically two or three, of the CDR regions that correspond to the non-human immunoglobulin, whereas all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody or antigen-binding fragment thereof can also comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Examples of methods used to generate humanized antibodies are described in U.S. Pat. No. 5,225,539; Roguska et al., Proc. Natl. Acad. Sci., USA, 91 (3):969-973 (1994), and Roguska et al., Protein Eng. 9 (10):895-904 (1996). In some aspects, a "humanized antibody" is a resurfaced antibody.

"Immunotherapy" refers to any medical intervention that induces, suppresses or enhances the immune system of a patient for the treatment of a disease. In some embodiments, immunotherapies activate a patient's innate and/or adaptive immune responses (e.g. T cells) to more effectively target and remove a pathogen or cure a disease, such as cancer or an immune disease.

"Effective amount" or "therapeutically effective amount" are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result. Such results may include, but are not limited to, the inhibition of virus infection as determined by any means suitable in the art.

As used herein "endogenous" refers to any material from or produced inside an organism, cell, tissue or system.

As used herein, the term "exogenous" refers to any material introduced from or produced outside an organism, cell, tissue or system.

The term "expression" as used herein is defined as the transcription and/or translation of a particular nucleotide sequence driven by its regulatory sequences.

"Parenteral" administration of an immunogenic composition includes, e.g., subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), or intrasternal injection, or infusion techniques.

The term "nucleic acid" or "polynucleotide" refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

As used herein, the terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

The term “promoter” as used herein is defined as a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

As used herein, the term “promoter/regulatory sequence” means a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

The term "polarization" is used herein to designate the phenotypic features and the functional features of the macrophages. The phenotype can be defined through the surface markers expressed by the macrophages. The functionality, can be defined for example based on the nature and the quantity of chemokines and/or cytokines expressed, in particular secreted, by the macrophages. Indeed, the macrophages present different phenotypic and functional features depending of their state, either anti-tumor Ml-type macrophage or pro-tumor M2-type macrophage. M2-type macrophages can be characterized by the expression of surface markers such as CD206, CD l ib, PD- Ll and CD200R and then secretion of cytokines such as CCL17. M1-type macrophages can be defined by the expression of surface markers such as CD86 and CCR7 and the secretion of cytokines such as G-CSF, GM-CSF, IFNg, IL-1a, IL-1B, IL2, IL3, IL5, IL7, IL12p70, IL16, IL27, CCL2, CXCL9, TARC, TIMP1, and TNF-α. In the context of the invention, anti-SFRP2 inhibitors induce polarization of a macrophage population from M2 to M1 phenotype.

It encompasses the meaning of the term "activation" usually used to mean the perturbation of macrophages with exogenous agents. Macrophages change their polarization states in response to growth-factors (CSF-1 and GM-CSF) and external stimuli such as microbes, microbial product and nucleotides derivatives, antibody-Fc receptor stimulation, glucocorticoids, phagocytosis.

The term “M1 phenotype” or “classically activated M1” as used herein, refers to macrophages that exhibit anti-tumor activities or markers known in the art to

be associated with the M1 phenotype such as, but not limited to, stimulation of $CD8^+$ T-Cells and/or Natural Killer cells, phagocytosis of tumor cells, secretion and/or expression of M1 associated cytokines (e.g., G-CSF, GM-CSF, IFNg, IL-1a, IL-1B, IL2, IL3, IL5, IL7, IL12p70, IL16, IL27, CCL2, CXCL9, TARC, TIMP1, and TNF-α), expression of M1 associated miRNAs (e.g., miRNAl55, miR-33) (see, e.g., Mosser, D. M., & Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. Nature Reviews Immunology, 8(12), 958-96; Murray, P. J., Allen, J. E., Biswas, S. K., Fisher, E. A., Gilroy, D. W., Goerdt, S., Wynn, T. A. (2014). Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity, 41(1), 14-20; and Liu, Y. C., Zou, X. B., Chai, Y. F., & Yao, Y. M. (2014). Macrophage polarization in inflammatory diseases. International Journal of Biological Sciences, 10(5), 520-5299, and references cited therein, each incorporated by reference for all purposes) and/or reduced expression/secretion of M2 associated factors or reduced M2 associated activities listed above compared to at least one reference sample, wherein the reference sample comprises a population of M2-activated macrophages. M2- activation in vitro is evoked by treatment with IL-4 and IL-13 (see, e.g., Liu, Y. C., Zou, X. B., Chai, Y. F., & Yao, Y. M. (2014). Macrophage polarization in inflammatory diseases. International Journal of Biological Sciences, 10(5), 520-529, incorporated by references for all purposes).

As used herein, the term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. The formulation can be sterile.

As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

As used herein, the term "SFRP2" refers to secreted-frizzled related protein 2 polypeptides including, but not limited to, native SFRP2 polypeptides and any naturally occurring variants thereof. As used herein, the term "human SFRP2" refers to a polypeptide comprising the amino acid sequence of:

MLQGPGSLLLLFLASHCCLGSARGLFLFGQPDFSYKRSNCKPIPVNLQLCHGIEY QNMRLPNLLGHETMKEVLEQAGAWIPLVMKQCHPDTKKFLCSLFAPVCLDDLD ETIQPCHSLCVQVKDRCAPVMSAFGFPWPDMLECDRFPQDNDLCIPLASSDHLLP ATEEAPKVCEACKNKNDDDNDIMETLCKNDFALKIKVKEITYINRDTKIILETKSK TIYKLNGVSERDLKKSVLWLKDSLQCTCEEMNDINAPYLVMGQKQGGELVITSV KRWQKGQREFKRISRSIRKLQC (SEQ ID NO: 12). An "SFRP2 polynucleotide," "SFRP2 nucleotide," or "SFRP2 nucleic acid" refers to a polynucleotide encoding any SFRP2, including those described above.

As used herein, the terms "subject" and "patient" are used interchangeably. The subject can be a mammal such as a non-human animal (e.g., cow, pig, horse, cat, dog, rat, mouse, monkey or other primate, etc.). In some aspects, the subject is a human.

The term "therapeutic" as used herein means a treatment. A therapeutic effect is obtained by reduction, suppression, remission, or eradication of a disease state.

The term "prophylaxis" as used herein means the prevention of or protective treatment for a disease or disease state.

By the term "specifically binds," as used herein, is meant an antibody or antigen binding fragment thereof, or a ligand, which recognizes and binds with a cognate binding partner present in a sample, but which antibody, antigen binding fragment thereof or ligand does not substantially recognize or bind other molecules in the sample.

As used herein, the terms "variable region" or "variable domain" are used interchangeably and are common in the art. The variable region typically refers to a portion of an antibody, generally, a portion of a light or heavy chain, typically about the amino-terminal 110 to 120 amino acids or 110 to 125 amino acids in the mature heavy chain and about 90 to 115 amino acids in the mature light chain, which differ extensively in sequence among antibodies and are used in the binding and specificity of a particular antibody for its particular antigen.

The terms "VL" and "VL domain" are used interchangeably to refer to the light chain variable region of an antibody.

The terms "VH" and "VH domain" are used interchangeably to refer to the heavy chain variable region of an antibody.

The "hypervariable regions" in each chain are held together in close proximity by FRs, and with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al, Sequences of Proteins of Immunological Interest, 1992; Chothia et al., Conformations of immunoglobulin hypervariable regions. Nature (1989) 342:877-83.). The term "hypervariable region" as used herein refers to the amino acid residues of an antibody, which are responsible for antigen binding. The hypervariable region generally comprises amino acid residues from a "complementary determining region" or "CDR", the latter being of highest sequence variability and/or involved in antigen recognition.

Ranges: throughout this disclosure, various aspects of the invention can 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, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Therapeutic Inhibitor Compositions and Methods of Use

In various embodiments, the present invention includes SFRP2 inhibitor compositions and methods of treating or preventing a disease or disorder, inducing macrophage polarization, reducing total tumor associated macrophage number, or a combination thereof where a diminished level or activity of SFRP2 is desired. One non-limiting example of a disease or disorder where a diminished level or activity of SFRP2 is desired which can be treated or prevented with the compositions and methods of the

invention includes cancer. In various embodiments, the SFRP2 inhibitor compositions and methods of treatment or prevention of the invention diminish the amount of SFRP2 polypeptide, the amount of SFRP2 protein, the amount of SFRP2 mRNA, the amount of SFRP2 enzymatic activity, or a combination thereof.

It will be understood by one skilled in the art, based upon the disclosure provided herein, that a decrease in the level of SFRP2 encompasses the decrease in SFRP2 expression, including transcription, translation, or both, and also encompasses promoting the degradation of SFRP2, including at the RNA level (e.g., RNAi, shRNA, etc.) and at the protein level (e.g., Ubiquitination, etc.) The skilled artisan will also appreciate, once armed with the teachings of the present invention, that a decrease in the level of SFRP2 includes a decrease in a SFRP2 activity (e.g., enzymatic activity, substrate binding activity, receptor binding activity, etc.). Thus, decreasing the level or activity of SFRP2 includes, but is not limited to, decreasing transcription, translation, or both, of a nucleic acid encoding SFRP2; and it also includes decreasing any activity of a SFRP2 polypeptide, or peptide fragment thereof, as well.

One skilled in the art, based upon the disclosure provided herein, would understand that the invention is useful in treating or preventing a disease or disorder, inducing macrophage polarization, reducing total tumor associated macrophage number, or a combination thereof in a subject in need thereof, whether or not the subject is also being treated with other medication or therapy. Further, the skilled artisan would further appreciate, based upon the teachings provided herein, that the disease or disorders treatable by the compositions and methods described herein encompass any disease or disorder where SFRP2 plays a role and where diminished SFRP2 level or activity will promote a positive therapeutic outcome. In various embodiments, the disease or disorder treatable or preventable using the compounds and methods of the invention is solid tumors. In some embodiments the solid tumor is breast cancer, pancreatic cancer, sarcomas, osteosarcomas, ovarian cancer, colon cancer, or lung cancer. In some embodiments the breast cancer is triple-negative breast cancer (TNBC).

In another embodiment, the SFRP2 inhibitor of the invention can be administered to a patient who is being treated with exogenous SFRP2, recombinant

SFRP2, and/or a SFRP2 activator, in order to control, titrate, diminish, or stabilize the level or activity of endogenous and/or exogenous SFRP2 in the patient.

The SFRP2 inhibitor compositions and methods of the invention that decrease the level or activity (e.g., enzymatic activity, substrate binding activity, receptor binding activity, etc.) of SFRP2, include, but should not be construed as being limited to, a chemical compound, a protein, a peptide, a peptidomimetic, an antibody, an antibody fragment, a monobody, an antibody mimetic, a ribozyme, a small molecule chemical compound, an short hairpin RNA, RNAi, an antisense nucleic acid molecule (e.g., siRNA, miRNA, etc.), a nucleic acid encoding an antisense nucleic acid molecule, a nucleic acid sequence encoding a protein, a SFRP2 receptor, or combinations thereof. In some embodiments, the inhibitor is an allosteric inhibitor. One of skill in the art would readily appreciate, based on the disclosure provided herein, that a SFRP2 inhibitor composition encompasses any chemical compound that decreases the level or activity of SFRP2. Additionally, a SFRP2 inhibitor composition encompasses a chemically modified compound, and derivatives, as is well known to one of skill in the chemical arts.

Examples of SFRP2 inhibitors include, but are not limited to, anti-SFRP2 antibodies as described in U.S. Patent Application No. 17/547,550, the contents of which is incorporated herein in its entirety.

The SFRP2 inhibitor compositions and methods of the invention that decrease the level or activity (e.g., enzymatic activity, substrate binding activity, receptor binding activity, etc.) of SFRP2 include antibodies, and fragments thereof. The antibodies of the invention include a variety of forms of antibodies including, for example, polyclonal antibodies, monoclonal antibodies, intracellular antibodies ("intrabodies"), Fv, Fab and F(ab)2, single chain antibodies (scFv), heavy chain antibodies (such as camelid antibodies), synthetic antibodies, chimeric antibodies, monobodies, and humanized antibodies. In one embodiment, the antibody of the invention is an antibody that specifically binds to SFRP2.

Further, one of skill in the art, when equipped with this disclosure and the methods exemplified herein, would appreciate that a SFRP2 inhibitor composition includes such inhibitors as discovered in the future, as can be identified by well-known criteria in the art of pharmacology, such as the physiological results of inhibition of

SFRP2 as described in detail herein and/or as known in the art. Therefore, the present invention is not limited in any way to any particular SFRP2 inhibitor composition as exemplified or disclosed herein; rather, the invention encompasses those inhibitor compositions that would be understood by the routineer to be useful as are known in the art and as are discovered in the future.

Further methods of identifying and producing SFRP2 inhibitor compositions are well known to those of ordinary skill in the art, including, but not limited, obtaining an inhibitor from a naturally occurring source. Alternatively, a SFRP2 inhibitor can be synthesized chemically. Further, the person of skill in the art would appreciate, based upon the teachings provided herein, that a SFRP2 inhibitor composition can be obtained from a recombinant organism. Compositions and methods for chemically synthesizing SFRP2 inhibitors and for obtaining them from natural sources are well known in the art and are described in the art.

One of skill in the art will appreciate that an inhibitor can be administered as a chemical compound, a protein, a peptide, a peptidomimetic, an antibody, an antibody fragment, an antibody mimetic, a ribozyme, a small molecule chemical compound, a short hairpin RNA, RNAi, an antisense nucleic acid molecule (e.g., siRNA, miRNA, etc.), a nucleic acid encoding an antisense nucleic acid molecule, a nucleic acid sequence encoding a protein, or a combination thereof. Numerous vectors and other compositions and methods are well known for administering a protein or a nucleic acid construct encoding a protein to cells or tissues. Therefore, the invention includes a method of administering a protein or a nucleic acid encoding a protein that is an inhibitor of SFRP2.

One of skill in the art will realize that diminishing the amount or activity of a molecule that itself increases the level or activity of SFRP2 can serve in the compositions and methods of the present invention to decrease the level or activity of SFRP2.

Antisense oligonucleotides are DNA or RNA molecules that are complementary to some portion of an RNA molecule. When present in a cell, antisense oligonucleotides hybridize to an existing RNA molecule and inhibit translation into a gene product. Inhibiting the expression of a gene using an antisense oligonucleotide is well known in the art (Marcus-Sekura, 1988, Anal. Biochem. 172:289), as are methods of

expressing an antisense oligonucleotide in a cell (Inoue, U.S. Pat. No. 5,190,931). The methods of the invention include the use of an antisense oligonucleotide to diminish the amount of SFRP2, or to diminish the amount of a molecule that causes an increase in the amount or activity of SFRP2, thereby decreasing the amount or activity of SFRP2.

Contemplated in the present invention are antisense oligonucleotides that are synthesized and provided to the cell by way of methods well known to those of ordinary skill in the art. As an example, an antisense oligonucleotide can be synthesized to be between about 10 and about 100, more preferably between about 15 and about 50 nucleotides long. The synthesis of nucleic acid molecules is well known in the art, as is the synthesis of modified antisense oligonucleotides to improve biological activity in comparison to unmodified antisense oligonucleotides (Tullis, 1991, U.S. Pat. No. 5,023,243).

Similarly, the expression of a gene may be inhibited by the hybridization of an antisense molecule to a promoter or other regulatory element of a gene, thereby affecting the transcription of the gene. Methods for the identification of a promoter or other regulatory element that interacts with a gene of interest are well known in the art, and include such methods as the yeast two hybrid system (Bartel and Fields, eds., In: The Yeast Two Hybrid System, Oxford University Press, Cary, N.C.).

Alternatively, inhibition of a gene expressing SFRP2, or of a gene expressing a protein that increases the level or activity of SFRP2, can be accomplished through the use of a ribozyme. Using ribozymes for inhibiting gene expression is well known to those of skill in the art (see, e.g., Cech et al., 1992, J. Biol. Chem. 267:17479; Hampel et al., 1989, Biochemistry 28: 4929; Altman et al., U.S. Pat. No. 5,168,053). Ribozymes are catalytic RNA molecules with the ability to cleave other single-stranded RNA molecules. Ribozymes are known to be sequence specific, and can therefore be modified to recognize a specific nucleotide sequence (Cech, 1988, J. Amer. Med. Assn. 260:3030), allowing the selective cleavage of specific mRNA molecules. Given the nucleotide sequence of the molecule, one of ordinary skill in the art could synthesize an antisense oligonucleotide or ribozyme without undue experimentation, provided with the disclosure and references incorporated herein.

Alternatively, inhibition of a gene expressing SFRP2, or of a gene expressing a protein that increases the level or activity of SFRP2, can be accomplished through the use of a short hairpin RNA or antisense RNA, including siRNA, miRNA, and RNAi. Given the nucleotide sequence of the molecule, one of ordinary skill in the art could synthesize a short hairpin RNA or antisense RNA without undue experimentation, provided with the disclosure and references incorporated herein.

The invention provides compositions that bind to SFRP2. In one embodiment, the SFRP2 binding agent inhibits SFRP2 levels or activity. Thus, in diseases and conditions where a reduction of SFRP2 activity would be beneficial, such inhibitory SFRP2 binding agents can potentially act as therapeutics.

The SFRP2 inhibitor compositions of the invention that decrease the level or activity (e.g., enzymatic activity, substrate binding activity, receptor binding activity, etc.) of SFRP2 include, but should not be construed as being limited to, a chemical compound, a protein, a peptide, a peptidomimetic, an antibody, an antibody fragment, an antibody mimetic, a ribozyme, a small molecule chemical compound, an short hairpin RNA, RNAi, an antisense nucleic acid molecule (e.g., siRNA, miRNA, etc.), a nucleic acid encoding an antisense nucleic acid molecule, a nucleic acid sequence encoding a protein, a competitive inhibitor, or combinations thereof. In some embodiments, the inhibitor is an allosteric inhibitor. One of skill in the art would readily appreciate, based on the disclosure provided herein, that a SFRP2 inhibitor composition encompasses a chemical compound that decreases the level or activity of SFRP2. Additionally, a SFRP2 inhibitor composition encompasses a chemically modified compound, and derivatives, as is well known to one of skill in the chemical arts.

The SFRP2 inhibitor compositions of the invention that decrease the level or activity (e.g., enzymatic activity, substrate binding activity, receptor binding activity, etc.) of SFRP2 include antibodies, and fragments thereof. The antibodies of the invention include a variety of forms of antibodies including, for example, polyclonal antibodies, monoclonal antibodies, intracellular antibodies ("intrabodies"), Fv, Fab and F(ab)2, single chain antibodies (scFv), heavy chain antibodies (such as camelid antibodies), synthetic antibodies, chimeric antibodies, and a humanized antibodies. In one embodiment, the antibody of the invention is an antibody that specifically binds to

SFRP2. In some embodiments, the antibodies of the invention are bispecific antibodies, where at least one specificity is to SFRP2. In some embodiments, the antibodies of the invention are bispecific antibodies, where the first specificity is to SFRP2 and the second specificity is to a second binding partner molecule that is carried and deployed to an anatomic location where SFRP2 binding is desired.

Antibodies, including SFRP2 binding fragments thereof, of the present invention include, in certain embodiments, antibody amino acid sequences encoded by any suitable polynucleotide, or any isolated or formulated antibody. Further, antibodies of the present disclosure comprise antibodies having the structural and/or functional features of SFRP2 binding. In one embodiment, the anti-SFRP2 antibody binds SFRP2 and, thereby partially or substantially alters at least one biological activity of SFRP2 (e.g., enzymatic activity, substrate binding activity, receptor binding activity, etc.).

In one embodiment, anti-SFRP2 antibodies of the invention immunospecifically bind at least one specified epitope specific to SFRP2, and do not specifically bind to other polypeptides, other than SFRP2. The at least one epitope can comprise at least one antibody binding region that comprises at least one portion of the SFRP2 protein. The term "epitope" as used herein refers to a protein determinant capable of binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

The binding portion of an antibody comprises one or more fragments of an antibody that retain the ability to specifically bind to binding partner molecule (e.g., SFRP2). It has been shown that the binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment

(Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. Binding portions can be produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins.

An antibody that binds to SFRP2 of the invention is an antibody that inhibits, blocks, or interferes with at least one SFRP2 activity (e.g., enzymatic activity, substrate binding activity, receptor binding activity, etc.), in vitro, in situ and/or in vivo. A suitable anti-SFRP2 antibody, specified portion, or variant can also optionally affect at least one SFRP2 activity or function, such as but not limited to, RNA, DNA or protein synthesis, and/or protein release.

In some embodiments, the anti-SFRP2 antibody comprises a humanized anti-secreted frizzled-related protein 2 (SFRP2) antibody or antigen binding fragment thereof which binds SFRP2, wherein said antibody or antigen binding fragment thereof comprises a complementary determining region (CDR) H1 comprising the amino acid sequence of SEQ ID NO:19, a CDR H2 comprising the amino acid sequence of SEQ ID NO:20, a CDR H3 comprising the amino acid sequence of SEQ ID NO:21, a CDR L1 comprising the amino acid sequence of SEQ ID NO:22, a CDR L2 comprising the amino acid sequence of SEQ ID NO:23, and a CDR L3 comprising the amino acid sequence of SEQ ID NO:24.

In some embodiments, the anti-SFRP2 antibody comprises a humanized anti-secreted frizzled-related protein 2 (SFRP2) antibody or antigen binding fragment thereof which binds SFRP2, wherein said antibody or antigen binding fragment thereof comprises a variable heavy (VH) chain polypeptide at least 90%, 95%, or 99% identical

to a polypeptide comprising the amino acid sequence of SEQ ID NO: 1, 2, 3, or 4; and further wherein said antibody or antigen binding fragment thereof comprises a variable light (VL) chain polypeptide at least 90%, 95%, or 99% identical to a polypeptide comprising the amino acid sequence of SEQ ID NO: 5, 6, 7, 8, or 9.

Table 1: COMPLEMENTARY DETERMINING REGION AMINO ACID SEQUENCES

SEQ ID NO	DESCRIPTION	SEQUENCE
19	CDRH1	RYWWH
20	CDRH2	RIDPNSGTTRFIEKFKT
21	CDRH3	WGPYYGYAMDY
22	CDRL1	SASSSVTYMH
23	CDRL2	DTSRLAP
24	CDRL3	HQWSTYPPT

Table 2: VH CHAIN AMINO ACID SEQUENCES

SEQ ID NO	AMINO ACID SEQUENCE
1	QVQLVQSGAELKKPGASVKVSCKASGFTFT**RYWWH**WV RQAPGKGLEWIG**RIDPNSGTTRFIEKFKT**RATITVDKSTST AYMHLSSLRSEDSAVYYCAR**WGPYYGYAMDY**WGQGTS VTVSS
2	QVQLVQSGAELKKPGASVKVSCKASGFTFT**RYWWH**WVRQ APGKGLEWIG**RIDPNSGTTRFIEKFKT**RATITVDKSTSTAY MELSSLRSEDSAVYYCAR**WGPYYGYAMDY**WGQGTSVTV SS
3	QVQLVQSGAEVKKPGASVKVSCKASGFTFT**RYWWH**WV RQAPGKGLEWIG**RIDPNSGTTRFIEKFKT**RATITVDKSTST AYMELSSLRSEDTAVYYCAR**WGPYYGYAMDY**WGQGTL VTVSS
4	QVQLVQSGAEVKKPGASVKVSCKASGFTFT**RYWWH**WV RQAPGKGLEWIG**RIDPNSGTTRFIEKFKT**RVTITVDKSTST AYMELSSLRSEDTAVYYCAR**WGPYYGYAMDY**WGQGTL VTVSS

Table 3: VL CHAIN AMINO ACID SEQUENCES

SEQ ID NO	AMINO ACID SEQUENCE
5	QIVLTQSPAILSLSPGERVTITC**SASSSVTYMH**WYQQKLG KAPKLWIY**DTSRLAP**GSPARFSGSGSGTDYTLTISSLETEDF ASYFC**HQWSTYPPT**FGQGTKLEIK
6	QIVLTQSPATLSLSPGERVTITC**SASSSVTYMH**WYQQKLGKA PKLWIY**DTSRLAP**GSPARFSGSGSGTDYTLTISSLESEDFASY FC**HQWSTYPPT**FGQGTKLEIK
7	QIVLTQSPATLSLSPGERVTITC**SASSSVTYMH**WYQQKPG KAPKLWIY**DTSRLAP**GSPARFSGSGSGTDYTLTISSLESEDF ASYFC**HQWSTYPPT**FGQGTKLEIK
8	QIVLTQSPATLSLSPGERVTITC**SASSSVTYMH**WYQQKPGKA PKLWIY**DTSRLAP**GSPARFSGSGSGTDYTLTISSLESEDFATY FC**HQWSTYPPT**FGQGTKLEIK
9	QIVLTQSPATLSLSPGERVTITC**SASSSVTYMH**WYQQKPGKA PKLLIY**DTSRLAP**GSPARFSGSGSGTDYTLTISSLESEDFATY FC**HQWSTYPPT**FGQGTKLEIK

Table 4: HEAVY CHAIN AND LIGHT CHAIN AMINO ACID SEQUENCES

SEQ ID NO	AMINO ACID SEQUENCE
15	QVQLVQSGAELKKPGASVKVSCKASGFTFTRYWWHWV RQAPGKGLEWIGRIDPNSGTTRFIEKFKTRATITVDKSTST AYMELSSLRSEDSAVYYCARWGPYYGYAMDYWGQGTS VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
16	QIVLTQSPATLSLSPGERVTITCSASSSVTYMHWYQQKPG KAPKLLIYDTSRLAPGSPARFSGSGSGTDYTLTISSLESEDF ATYFCHQWSTYPPTFGQGTKLEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC

In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the

amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 5, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 6, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 7, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 8, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 9, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 2 and SEQ ID NO: 5, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 2 and SEQ ID NO: 6, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 2 and SEQ ID NO: 7, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 2 and SEQ ID NO: 8, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 2 and SEQ ID NO: 9, respectively.

In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 3 and SEQ ID NO: 5, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid

sequences of SEQ ID NO: 3 and SEQ ID NO: 6, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 3 and SEQ ID NO: 7, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 3 and SEQ ID NO: 8, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 3 and SEQ ID NO: 9, respectively.

In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 4 and SEQ ID NO: 5, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 4 and SEQ ID NO: 6, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 4 and SEQ ID NO: 7, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 4 and SEQ ID NO: 8, respectively. In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 4 and SEQ ID NO: 9, respectively.

In some embodiments, the humanized anti-SFRP2 comprises a variable heavy (VH) chain polypeptide and a variable light (VL) chain polypeptide comprising the amino acid sequences of SEQ ID NO: 15 and SEQ ID NO: 16, respectively.

In some aspects, an anti-SFRP2 antibody or antigen binding fragment thereof comprises a chimeric antibody. In some aspects, an anti-SFRP2 antibody or antigen binding fragment thereof comprises a chimeric antibody specific for binding to an epitope of SFRP2. In some embodiments, the epitope of SFRP2 is

NDFALKIKVKEITYINRDT (SEQ ID NO:14). In some aspects, a chimeric anti-SFRP2 antibody comprises a VH chain polypeptide of:

QVQLQQPGAELVQPGASVMLSCKASGFTFTRYWWHWVRQTPGR GLEWIGRIDPNSGTTRFIEKFKTKATLTVDKPSSTAYMHLSSLTSEDSAVYYCARW GPYYGYAMDYWGPGTSVTVSS (SEQ ID NO: 10) and a VL chain polypeptide of:

QIVLTQSPAIMSASPGQKVTITCSASSSVTYMHWYQQKLGSSPKLW IYDTSRLAPGSPARFSGSGSGTSYSLTISSMETEDAASYFCHQWSTYPPTFGTGTKL EIQ (SEQ ID NO: 11). In some aspects, a chimeric anti-SFRP2 antibody comprises a VH chain polypeptide of SEQ ID NO: 10 and a VL chain polypeptide of:

QIVLTQSPAIMSASPGQKVTITCSASSSVTYMHWYQQKLGSSPKLW IYDTSRLAPGSPARFSGSGSGTSYSLTISSMETEDAASYFCHQWSTYPPTFGTGTKL EIK (SEQ ID NO: 13). In some aspects, a chimeric anti-SFRP2 antibody comprises human constant regions, such as any of those discussed herein. In some instances, a chimeric anti-SFRP2 antibody comprises an IgGI constant region.

In some embodiments, the present invention relates to a composition comprising at least one nucleic acid molecule encoding at least one selected from the group consisting of: a CDR, VH, VL, heavy chain or light chain of an anti-SFRP2 antibody, as described herein. For example in one embodiment, the nucleic acid molecule encodes at least one CDR selected from the group consisting of: SEQ ID NOs: 19-24. In one embodiment, the nucleic acid molecule encodes a VH selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 10. In one embodiment, the nucleic acid molecule encodes a VL selected from the group consisting of: SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 11 and SEQ ID NO: 13. In one embodiment, the nucleic acid molecule encodes a heavy chain comprising SEQ ID NO: 15. In one embodiment, the nucleic acid molecule encodes a light chain comprising SEQ ID NO: 16.

In some embodiments, the present invention relates to a composition comprising a combination of nucleic acid molecules. In some embodiments, the composition comprises a combination of nucleic acid molecules encoding the VH and the VL or the heavy chain and the light chain of a anti-SFRP2 antibody described herein.

In some embodiments, the present invention relates to a composition comprising a nucleic acid molecule encoding a anti-SFRP2 antibody. In some embodiments, the composition comprising a nucleic acid molecule encoding a anti-SFRP2 antibody comprises at least one nucleic acid molecule encoding a light chain amino acid sequence, or a fragment thereof, and at least one nucleic acid molecule encoding a heavy chain amino acid sequence, or a fragment thereof.

In some embodiments, the anti-SFRP2 antibody heavy chain comprises a sequence of SEQ ID NO: 15. In some aspects, the heavy chain is encoded by a polynucleotide sequence of SEQ ID NO:17:

caggtccaactggtgcagtctggggctgagcttaaaaagcctggggcttcagtgaaggtgtcctgcaaggcttctggcttcacct
tcacccgctactggtggcactgggtgcgccaggcccctggaaagggccttgagtggattggaaggattgatcctaatagtggta
ccactcgcttcatcgagaagttcaagacccgcgccacaatcactgtagacaaatccaccagcacagcctacatggaactcagca
gcctgcgctctgaggactctgcggtctattattgtgcaagatggggaccttattacggctatgctatggactactggggtcaagga
acctcagtcaccgtctcctcagcctccaccaagggcccatcggtcttcccccctggcaccctctagcaagagcacctctgggggc
acagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcg
gcgtgcacaccttcccggctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttggg
cacccagacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaatcttgtgaca
aaactcacacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggaca
ccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggt
acgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcg
tcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatc
gagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgacc
aagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagc
cggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctatagcaagctcaccgtggacaaga
gcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctcc
ctgtctcccgggaaatga.

In some embodiments, the anti-SFRP2 antibody light chain comprises a sequence of SEQ ID NO: 16. In some aspects, the light chain is encoded by a polynucleotide sequence of SEQ ID NO:18:

Caaattgttctcacccagtctccagcaaccctgtctttgtctcctggggaaagagtcaccataacctgcagtgccagctcaagtgt
aacctacatgcactggtaccagcagaagccaggcaaagcccccaaactcttgatttatgacacatcccggctggctcctggatct

cctgctcgcttctccggcagtgggtctgggaccgactacaccctcacaatcagcagcctagagtctgaagatttcgccacttattt
ctgccatcagtggagtacctacccacccacgttcggtcaggggaccaagctggagatcaaacgaactgtggctgcaccatctgt
cttcatcttcccgccatctgatgagcagcttaagtccggaactgctagcgttgtgtgcctgctgaataacttctatcccagagaggc
caaagtacagtggaaggtggataacgccctccaatcgggaaactcccaggagagtgtcacagagcaggacagcaaggacag
cacctacagcctcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccat
cagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttag.

In some embodiments, the composition comprises a combination of nucleic acid molecules encoding a first and a second anti-SFRP2 antibody or fragment thereof, wherein the first and second anti-SFRP2 antibody or fragment thereof bind to different epitopes of SFRP2. In some embodiments, the anti-SFRP2 antibody or fragment thereof comprises a light chain comprising SEQ ID NO:18 and a heavy chain comprising SEQ ID NO:17.

Method of Preventing or Treating a Disease or Disorder

The present invention further relates, in part, to methods of preventing or treating diseases or disorders associated with an increased level of SFRP2 in a subject in need thereof. In one aspect, the method comprises administering a therapeutic agent to the subject. In some embodiments, the therapeutic agent reduces the level (e.g., activity, amount, concentration, expression, level, etc.) of SFRP2 according to the method of the present invention. In some embodiments, the therapeutic agent is administered to a subject diagnosed as having a disease or disorder associated with an increased level of SFRP2.

In one aspect, the present invention relates to a method of preventing or treating a disease or disorder associated with an increased level of SFRP2 in a subject in need thereof. In one embodiment, the method comprises administering a treatment to reduce the level (e.g., activity, amount, concentration, expression, level, etc.) of SFRP2 according to the method of the present invention in the subject. In one embodiment, the treatment comprises inhibiting SFRP2. In one embodiment, the treatment comprises administering a therapeutically effective amount of an inhibitor of SFRP2. For example, in some embodiments, the inhibitor of SFRP2 is an antibody, nucleic acid, peptide, small molecule, antagonist, aptamer, peptidomimetic, or a combination thereof.

In one aspect, the present invention relates to a method of preventing or treating solid tumors in a subject in need thereof. In some embodiments the solid tumor is breast cancer, pancreatic cancer, sarcomas, osteosarcomas, ovarian cancer, colon cancer, or lung cancer. In some embodiments the breast cancer is triple-negative breast cancer (TNBC). In one embodiment, the method comprises administering a treatment to decrease the level (e.g., activity, amount, concentration, expression, level, etc.) of SFRP2 in the subject.

In one embodiment, the treatment comprises administering a therapeutically effective amount of an inhibitor of SFRP2. For example, in some embodiments, the inhibitor of SFRP2 is a nucleic acid, an antisense nucleic acid molecule (e.g., siRNA), a peptide, an antibody, a small molecule, or a combination thereof.

In some embodiments, the method of preventing or treating solid tumors comprises administering a treatment to the subject for inhibiting the level or activity of SFRP2 or any combination thereof, or administering an antibody that binds to SFRP2, or a combination thereof.

In one embodiment, the present invention provides a method comprising administering to a subject an inhibitor of SRFP2. In one embodiment, the subject has cancer. In some embodiments, the solid tumor is breast cancer, pancreatic cancer, sarcomas, osteosarcomas, ovarian cancer, colon cancer, or lung cancer. In one embodiment, the subject has breast cancer. In one embodiment, the subject has triple-negative breast cancer.

Examples of SFRP2 inhibitors include, but are not limited to, anti-SFRP2 antibodies as described in U.S. Patent Application No. 17/547,550, the contents of which is incorporated herein in its entirety.

One of skill in the art will appreciate that inhibitors of SFRP2 can be administered acutely (e.g., over a short period of time, such as a day, a week or a month) or chronically (e.g., over a long period of time, such as several months or a year or more). One of ordinary skill in the art will appreciate, based on the disclosure provided herein, that SFRP2 inhibitor compositions can be used to treat or prevent a disease or disorder, inducing macrophage polarization, reducing total tumor associated macrophage number, or a combination thereof in a subject in need thereof.

It will be appreciated by one of skill in the art, when armed with the present disclosure including the methods detailed herein, that the invention is not limited to treatment of a disease or disorder, such as cancer, that is already established. Particularly, the disease or disorder need not have manifested to the point of detriment to the subject; indeed, the disease or disorder need not be detected in a subject before treatment is administered. That is, significant disease or disorder does not have to occur before the present invention may provide benefit. Therefore, the present invention includes a method for preventing a disease or disorder, inducing macrophage polarization, reducing total tumor associated macrophage number, or a combination thereof in a subject, in that a SFRP2 inhibitor composition, as discussed previously elsewhere herein, can be administered to a subject prior to the onset of the disease or disorder, thereby preventing the disease or disorder from developing. The preventive methods described herein also include the treatment of a subject that is in remission for the prevention of a recurrence of a disease or disorder.

One of skill in the art, when armed with the disclosure herein, would appreciate that the prevention of a disease or disorder encompasses administering to a subject a SFRP2 inhibitor composition as a preventative measure against the disease or disorder, including cancer. As more fully discussed elsewhere herein, methods of decreasing the level or activity of SFRP2 encompass a wide plethora of techniques for decreasing not only SFRP2 activity, but also for decreasing expression of a nucleic acid encoding SFRP2, including either a decrease in transcription, a decrease in translation, or both.

Additionally, as disclosed elsewhere herein, one skilled in the art would understand, once armed with the teaching provided herein, that the present invention encompasses a method of preventing a wide variety of diseases, disorders and pathologies where a decrease in expression and/or activity of SFRP2 mediates, treats or prevents the disease, disorder or pathology. Methods for assessing whether a disease relates to the levels or activity of SFRP2 are known in the art. Further, the invention encompasses treatment or prevention of such diseases discovered in the future.

The invention encompasses administration of an inhibitor of SFRP2 to practice the methods of the invention; the skilled artisan would understand, based on the

disclosure provided herein, how to formulate and administer the appropriate SFRP2 inhibitor to a subject. However, the present invention is not limited to any particular method of administration or treatment regimen.

Method of Inducing Macrophage Polarization

The present invention further relates, in part, to methods of inducing macrophage polarization, in particular the ability to promote the polarization of macrophages into a pro-inflammatory M1-type macrophage, in a subject in need thereof. In one aspect, the method comprises administering a SFRP2 inhibitor to a subject to induce tumor associated macrophage (TAM) polarization to M1 phenotype.

Macrophage polarization is a process by which macrophages adopt different functional programs in response to the signals from their microenvironment. This ability is connected to their multiple roles in the organism: they are powerful effector cells of the innate immune system, but also important in removal of cellular debris, embryonic development and tissue repair.

Macrophage phenotypes are broadly divided into 2 groups: M1 (classically-activated macrophages) and M2 (alternatively-activated macrophages). This broad classification is based on in vitro studies, in which cultured macrophages were treated with molecules that stimulated their phenotype switching to particular state. M1 macrophages are pro-inflammatory, important in direct host-defense against pathogen, such as phagocytosis and secretion of pro-inflammatory cytokines and microbicidal molecules. M2 macrophages have quite the opposite function: regulation of the resolution phase of inflammation and the repair of damaged tissues. See, e.g., Wynn, T. A., Chawla, A., & Pollard, J. W. (2013). Origins and Hallmarks of Macrophages: Development, Homeostasis, and Disease. Nature, 496(7446), 445-455; Mills, C. D., Kincaid, K., Alt, J. M., Heilman, M. J., & Hill, A. M. (2000). M-l/M- 2 Macrophages and the Thl/Th2 Paradigm. The Journal of Immunology, 164(12), 6166- 6173.

The inhibitor of the present invention selectively promotes a tissue-resident microenvironment to treat diseases like cancer by polarizing macrophages to an M1 phenotype in the context of this disease.

One aspect of the present invention is the use of a SFRP2 inhibitor to promote macrophage polarization, in particular the ability to promote the polarization of macrophages into a pro-inflammatory M1-type macrophage, in a subject (e.g., a human) in need thereof. Macrophages mature in tissues and are activated in a dynamic response to combinations of stimuli to acquire specialized functional phenotypes that, in certain cases, are detrimental to the individual. As for the lymphocyte system, a dichotomy has been proposed for macrophage activation: classic (M1) vs. alternative (M2). Although several intermediate functional states have been observed, M1 and M2 subtypes remain relevant to describe the extremes on a continuum of macrophage states, M1 being the most pro-inflammatory state and M2 being more associated with a decrease of inflammation.

By "macrophage polarization", it is herein meant that the SFRP2 inhibitor modifies the balance between the different subtypes of macrophages in the treated individual, at least at the phenotypic and/or functional level. Hence, according to the present invention, the treatment of a subject with a SFRP2 inhibitor leads to a modification in the profile of surface markers expressed by the subject's macrophage. An exemplary modification in the profile of surface markers can include, but is not limited to, the increase in M1 markers such as G-CSF, GM-CSF, IFNg, IL-1a, IL-1B, IL2, IL3, IL5, IL7, IL12p70, IL16, IL27, CCL2, CXCL9, TARC, TIMP1, and TNF-α.

According to a particular embodiment of the present invention, macrophage polarization by the SFRP2 inhibitor includes an increase of pro-inflammatory M1-type macrophage polarization. In some embodiments, the SFRP2 inhibitor can induce the emergence of more macrophages exhibiting a M1 phenotypic polarization and an increase of the proportion of macrophages overexpressing cellular markers such as CD86 and CCR7 and/or producing cytokines such as G-CSF, GM-CSF, IFNg, IL-1a, IL-1B, IL2, IL3, IL5, IL7, IL12p70, IL16, IL27, CCL2, CXCL9, TARC, TIMP1, and TNF-α. In some embodiments, the SFRP2 inhibitor induces an inhibition of M2 phenotypic polarization of macrophages, leading to a decrease of the proportion of macrophages overexpressing cellular markers such as CD206, CD1 lb, PD-L1 and/or CD200R and/or producing cytokines such as CCL17. The SFRP2 inhibitor can thus

modulate the macrophage polarization at both phenotypic level (expression of cellular surface markers) and functional level (production of chemokines and cytokines).

Another aspect of the present invention is the use of a SFRP2 inhibitor to reduce the total number of tumor associated macrophages in a subject.

Among the compositions which can be used according to the present invention, one can utilize small chemical molecules, nucleotides, polypeptides, antagonist peptides, antibodies and fragments thereof, especially anti-SFRP2 antibodies such as those used in the experiments described below, any other antibody selected amongst the many anti-SFRP2 commercially available antibodies or any other (new) anti-SFRP2 antagonist antibody, fragments of antibodies, aptamers targeting SFRP2, etc.

Particularly, the SFRP2 inhibitor is selected from the group consisting of an antagonist peptide, an anti-SFRP2 antibody, in particular an anti-SFRP2 antagonist antibody, a nucleic acid encoding such compound, and a compound able to inhibit the expression of the SFRP2 protein, in particular a siRNA. Preferably, an SFRP2 inhibitor is an antagonist peptide or an anti-SFRP2 antibody, in particular an anti-SFRP2 antagonist antibody.

Inducing polarization of macrophages to M1 pro-inflammatory macrophages or reducing the total number of tumor associated macrophages can be useful in a number of pathologies or situations. As described throughout, this modification is particularly useful in the context of cancers, to restore an anti-tumor activity of macrophages.

In some embodiments, the SFRP2 inhibitor is used to treat a subject who has a solid tumor selected from the group consisting of breast cancer, pancreatic cancer, sarcomas, osteosarcomas, ovarian cancer, colon cancer, or lung cancer. In some embodiments, the breast cancer is triple negative breast cancer.

Method of Assessing the Prognosis, Assessing the Effectiveness, or Alleviating the Toxicity of Treatment of a Disease or Disorder

The present invention further relates, in part, to a method of assessing the prognosis or assessing the effectiveness of treatment of a disease or disorder associated with SFRP2 in a subject in need thereof.

In one aspect, the present invention provides a method of assessing the prognosis or assessing the effectiveness of treatment of a disease or disorder in a subject, the method comprising administering to a subject an inhibitor of SFRP2 for the treatment or prevention of a disease or disorder, and measuring a response selected from the group consisting of: a change in the level of interferon-gamma (IFN-γ), a change in macrophage polarization, or a change in the number of tumor associated macrophages. In one aspect, the present invention provides a method of assessing the prognosis or assessing the effectiveness of treatment for solid tumors, the method comprising measuring a response selected from the group consisting of: a change in the level of interferon-gamma (IFN-γ), a change in macrophage polarization, or a change in the number of tumor associated macrophages. In some embodiments the solid tumor is breast cancer.

The present invention further relates, in part, to a method of assessing the prognosis or assessing the effectiveness of treatment of a disease or disorder associated with the level or activity of SFRP2 in a subject in need thereof. In one aspect, the present invention provides a method of assessing the prognosis or assessing the effectiveness of treatment for solid tumors in a subject, the method comprising assessing the level or activity of SFRP2 in the subject.

In one embodiment, the subject is effectively treated if the level of interferon-gamma (IFN-γ) is increased in a sample obtained from a subject following administration of the SFRP2 inhibitor compared to the level of interferon-gamma (IFN-γ) prior to administration. In one embodiment, the subject is effectively treated if macrophage polarization to a classically activated M1 is increased in a sample obtained from a subject following administration of the SFRP2 inhibitor compared to the level of macrophage polarization prior to administration. In one embodiment, the subject is effectively treated if the number of tumor associated macrophages is decreased in a sample obtained from a subject following administration of the SFRP2 inhibitor compared to the number of tumor associated macrophages prior to administration. In some embodiments, if the subject is effectively treated administration of the SFRP2 inhibitor is continued.

Information obtained from the methods of the invention described herein can be used alone, or in combination with other information (e.g., age, family history,

disease status, disease history, vital signs, blood chemistry, PSA level, Gleason score, primary tumor staging, lymph node staging, metastasis staging, expression of other gene signatures relevant to outcomes of a disease or disorder, such as autoimmune disease or disorder, cancer, inflammatory disease or disorder, metabolic disease or disorder, neurodegenerative disease or disorder, organ tissue rejection, organ transplant rejection, or any combination thereof, etc.) from the subject or from the biological sample obtained from the subject.

Pharmaceutical Compositions

The present invention also provides various pharmaceutical compositions comprising an inhibitor of the present invention. In some embodiments, the composition of the invention inhibits SFRP2.

In various aspects, the composition comprises: one or more inhibitor of the present invention and one or more stabilizers. In various embodiments, the stabilizer to compound weight ratio is less than 50%. In one embodiment, the stabilizer comprises a biocompatible polymer. Examples of stabilizers include, but are not limited to, biocompatible polymer, a biodegradable polymer, a multifunctional linker, starch, modified starch, and starch derivatives, gums, including but not limited to polymers, polypeptides, albumin, amino acids, thiols, amines, carboxylic acid and combinations or derivatives thereof, citric acid, xanthan gum, alginic acid, other alginates, benitoniite, veegum, agar, guar, locust bean gum, gum arabic, quince psyllium, flax seed, okra gum, arabinoglactin, pectin, tragacanth, scleroglucan, dextran, amylose, amylopectin, dextrin, etc., cross-linked polyvinylpyrrolidone, ion-exchange resins, potassium polymethacrylate, carrageenan (and derivatives), gum karaya and biosynthetic gum, polycarbonates (linear polyesters of carbonic acid); microporous materials (bisphenol, a microporous poly(vinylchloride), micro-porous polyamides, microporous modacrylic copolymers, microporous styrene-acrylic and its copolymers); porous polysulfones, halogenated poly(vinylidene), polychloroethers, acetal polymers, polyesters prepared by esterification of a dicarboxylic acid or anhydride with an alkylene polyol, poly(alkylenesulfides), phenolics, polyesters, asymmetric porous polymers, cross-linked olefin polymers, hydrophilic microporous homopolymers, copolymers or interpolymers

having a reduced bulk density, and other similar materials, poly(urethane), cross-linked chain-extended poly(urethane), poly(imides), poly(benzimidazoles), collodion, regenerated proteins, semi-solid cross-linked poly(vinylpyrrolidone), monomeric, dimeric, oligomeric or long-chain, copolymers, block polymers, block co-polymers, polymers, PEG, dextran, modified dextran, polyvinylalcohol, polyvinylpyrollidone, polyacrylates, polymethacrylates, polyanhydrides, polypeptides, albumin, alginates, amino acids, thiols, amines, carboxylic acids, or combinations thereof.

The compositions may be formulated in a pharmaceutically acceptable excipient, such as wetting agents, buffers, disintegrants, binders, fillers, flavoring agents and liquid carrier media such as sterile water, water/ethanol etc. The compositions should be suitable for administration either by topical administration or injection or inhalation or catheterization or instillation or transdermal introduction into any of the various body cavities including the alimentary canal, the vagina, the rectum, the bladder, the ureter, the urethra, the mouth, etc. For oral administration, the pH of the composition is preferably in the acid range (e.g., 2 to 7) and buffers or pH adjusting agents may be used. The contrast media may be formulated in conventional pharmaceutical administration forms, such as tablets, capsules, powders, solutions, dispersion, syrups, suppositories etc.

The compositions of the invention can be formulated and administered to a subject, as now described. The invention encompasses the preparation and use of pharmaceutical compositions comprising the compositions of the invention useful for the delivery of a therapeutic agent to a cell. The invention also encompasses the preparation and use of pharmaceutical compositions comprising the compositions of the invention useful for the treatment of a disease or disorder. The invention also encompasses the preparation and use of pharmaceutical compositions comprising the compositions of the invention useful for improved cell penetration.

Such a pharmaceutical composition may consist of the active ingredient alone, in a form suitable for administration to a subject, or the pharmaceutical composition may comprise the active ingredient and one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The active ingredient may be present in the pharmaceutical composition in the form of a

physiologically acceptable ester or salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

In various embodiments, the pharmaceutical compositions useful in the methods of the invention may be administered, by way of example, systemically, parenterally, or topically, such as, in oral formulations, inhaled formulations, including solid or aerosol, and by topical or other similar formulations. In addition to the appropriate therapeutic composition, such pharmaceutical compositions may contain pharmaceutically acceptable carriers and other ingredients known to enhance and facilitate drug administration. Other possible formulations, such as nanoparticles, liposomes, resealed erythrocytes, and immunologically based systems may also be used to administer an appropriate modulator thereof, according to the methods of the invention.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

Pharmaceutical compositions that are useful in the methods of the invention may be prepared, packaged, or sold in formulations suitable for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, intravenous, ophthalmic, intrathecal and other known routes of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient (e.g., a SFRP2 inhibitor), and immunologically-based formulations.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a “unit dose” is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

The relative amounts of the active ingredient (e.g., the SFRP2 inhibitor), the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient. In various embodiments, the composition comprises at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 11%, at least about 12%, at least about 13%, at least about 14%, at least about 15%, at least about 16%, at least about 17%, at least about 18%, at least about 19%, at least about 20%, at least about 21%, at least about 22%, at least about 23%, at least about 24%, at least about 25%, at least about 26%, at least about 27%, at least about 28%, at least about 29%, at least about 30%, at least about 31%, at least about 32%, at least about 33%, at least about 34%, at least about 35%, at least about 36%, at least about 37%, at least about 38%, at least about 39%, at least about 40%, at least about 41%, at least about 42%, at least about 43%, at least about 44%, at least about 45%, at least about 46%, at least about 47%, at least about 48%, at least about 49%, at least about 50%, at least about 51%, at least about 52%, at least about 53%, at least about 54%, at least about 55%, at least about 56%, at least about 57%, at least about 58%, at least about 59%, at least about 60%, at least about 61%, at least about 62%, at least about 63%, at least about 64%, at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% (w/w) active ingredient.

In addition to the active ingredient, a pharmaceutical composition of the invention may further comprise one or more additional pharmaceutically active agents.

Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology.

A formulation of a pharmaceutical composition of the invention suitable for oral administration may be prepared, packaged, or sold in the form of a discrete solid dose unit including, but not limited to, a tablet, a hard or soft capsule, a cachet, a troche, or a lozenge, each containing a predetermined amount of the active ingredient. Other formulations suitable for oral administration include, but are not limited to, a powdered or granular formulation, an aqueous or oily suspension, an aqueous or oily solution, or an emulsion.

A tablet comprising the active ingredient (e.g., a SFRP2 inhibitor) may, for example, be made by compressing or molding the active ingredient, optionally with one or more additional ingredients. Compressed tablets may be prepared by compressing, in a suitable device, the active ingredient in a free-flowing form such as a powder or granular preparation, optionally mixed with one or more of a binder, a lubricant, an excipient, a surface active agent, and a dispersing agent. Molded tablets may be made by molding, in a suitable device, a mixture of the active ingredient, a pharmaceutically acceptable carrier, and at least sufficient liquid to moisten the mixture. Pharmaceutically acceptable excipients used in the manufacture of tablets include, but are not limited to, inert diluents, granulating and disintegrating agents, binding agents, and lubricating agents. Known dispersing agents include, but are not limited to, potato starch and sodium starch glycolate. Known surface active agents include, but are not limited to, sodium lauryl sulphate. Known diluents include, but are not limited to, calcium carbonate, sodium carbonate, lactose, microcrystalline cellulose, calcium phosphate, calcium hydrogen phosphate, and sodium phosphate. Known granulating and disintegrating agents include, but are not limited to, corn starch and alginic acid. Known binding agents include, but are not limited to, gelatin, acacia, pre-gelatinized maize starch, polyvinylpyrrolidone, and hydroxypropyl methylcellulose. Known lubricating agents include, but are not limited to, magnesium stearate, stearic acid, silica, and talc.

Tablets may be non-coated or they may be coated using known methods to achieve delayed disintegration in the gastrointestinal tract of a subject, thereby providing sustained release and absorption of the active ingredient. By way of example, a material

such as glyceryl monostearate or glyceryl distearate may be used to coat tablets. Further by way of example, tablets may be coated using methods described in U.S. Pat. Nos. 4,256,108; 4,160,452; and 4,265,874 to form osmotically-controlled release tablets. Tablets may further comprise a sweetening agent, a flavoring agent, a coloring agent, a preservative, or some combination of these in order to provide pharmaceutically elegant and palatable preparation.

Hard capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such hard capsules comprise the active ingredient, and may further comprise additional ingredients including, for example, an inert solid diluent such as calcium carbonate, calcium phosphate, or kaolin.

Soft gelatin capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such soft capsules comprise the active ingredient, which may be mixed with water or an oil medium such as peanut oil, liquid paraffin, or olive oil.

Liquid formulations of a pharmaceutical composition of the invention which are suitable for oral administration may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

Liquid suspensions may be prepared using conventional methods to achieve suspension of the active ingredient in an aqueous or oily vehicle. Aqueous vehicles include, for example, water and isotonic saline. Oily vehicles include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin. Liquid suspensions may further comprise one or more additional ingredients including, but not limited to, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent.

Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, and hydroxypropylmethylcellulose. Known dispersing or wetting agents

include, but are not limited to, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (e.g. polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include, but are not limited to, lecithin and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl-para-hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. The

oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

Methods for impregnating or coating a material with a chemical composition are known in the art, and include, but are not limited to methods of depositing or binding a chemical composition onto a surface, methods of incorporating a chemical composition into the structure of a material during the synthesis of the material (i.e., such as with a physiologically degradable material), and methods of absorbing an aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

Parenteral administration of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of an individual and administration of the pharmaceutical composition through the breach in the tissue. Parental administration can be local, regional or systemic. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, intravenous, intraocular, intravitreal, subcutaneous, intraperitoneal, intramuscular, intradermal, intrasternal injection, and intratumoral.

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a

preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e., powder or granular) form for reconstitution with a suitable vehicle (e.g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Formulations suitable for topical administration include, but are not limited to, liquid or semi-liquid preparations such as liniments, lotions, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically-administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 to about 7 nanometers, and preferably from about 1 to about 6 nanometers. Such compositions are conveniently in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder or using a self-propelling solvent/powder-dispensing container such as a device comprising the active ingredient dissolved or suspended in a low-boiling propellant in a sealed container. Preferably, such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nanometers and at least 95% of the particles by number have a diameter less than 7 nanometers. More preferably, at least 95% of the particles by weight have a diameter greater than 1 nanometer and at least 90% of the particles by number have a diameter less than 6 nanometers. In some embodiments, dry powder compositions include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

Low boiling propellants generally include liquid propellants having a boiling point of below 65 °F at atmospheric pressure. Generally, the propellant may constitute 50 to 99.9% (w/w) of the composition, and the active ingredient may constitute 0.1 to 20% (w/w) of the composition. The propellant may further comprise additional ingredients such as a liquid non-ionic or solid anionic surfactant or a solid diluent (in some embodiments having a particle size of the same order as particles comprising the active ingredient).

Pharmaceutical compositions of the invention formulated for pulmonary delivery may also provide the active ingredient in the form of droplets of a solution or suspension. Such formulations may be prepared, packaged, or sold as aqueous or dilute alcoholic solutions or suspensions, optionally sterile, comprising the active ingredient, and may conveniently be administered using any nebulization or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, or a preservative such as methylhydroxybenzoate. The

droplets provided by this route of administration preferably have an average diameter in the range from about 0.1 to about 200 nanometers.

The formulations described herein as being useful for pulmonary delivery are also useful for intranasal delivery of a pharmaceutical composition of the invention.

Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 to 500 micrometers.

Such a formulation is administered in the manner in which snuff is taken i.e. by rapid inhalation through the nasal passage from a container of the powder held close to the nares. Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of the active ingredient, and may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conventional methods, and may, for example, contain 0.1 to 20% (w/w) active ingredient, the balance comprising an orally dissolvable or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder or an aerosolized or atomized solution or suspension comprising the active ingredient. Such powdered, aerosolized, or aerosolized formulations, when dispersed, preferably have an average particle or droplet size in the range from about 0.1 nanomaters to about 2000 micrometers, and may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for ophthalmic administration. Such formulations may, for example, be in the form of eye drops including, for example, a 0.1-1.0% (w/w) solution or suspension of the active ingredient in an aqueous or oily liquid carrier. Such drops may further comprise buffering agents, salts, or one or more other of the additional ingredients described herein. Other opthalmically-administrable

formulations which are useful include those which comprise the active ingredient in microcrystalline form or in a liposomal preparation.

As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" which may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Genaro, ed., 1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa.

Administration of the compounds of the present invention or the compositions thereof may be continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the agents of the invention may be essentially continuous over a preselected period of time or may be in a series of spaced doses. Both local and systemic administration is contemplated. The amount administered will vary depending on various factors including, but not limited to, the composition chosen, the particular disease, the weight, the physical condition, and the age of the mammal, and whether prevention or treatment is to be achieved. Such factors can be readily determined by the clinician employing animal models or other test systems which are well known to the art.

One or more suitable unit dosage forms having the therapeutic agent(s) of the invention, which, as discussed below, may optionally be formulated for sustained release (for example using microencapsulation, see WO 94/07529, and U.S. Pat. No. 4,962,091 the disclosures of which are incorporated by reference herein), can be administered by a variety of routes including parenteral, including by intravenous and intramuscular routes, as well as by direct injection into the diseased tissue. For example,

the therapeutic agent may be directly injected into the muscle. The formulations may, where appropriate, be conveniently presented in discrete unit dosage forms and may be prepared by any of the methods well known to pharmacy. Such methods may include the step of bringing into association the therapeutic agent with liquid carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combinations thereof, and then, if necessary, introducing or shaping the product into the desired delivery system.

When the therapeutic agents of the invention are prepared for administration, they are preferably combined with a pharmaceutically acceptable carrier, diluent or excipient to form a pharmaceutical formulation, or unit dosage form. The total active ingredients in such formulations include from 0.1 to 99.9% by weight of the formulation. A "pharmaceutically acceptable" is a carrier, diluent, excipient, and/or salt that is compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof. The active ingredient for administration may be present as a powder or as granules; as a solution, a suspension or an emulsion.

Pharmaceutical formulations containing the therapeutic agents of the invention can be prepared by procedures known in the art using well known and readily available ingredients. The therapeutic agents of the invention can also be formulated as solutions appropriate for parenteral administration, for instance by intramuscular, subcutaneous or intravenous routes.

The pharmaceutical formulations of the therapeutic agents of the invention can also take the form of an aqueous or anhydrous solution or dispersion, or alternatively the form of an emulsion or suspension.

Thus, the therapeutic agent may be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be presented in unit dose form in ampules, pre-filled syringes, small volume infusion containers or in multi-dose containers with an added preservative. The active ingredients may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredients may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

It will be appreciated that the unit content of active ingredient or ingredients contained in an individual aerosol dose of each dosage form need not in itself constitute an effective amount for treating the particular indication or disease since the necessary effective amount can be reached by administration of a plurality of dosage units. Moreover, the effective amount may be achieved using less than the dose in the dosage form, either individually, or in a series of administrations.

The pharmaceutical formulations of the present invention may include, as optional ingredients, pharmaceutically acceptable carriers, diluents, solubilizing or emulsifying agents, and salts of the type that are well-known in the art. Specific non-limiting examples of the carriers and/or diluents that are useful in the pharmaceutical formulations of the present invention include water and physiologically acceptable buffered saline solutions, such as phosphate buffered saline solutions pH 7.0-8.0.

In general, water, suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration contain the active ingredient, suitable stabilizing agents and, if necessary, buffer substances. Antioxidizing agents such as sodium bisulfate, sodium sulfite or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used are citric acid and its salts and sodium Ethylenediaminetetraacetic acid (EDTA). In addition, parenteral solutions can contain preservatives such as benzalkonium chloride, methyl or propyl-paraben and chlorobutanol. Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, a standard reference text in this field.

The active ingredients of the invention may be formulated to be suspended in a pharmaceutically acceptable composition suitable for use in mammals and in particular, in humans. Such formulations include the use of adjuvants such as muramyl dipeptide derivatives (MDP) or analogs that are described in U.S. Patent Nos. 4,082,735; 4,082,736; 4,101,536; 4,185,089; 4,235,771; and 4,406,890. Other adjuvants, which are useful, include alum (Pierce Chemical Co.), lipid A, trehalose dimycolate and dimethyldioctadecylammonium bromide (DDA), Freund's adjuvant, and IL 12. Other components may include a polyoxypropylene-polyoxyethylene block polymer

(Pluronic®), a non-ionic surfactant, and a metabolizable oil such as squalene (U.S. Patent No. 4,606,918).

Additionally, standard pharmaceutical methods can be employed to control the duration of action. These are well known in the art and include control release preparations and can include appropriate macromolecules, for example polymers, polyesters, polyamino acids, polyvinyl, pyrolidone, ethylenevinylacetate, methyl cellulose, carboxymethyl cellulose or protamine sulfate. The concentration of macromolecules as well as the methods of incorporation can be adjusted in order to control release. Additionally, the agent can be incorporated into particles of polymeric materials such as polyesters, polyamino acids, hydrogels, poly (lactic acid) or ethylenevinylacetate copolymers. In addition to being incorporated, these agents can also be used to trap the compound in microcapsules.

Accordingly, the composition of the present invention may be delivered via various routes and to various sites in a mammal body to achieve a particular effect (see, e.g., Rosenfeld et al., 1991; Rosenfeld et al., 1991a; Jaffe et al., supra; Berkner, supra). One skilled in the art will recognize that although more than one route can be used for administration, a particular route can provide a more immediate and more effective reaction than another route. In one embodiment, the composition described above is administered to the subject by subretinal injection. In other embodiments, the composition is administered by intravitreal injection. Other forms of administration that may be useful in the methods described herein include, but are not limited to, direct delivery to a desired organ (e.g., the eye), oral, inhalation, intranasal, intratracheal, intravenous, intramuscular, subcutaneous, intradermal, and other parental routes of administration. Additionally, routes of administration may be combined, if desired. In another embodiments, route of administration is subretinal injection or intravitreal injection.

The active ingredients of the present invention can be provided in unit dosage form wherein each dosage unit, e.g., a teaspoonful, tablet, solution, or suppository, contains a predetermined amount of the composition, alone or in appropriate combination with other active agents. The term “unit dosage form” as used herein refers to physically discrete units suitable as unitary dosages for human and mammal subjects,

each unit containing a predetermined quantity of the compositions of the present invention, alone or in combination with other active agents, calculated in an amount sufficient to produce the desired effect, in association with a pharmaceutically acceptable diluent, carrier, or vehicle, where appropriate. The specifications for the unit dosage forms of the present invention depend on the particular effect to be achieved and the particular pharmacodynamics associated with the composition in the particular host.

The pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of at least about 1 ng/kg, at least about 5 ng/kg, at least about 10 ng/kg, at least about 25 ng/kg, at least about 50 ng/kg, at least about 100 ng/kg, at least about 500 ng/kg, at least about 1 μg/kg, at least about 5 μg/kg, at least about 10 μg/kg, at least about 25 μg/kg, at least about 50 μg/kg, at least about 100 μg/kg, at least about 500 μg/kg, at least about 1 mg/kg, at least about 5 mg/kg, at least about 10 mg/kg, at least about 25 mg/kg, at least about 50 mg/kg, at least about 100 mg/kg, at least about 200 mg/kg, at least about 300 mg/kg, at least about 400 mg/kg, and at least about 500 mg/kg of body weight of the subject.

In some embodiments, the pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of no more than about 1 ng/kg, no more than about 5 ng/kg, no more than about 10 ng/kg, no more than about 25 ng/kg, no more than about 50 ng/kg, no more than about 100 ng/kg, no more than about 500 ng/kg, no more than about 1 μg/kg, no more than about 5 μg/kg, no more than about 10 μg/kg, no more than about 25 μg/kg, no more than about 50 μg/kg, no more than about 100 μg/kg, no more than about 500 μg/kg, no more than about 1 mg/kg, no more than about 5 mg/kg, no more than about 10 mg/kg, no more than about 25 mg/kg, no more than about 50 mg/kg, no more than about 100 mg/kg, no more than about 200 mg/kg, no more than about 300 mg/kg, no more than about 400 mg/kg, and no more than about 500 mg/kg of body weight of the subject. Also contemplated are dosage ranges between any of the doses disclosed herein.

Typically, dosages which may be administered in a method of the invention to a subject, in some embodiments a human, range in amount from 0.5 μg to about 100 g per kilogram of body weight of the subject. While the precise dosage administered will vary depending upon any number of factors, including but not limited

to, the type of subject and type of disease state being treated, the age of the subject and the route of administration. In some embodiments, the dosage of the compound will vary from about 1 μg to about 10 mg per kilogram of body weight of the subject. In other embodiments, the dosage will vary from about 3 μg to about 1 mg per kilogram of body weight of the subject.

The compositions may be administered to a subject as frequently as several times daily, or it may be administered less frequently, such as once a day, twice a day, thrice a day, once a week, twice a week, thrice a week, once every two weeks, twice every two weeks, thrice every two weeks, once a month, twice a month, thrice a month, or even less frequently, such as once every several months or even once or a few times a year or less. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type and age of the subject, etc. The formulations of the pharmaceutical compositions may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

Individuals to which administration of the pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as non-human primates, cattle, pigs, horses, sheep, cats, and dogs.

These compositions described herein are by no means all inclusive, and further modifications to suit the specific application will be apparent to the ordinary skilled artisan. Moreover, the effective amount of the compositions can be further approximated through analogy to compounds known to exert the desired effect.

EXEMPLARY EMBODIMENTS

This invention provides the following non-limiting embodiments.

Embodiment 1 is a method of inducing macrophage polarization in a subject having a solid tumor, the method comprising administering to the subject an inhibitor of SFRP2.

Embodiment 2 is the method of embodiment 1, wherein the inhibitor is selected from the group consisting of a chemical compound, a protein, a peptide, a peptidomimetic, an antibody, an antibody fragment, an antibody mimetic, a chimeric antibody, a bispecific antibody, a ribozyme, a small molecule chemical compound, a monobody, a short hairpin RNA, RNAi, siRNA, miRNA, or a nucleic acid encoding an antisense nucleic acid molecule.

Embodiment 3 is the method of embodiment 1 or 2, wherein the inhibitor is an antibody.

Embodiment 4 is the method of any one of embodiments 1 through 3, wherein the antibody is an anti-SFRP2 antibody.

Embodiment 5 is the method of any one of embodiments 1 through 4, wherein the anti-SFRP2 antibody comprises a complementary determining region (CDR) H1 comprising the amino acid sequence of SEQ ID NO:19, a CDR H2 comprising the amino acid sequence of SEQ ID NO:20, a CDR H3 comprising the amino acid sequence of SEQ ID NO:21, a CDR LI comprising the amino acid sequence of SEQ ID NO:22, a CDR L2 comprising the amino acid sequence of SEQ ID NO:23, and a CDR L3 comprising the amino acid sequence of SEQ ID NO:24.

Embodiment 6 is the method of any one of embodiments 1 through 5, wherein the solid tumor is breast cancer.

Embodiment 7 is the method of any one of embodiments 1 through 6, wherein the breast cancer is triple negative metastatic breast cancer.

Embodiment 8 is the method of any one of embodiments 1 through 7, wherein the macrophage is polarized into a classically activated M1.

Embodiment 9 is the method of any one of embodiments 1 through 8, wherein the classically activated M1 expresses at least one M1 marker, wherein the M1 marker is selected from the group consisting of G-CSF, GM-CSF, IFNg, IL-1a, IL-1B, IL2, IL3, IL5, IL7, IL12p70, IL16, IL27, CCL2, CXCL9, TARC, TIMP1, or TNF-α.

Embodiment 10 is a method of reducing the number of tumor associated macrophages in a subject, the method comprising administering to the subject an inhibitor of SFRP2.

Embodiment 11 is the method of embodiment 10, wherein the inhibitor is selected from the group consisting of: a chemical compound, a protein, a peptide, a peptidomimetic, an antibody, an antibody fragment, an antibody mimetic, a chimeric antibody, a bispecific antibody, a ribozyme, a small molecule chemical compound, a monobody, a short hairpin RNA, RNAi, siRNA, miRNA, or a nucleic acid encoding an antisense nucleic acid molecule.

Embodiment 12 is the method of embodiment 10 or 11, wherein the inhibitor is an antibody.

Embodiment 13 is the method of any one of embodiments 10 through 12, wherein the antibody is an anti-SFRP2 antibody.

Embodiment 14 is the method of any one of embodiments 10 through 13, wherein the anti-SFRP2 antibody comprises a complementary determining region (CDR) H1 comprising the amino acid sequence of SEQ ID NO:19, a CDR H2 comprising the amino acid sequence of SEQ ID NO:20, a CDR H3 comprising the amino acid sequence of SEQ ID NO:21, a CDR LI comprising the amino acid sequence of SEQ ID NO:22, a CDR L2 comprising the amino acid sequence of SEQ ID NO:23, and a CDR L3 comprising the amino acid sequence of SEQ ID NO:24.

Embodiment 15 is the method of any one of embodiments 10 through 14, wherein the subject has a solid tumor.

Embodiment 16 is a method comprising: a) administering to a subject an inhibitor of SFRP2 for the treatment or prevention of a disease or disorder, and b) measuring a response selected from the group consisting of: a change in the level of interferon-gamma (IFN-γ), a change in macrophage polarization, or a change in the number of tumor associated macrophages.

Embodiment 17 is the method of embodiment 16, wherein the level of interferon-gamma (IFN-γ) is increased, thereby indicating that the inhibitor is effective in treating the disease or disorder in the subject.

Embodiment 18 is the method of embodiment 16 or 17, wherein macrophage polarization to a classically activated M1 is increased, thereby indicating that the inhibitor is effective in treating the disease or disorder in the subject.

Embodiment 19 is the method of any one of embodiments 16 through 18, wherein the number of tumor associated macrophages is decreased, thereby indicating that the inhibitor is effective in treating the disease or disorder in the subject.

EXPERIMENTAL EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the present invention and practice the claimed methods. The following working examples therefore, specifically point out certain embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

Example 1: A humanized monoclonal antibody to secreted frizzled related protein 2 polarizes macrophages into classically activated M1.

Secreted frizzled-related protein 2 (SFRP2) is emerging as a promising novel target in the battle against TNBC. SFRP2 plays a dual role- it induces angiogenesis and simultaneously shields tumors from apoptosis. Additionally, it promotes tumor migration, contributing to aggressive behavior (Siamakpour-Reihani S, et al., PLoSOne 2011;6(6):e20412). SFRP2 acts as a key mediator of the interactions between breast cancer cells and lung alveolar type 1 cells. This interaction promotes lung metastases, making SFRP2 a critical player in breast cancer (Montagner M, et al., Nat Cell Biol 2020;22(3):289-96). While some members of the SFRP family (like SFRP1/3/5) act as tumor suppressors in breast cancer, SFRP2 promotes breast tumor growth (Wu ZH, et al.,

Cell Transplant 2020;29:963689720962479). *SFRP2* mRNA is overexpressed in breast tumor vessels compared to normal human breast tumor endothelium (Bhati R, et al., Am J Pathol 2008;172(5):1381-90); immunohistochemistry confirms this finding (Courtwright A, et al., Cancer Res 2009). Elevated serum SFRP2 levels in humans correlate with breast cancer and serve as a prognostic marker for survival (Huang C, et al., Dis Markers 2019;2019:6149381). Wu et al performed a comprehensive analysis of SFRPs in breast cancer using the ONCOMINE databases. Their findings revealed intriguing patterns related to SFRP2 expression. In one dataset within ONCOMINE, *SFRP2* expression was 4.89-fold higher in the ductal breast carcinoma tissues compared to normal tissues. Similarly, in the context of invasive ductal breast carcinoma, SFRP2 exhibited a 4.202-fold increase compared to normal breast tissue. In a separate data set, SFRP2 was upregulated 19-fold in invasive breast cancer compared to normal tissues (Wu ZH, et al., Cell Transplant 2020;29:963689720962479). In summary, the wealth of evidence points to SFRP2 as a promising therapeutic target in the complex landscape of breast cancer.

SFRP2 orchestrates the functions of multiple cell types in cancer biology. It is a Wnt signaling protein that interacts with frizzled-5 (FZD5) and activates the calcineurin/ nuclear factor of activated T cells (NFAT) pathway in endothelial cells to stimulate angiogenesis. In tumor cells, activation of NFAT by SFRP2 amplifies migration and reduces apoptosis (Siamakpour-Reihani S, et al., PLoSOne 2011;6(6):e20412; Courtwright A, et al., Cancer Res 2009; Peterson YK, et al., Angiogenesis 2017;20(4):615-28). In activated T-cells, SFRP2 increases CD38 and PD-1 mRNA and protein. CD38 regulates NAD+ levels, which directs T-cell fate and metabolic pathways. CD38 is responsible for aggregation and polarization of macrophages (Li W, et al., Front Oncol 2022;12:775649). In select tumors CD38 upregulation induces resistance to PD-1/PD-L1 blocking antibodies, which can be overcome by co-inhibition of CD38 and PD-L1 to improve antitumor immune response (Chen L, et al., Cancer Discov 2018;8(9):1156-75), and the combination of hSFRP2 monoclonal antibodies (mAb) and PD-1 mAb was synergistic in inhibiting the growth of osteosarcoma metastases (Nasarre P, et al., Cancers (Basel) 2021;13(11)). The inquiry into CD38 expressed in breast cancer remains an uncharted territory. While the role of SFRP2 in T-cell exhaustion has been described (Nasarre P, et al., Cancers (Basel) 2021;13(11)), its presence within

macrophages and potential impact on macrophage polarization, and whether it plays a role in macrophage polarization is unknown. Its use as a cancer therapeutic is described herein.

SFRP2 plays a major role in regulation of CD38 and PD-1 in activated T-cells in other tumors

SFRP2 was previously shown to increase CD38 and PD-1 mRNA and protein expression in activated T-cells in vitro; while hSFRP2 mAb reduces CD38 and PD-1 in tumor infiltrating lymphocytes (TILS) from metastatic osteosarcoma in vivo. When combined with a PD-1 mAb, hSFRP2 mAb shows a synergistic effect in reducing the number of metastatic nodules. Unlike CD38, which is ubiquitously expressed, SFRP2 expression is restricted to tumors and tumor microenvironment with lower expression in normal tissue (Bhati R, et al., Am J Pathol 2008;172(5):1381-90; Tsuruta JK, et al., PLoS One 2014;9(1):e86642; Tsuruta JK, et al., PLoS One 2017;12(3):e0174281).

Activated T-cells to increase expression of PD-1 and CD38 mRNA and protein

The role of SFRP2 is only starting to be explored in the tumor immune microenvironment. In metastatic osteosarcoma, it was found that SFRP2 contributed to T-cell exhaustion by increasing PD-1 and CD38, and the combination of hSFRP2 mAb and a PD-1 mAb was synergistic at inhibiting metastatic osteosarcoma (Garcia D, et al., Ann Surg Oncol 2019;26(13):4782-90).

A new humanized antibody against SFRP2 (hSFRP2 mAb) that effectively blocked the growth of primary Hs578t xenografts in mice was previously developed, and a significant decrease in tumor volume compared to IgG1 control was found (Garcia D, et al., Ann Surg Oncol 2019;26(13):4782-90). The time course T-cell study showed that hSFRP2 mAb did not trigger any immune reactions in healthy donors. The pharmacokinetic studies showed a half-life of 4.1 days. Moreover, hSFRP2 mAb treatment did not cause any weight loss, fatigue, or liver or kidney damage (Garcia D, et al., Ann Surg Oncol 2019;26(13):4782-90). Chemotherapy remains the standard of care for TNBC treatment, however TNBC is associated with high recurrence rates, high

incidence of distant metastases, and poor overall survival (Dent R, et al., Clin Cancer Res 2007;13(15 Pt 1):4429-34).

Understanding these mechanisms is crucial for developing effective strategies to overcome drug resistance in TNBC and improve patient outcomes. This study describes SFRP2 localization within the tumor immune microenvironment, specifically tumor associated macrophages, and effects that hSFRP2 mAb invokes in tumor associated macrophages.

The results of the experiments are now described.

SFRP2 and CD38 proteins are expressed in a breast cancer tissue microarray (TMA)

To demonstrate the expression of SFRP2 in TNBC, an formalin-fixed paraffin-embedded (FFPE) tissue microarray (TMA) containing 88 cores of human TNBC (US Biomax Inc.) was analyzed for SFRP2 protein via immunohistochemistry (IHC). The TMA was scanned and staining intensity was quantified using spatial analysis via Phenoptrerports Open Source R package. SFRP2/DAB stained TMA was imaged at 20X magnification using the Vectra® Polaris™ Automated Quantitative Pathology Imaging System (Akoya Biosciences, Marlborough, MA) and analyzed using inForm® Tissue Analysis Software (v[2.6.0], Akoya Biosciences, Marlborough, MA). 13 representative cores were used to train the software. The cores were first segmented into tissue types: stroma, epithelium, and blank space. The cores were further segmented into cell nucleus, cytoplasm, and membrane. Lastly, the software was trained to identify the positive and negative phenotype for each cell. An algorithm was then generated based on these 13 cores. Using this software generated algorithm, all 88 cores underwent phenotyping and subsequent cell segmentation to determine SFRP2 positivity of every cell seen in each core at 20X. The percentage of SFRP2 positively staining cells in both stroma and tumor for each tissue core was calculated via analysis by PhenoptrReports Open Source R Package (akoyabio.github.io/phenoptrReports/index.html, Akoya Biosciences, Marlborough, MA).

Results for SFRP2 staining were analyzed as previously described for analyzing estrogen and progesterone receptor positivity (Baker GM, et al., J Pathol

Inform 2022;13:100118); categories are 0-absence of staining; low positive (0-10%), and positive >10%. In addition, the role of CD38 in breast cancer has not been well studied compared to other tumors. Its expression was evaluated in TNBC. CD38 protein was measured using an FFPE TMA containing 88 cores of human TNBC. Using IHC with antibodies to CD38, the TMA slide was analyzed by inForm software for percent CD38 positivity. Each core was categorized into negative (<1%), low positive (1-10%), or positive (>10%) based on the established guidelines for ER/PR scoring (Baker GM, et al., J Pathol Inform 2022;13:100118).

The TMA was scanned, and staining intensity was quantified using spatial analysis via Phenoptrerports Open Source R package. SFRP2/DAB stained TMA was imaged at 20X magnification using the Vectra® Polaris™ Automated Quantitative Pathology Imaging System and analyzed using inForm® Tissue Analysis Software (v[2.6.0]). 83 of 88 cores stained positive for SFRP2, 4 cores-stained low positive, and 1 core was not evaluable due to tissue folding. To quantify SFRP2 and CD38 protein levels, a formalin-fixed paraffin-embedded (FFPE) TMA containing 88 cores from 44 human breast cancers (each core in the TMA is duplicated) was utilized. Using IHC with SFRP2 or CD38 antibodies, the TMA slide was analyzed using inForm software to calculate the percentage of SFRP2 or CD38-positive cells. SFRP2 staining was present in all breast tumors, with no statistical difference between subtypes when analyzed by two-way analysis of variance (ANOVA) (Figure 1A through Figure 1D). The results displayed are the mean of the duplicate cores. This confirms that SFRP2 is abundantly expressed in human TNBC. Similarly, CD38 staining was present in all breast tumors, with no statistical difference between subtypes when analyzed by ANOVA (Figure 1E through Figure 1H). Next, western blot analysis was performed on TNBC murine and human breast cancer cell lines (PY8119, E0177.LMB, and MDA-MB-231) for SFRP2, CD38, and PDL-1. Notably, these proteins were detected in all three cell lines (Figure 1I). Finally, gene expression in human hematopoietic cell types of CD38 (Figure 1J) and SFPR2 (Figure 1K) was analyzed. Expression of CD38 is high in blood and immune cells (B cells, T-cells, granulocytes, macrophages, Kupfer cells, and erythroid cells), while expression of SFRP2 is minimal across all studied cell types. Human Protein Atlas is

available from http:// www.proteinatlas.org. SFRP2 and CD38 co-localize in the tumor microenvironment in human TNBC.

SFRP2 co-localizes with tumor associated macrophages, tumor infiltrating lymphocytes, tumors cells and CD38 by multiplex IHC in human tumors

To delve deeper into the localization of SFRP2 and CD38 within the breast tumor immune microenvironment, multiplex IHC was conducted on four human TNBC FFPE sections. Following staining, a spatial analysis software was employed to ascertain the percentage positivity of each marker (CD68, SFRP2, CD19, CD3, Cytokeratin, and CD38). The analysis shows a strong co-localization of SFRP2 and CD38 in the tumor microenvironment. Of the cells that stained positively for CD38, 98.15% ± 1.35% also stained positively for SFRP2. SFRP2 was preferentially localized in tumor cells, TAMs, TILs, and B-cells. The percentage of cytokeratin+ tumor cells positive for SFRP2 was 87.57% ± 8.11% (n=4). The percentage of CD68+ TAM cells positive for SFRP2 was 90.34% ± 5.59% (n=4). The percentage of CD3+ TAM cells positive for SFRP2 was 96.38% ± 2.21% (n=4). The percentage of CD19+ B-cells positive for SFRP2 was 91.98% ± 7.72% (n=4) (Figure 2A).

Of the total number of cells stained positively for SFRP2, 46.15% ± 14.37% also stained positively for CD38. CD38 was preferentially localized in tumor cells, TAMs, TILs, and B-cells. The percentage of cytokeratin+ tumor cells staining positively for CD38 was 39.75% ± 20.17% (n=4). The percentage of CD68+ TAM cells staining positively for CD38 was 43.16% ± 14.94% (n=4). The percentage of CD3+ TAM cells staining positively for CD38 was 43.36% ± 9.05% (n=4). The percentage of CD19+ B-cells staining positive for CD38 was 42.38% ± 19.48% (n=4) (Figure 2B). Representative images are shown for SFRP2 and CD38 co-localizing in tumors (Figure 2C through Figure 2F), TAMs (Figure 2G through Figure 2J), and TILs (Figure 2K through Figure 2N). Therefore, targeting SFRP2 in breast cancer could lower CD38 and overcome resistance to checkpoint inhibitors without the toxicity of directly targeting CD38.

hSFRP2 mAb induces IFN-γ protein and mRNA in TAMs

IFN-Ɣ is a crucial cytokine in the immune response. It can activate macrophages to a more pro-inflammatory state (M1), which is associated with anti-tumor activity. Therefore, modulating IFN-Ɣ expression could enhance the immune system's ability to inhibit tumor growth. Because SFRP2 is localized in TAMs, the next question was whether the hSFRP2 mAb affects IFN-Ɣ production in breast tumor TAMs. A single-cell suspension of TAMs was analyzed using flow cytometry and showed a pre-selection of CD11b+ F4/80+ cells at 9.2%, which increased to 42% after TAM enrichment.

TAMs were resuspended and treated with hSFRP2 mAb (10 μM) and IgG1 control (10 μM) for one hour or 24 hours. The cells were collected and then lysed for western blot and qRT-PCR analyses. The dosimetry analyses revealed an increase in IFN-Ɣ after treatment with hSFRP2 mAb by western blot in TAMs treated for both 1 hour (by 1.71 fold) and 24 hours (by 2.19 fold) with hSFPR2 mAb relative to IgG1 control (Figure 3A); and a 2.35 ± 0.08-fold increase in IFN-Ɣ mRNA in hSFRP2 mAb treated TAMs when compared to IgG1 control treated TAMs ($n=3$, $p<.02$) (Figure 3B).

SFRP2 gene expression is inversely correlated with IFN-Ɣ mRNA levels in human breast tumor samples from patients

To further evaluate the relationship between SFRP2 and IFN-Ɣ expression, data from The Cancer Genome Atlas (TCGA) was obtained through the Human Protein Atlas. This study involved 1075 breast cancer patients from the TCGA database with available data on SFRP2 and IFN-Ɣ mRNA expression levels. The least squares linear regression results revealed a significant negative association between SFRP2 and IFN-Ɣ mRNA expression ($p<0.0001$), corroborating the in vitro findings (Figure 3C).

TAMs treated with hSFRP2 mAb increase T-cell proliferation, a marker for immunogenicity in the microenvironment

To assess the immunogenic potential of the hSFRP2 mAb in the microenvironment, TAM enriched single cell suspension was plated and treated for 1 hour with hSFRP2 mAb or IgG1 control. The TAMs were washed and co-cultured with T-cells with the following groups: 1) T-cells alone (negative control), 2) T-cells with T-

cell receptor (TCR) activation using anti-CD3 (2 μg/mL) and anti-CD28 (5 μg/mL)-coated wells) as the positive control, 3) T-cells with TCR co-cultured with IgG1 treated TAMs, and 4) T-cells with TCR activation co-cultured with hSFRP2 mAb treated TAMs. T-cell proliferation was quantified using flow cytometry for CD25 (an early T-cell proliferation marker) and CD69 (a late T-cell proliferation marker). Compared to the negative control, TCR, as the positive control, increased the percentage of CD25 and CD69 (Figure 3D and Figure 3E). In the group of T-cells co-cultured with control IGg1 mAb-treated TAMs, the percentage of CD25 was 9.33 ± 0.15, which increased to 16.2 ± 1.02 in the hSFRP2 mAb-treated TAMs (n=3, $p<.05$) (Figure 3D). Similarly, In the group of T-cells co-cultured with control IGg1 mAb-treated TAMs, the percentage of CD69 was 9.2 ± 0.32, which increased to 18.9 ± 2.43 in the hSFRP2 mAb treated TAMs (n=3, $p<.05$) (Figure 3E) These results show that both early and late T-cell activation markers increase significantly when co-cultured within TAMs that were pretreated with hSFRP2 mAb compared to TAMs pretreated with IgG1 control.

hSFRP2 mAb inhibits TNBC metastatic spread and increases tumor apoptosis

To evaluate the effectiveness of hSFRP2 mAb against metastatic breast cancer, the EO771.LMB cell line was utilized in a mouse metastasis model. Tumor cells were injected in the tale vein of 30 female C57BL/6 mice with hSFRP2 mAb treatment commencing 48h post-injection. The treatment regimen spanned over 28 days, with hSFRP2 mAb administered at 8 mg/kg every 3 days to half the mice, while the other half received an IgG1 control (8 mg/kg) every 3 days. No significant changes in weight or activity levels were observed throughout the treatment. On the 29th day, the mice were humanely euthanized, and the lungs were resected. In the hSFRP2 mAb-treated group, the lungs displayed a significantly reduced number of surface metastases (4.9 ± 1.1) in comparison to the IgG1 control-treated group (8.9 ±2.8, $p<0.05$). This finding underscores the effectiveness of hSFRP2 mAb as a promising monotherapy for metastatic triple-negative breast cancer (Figure 4A). The apoptotic impact of hSFRP2 on EO771.LMB metastases were investigated using TUNEL assays performed on FFPE lung sections. The analysis revealed a significant increase in the number of apoptotic cells in the lungs isolated from the group treated with hSFRP2 mAb in comparison to the IgG1

control treatment group (Figure 4B). The count of apoptotic cells per high power field (HPF) was 9.8 ± 1.8 for the IgG1 control group and 21.0 ± 4.6 for the hSFRP2 mAb group (n=15 per group, p<.01). These findings indicate that hSFRP2 mAb enhances apoptosis in EO771.LMB cells within pulmonary metastases.

In a second study to assess the efficacy of hSFRP2 mAb in inhibiting the growth of metastatic TNBC, the PY8119 cell line was employed within a murine metastasis model. For 21 days, two groups of mice were administered treatments: one with hSFRP2 mAb (8 mg/kg every three days) and the other with an IgG1 control (8 mg/kg every three days), each group comprising 10 mice. Following treatment, on day 22, the mice were humanely euthanized to evaluate the lungs for metastatic lesions. The visual count revealed a significant reduction in the number of surface metastases in the hSFRP2 mAb group, compared to the IgG1 control (n=15, p<0.05, Figure 4C). Further analysis using a TUNEL assay on formalin-fixed paraffin-embedded (FFPE) lung sections indicated a higher rate of apoptosis in the metastases from the hSFRP2 mAb group (n=10, p=0.001, Figure 4D). This increase in apoptotic cell count in the hSFRP2 mAb-treated lungs suggests that the antibody enhances the apoptosis of PY8119 cells in pulmonary metastases, highlighting its potential as a treatment for metastatic TNBC.

M1/M2 TAM polarization induced by hSFRP2 mAb treatment in breast cancer metastases

TAMs play a crucial role in the pathophysiology of TNBC and are broadly classified as M1 and M2. M1 macrophages exhibit pro-inflammatory and anti-tumor activity, while M2 macrophages display immunosuppressive (pro-tumoral) activity. A higher M1/M2 ratio indicates a dominance of pro-inflammatory M1-like TAMs, which boost T-cell immune responses to tumors and improve immunotherapy effectiveness. Conversely, a lower M1/M2 ratio suggests an increase in immunosuppressive M2-like TAMs, aiding tumor escape, weakening immune responses, and facilitating drug resistance. Increasing the M1/M2 TAM ratio is a potential therapeutic strategy to overcome resistance to antitumor drugs. To study whether hSFRP2 mAb affects the M1/M2 ratio in the tumor microenvironment of TNBC lung metastases, FFPE serial lung slides containing EO771.LMB metastases from mice treated with hSFRP2 mAb and IgG1

control were stained using IHC with antibodies to CD163 (M2 marker), CD86 (M1 Marker), and F4/80 (macrophage marker). Only cells that stained positively for both CD163 and F4/80 were counted as M2 positive, and only cells that stained positively for both CD86 and F4/80 were counted as M1 positive. Spatial analysis software showed that lungs extracted from E0771 mice treated with IgG1 had an M1/M2 ratio of 1.05 ± 0.07, which increased in the hSFRP2 mAb treated mouse lungs to 1.56 ± 0.08 ($p=0.028$, Figure 4E). Likewise, lungs extracted from PY8119 mice treated with IgG1 had an M1/M2 ratio of 0.42 ± 0.18, which increased in the hSFRP2 mAb treated mouse lungs to 1.15 ± 0.15 ($n=3$, $p=0.036$, Figure 4F).

Detection of IFN-ϒ protein in the serum of hSFRP2 mAb-treated TNBC mice

The levels of IFN-ϒ in the serum from mice with metastatic EO771.LMB or PY8119 TNBC treated with the hSFRP2 mAb or IGg1 was measured by ELISA. Treatment with the hSFRP2 mAb increased the serum concentration of IFN-ϒ protein in both EO771.LMB ($p<0.0001$, $n=5$) and PY8119 ($p<0.0001$, $n=7$) tumor-bearing mice compared to those treated with IgG1 (Figure 4G).

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed is:

1. A method of inducing macrophage polarization in a subject having a solid tumor, the method comprising administering to the subject an inhibitor of SFRP2.

2. The method of claim 1, wherein the inhibitor is selected from the group consisting of a chemical compound, a protein, a peptide, a peptidomimetic, an antibody, an antibody fragment, an antibody mimetic, a chimeric antibody, a bispecific antibody, a ribozyme, a small molecule chemical compound, a monobody, a short hairpin RNA, RNAi, siRNA, miRNA, or a nucleic acid encoding an antisense nucleic acid molecule.

3. The method of claim 2, wherein the inhibitor is an antibody.

4. The method of claim 3, wherein the antibody is an anti-SFRP2 antibody.

5. The method of claim 4, wherein the anti-SFRP2 antibody comprises a complementary determining region (CDR) H1 comprising the amino acid sequence of SEQ ID NO:19, a CDR H2 comprising the amino acid sequence of SEQ ID NO:20, a CDR H3 comprising the amino acid sequence of SEQ ID NO:21, a CDR LI comprising the amino acid sequence of SEQ ID NO:22, a CDR L2 comprising the amino acid sequence of SEQ ID NO:23, and a CDR L3 comprising the amino acid sequence of SEQ ID NO:24.

6. The method of claim 1, wherein the solid tumor is breast cancer.

7. The method of claim 6, wherein the breast cancer is triple negative metastatic breast cancer.

8. The method of claim 1, wherein the macrophage is polarized into a classically activated M1.

9. The method of claim 8, wherein the classically activated M1 expresses at least one M1 marker, wherein the M1 marker is selected from the group consisting of G-CSF, GM-CSF, IFNg, IL-1a, IL-1B, IL2, IL3, IL5, IL7, IL12p70, IL16, IL27, CCL2, CXCL9, TARC, TIMP1, or TNF-α.

10. A method of reducing the number of tumor associated macrophages in a subject, the method comprising administering to the subject an inhibitor of SFRP2.

11. The method of claim 10, wherein the inhibitor is selected from the group consisting of: a chemical compound, a protein, a peptide, a peptidomimetic, an antibody, an antibody fragment, an antibody mimetic, a chimeric antibody, a bispecific antibody, a ribozyme, a small molecule chemical compound, a monobody, a short hairpin RNA, RNAi, siRNA, miRNA, or a nucleic acid encoding an antisense nucleic acid molecule.

12. The method of claim 11, wherein the inhibitor is an antibody.

13. The method of claim 12, wherein the antibody is an anti-SFRP2 antibody.

14. The method of claim 13, wherein the anti-SFRP2 antibody comprises a complementary determining region (CDR) H1 comprising the amino acid sequence of SEQ ID NO:19, a CDR H2 comprising the amino acid sequence of SEQ ID NO:20, a CDR H3 comprising the amino acid sequence of SEQ ID NO:21, a CDR LI comprising the amino acid sequence of SEQ ID NO:22, a CDR L2 comprising the amino acid sequence of SEQ ID NO:23, and a CDR L3 comprising the amino acid sequence of SEQ ID NO:24.

15. The method of claim 10, wherein the subject has a solid tumor.

16. A method comprising:

a) administering to a subject an inhibitor of SFRP2 for the treatment or prevention of a disease or disorder, and

b) measuring a response selected from the group consisting of: a change in the level of interferon-gamma (IFN-γ), a change in macrophage polarization, or a change in the number of tumor associated macrophages.

17. The method of claim 16, wherein the level of interferon-gamma (IFN-γ) is increased, thereby indicating that the inhibitor is effective in treating the disease or disorder in the subject.

18. The method of claim 16, wherein macrophage polarization to a classically activated M1 is increased, thereby indicating that the inhibitor is effective in treating the disease or disorder in the subject.

19. The method of claim 16, wherein the number of tumor associated macrophages is decreased, thereby indicating that the inhibitor is effective in treating the disease or disorder in the subject.