

ANTI-CANCER TGFB2 AGENTS WITH IMMUNOTHERAPEUTICS

SEQUENCE LISTING

[0001] This application includes a sequence listing submitted electronically as an ST.26 file created on May 15, 2024, named 018988-012WO1_SL.xml, which is 120,193 bytes in size.

TECHNICAL FIELD

[0002] This invention relates to agents, compositions, and methods for use in treating or ameliorating the symptoms of cancer. Exemplary synergistic therapies include combinations of active agents for inhibiting or suppressing expression of TGF- β 2, immune checkpoint inhibitor agents, and interleukin immunotherapeutic agents. One or more biomarkers can be used to select subjects who benefit from the agents, compositions, and methods.

BACKGROUND

[0003] Cancer is a complex pathology involving multiple variant cellular pathways. Because of this complexity, many anti-cancer drugs have limited or partial therapeutic effectiveness.

[0004] Drawbacks of conventional therapies include lack of efficacy as determined by overall survival.

[0005] Further drawbacks of conventional therapies include significant unwanted side effects such as killing healthy cells in addition to killing cancer cells.

[0006] Additional drawbacks of anti-cancer agents include high toxicity at required levels of therapeutic administration.

[0007] What is needed are agents, compositions and methods for cancer diseases to increase efficacy and reduce toxicity and unwanted side effects.

[0008] Therapeutic compositions of different agents are needed to supply significant anti-tumor effects and cancer immunotherapeutic effects and which can improve efficacy, reduce side effects and reduce adverse health effects. There is a need for improved guidance for use of such compositions by using appropriate biomarkers to select synergistic effects of the agents and compositions.

[0009] There is an urgent need for new methods, agents and uses to combine strategies for cancer immunotherapy with strategies for direct anti-tumor attack for treating various cancers.

The need for therapy includes therapeutic compositions which combine cancer T-cell and immunotherapies with potent anti-cancer agents.

BRIEF SUMMARY

[0010] This invention provides agents, compositions, and methods for use in treating or ameliorating the symptoms of cancer. The compositions can promote anti-tumor effects over a range of different cancers. Synergistic pharmaceutical therapies of this invention include use of potent, direct anti-tumor agents along with cancer immunotherapeutic agents. Cancer immunotherapeutic modalities of this disclosure include combination therapies with immune checkpoint inhibitor agents and protein immunotherapeutic agents. The agents, compositions, and methods of this invention may combine strategies for cancer immunotherapy with strategies for direct anti-tumor attack for treating various cancers.

[0011] In some embodiments, compositions and methods of this invention can increase efficacy, as well as reduce toxic side effects and adverse health effects in cancer treatment.

[0012] In further embodiments, methods and therapeutic strategies of this invention can include increased guidance for successful patient outcomes using appropriate biomarkers to select synergistic effects of the compositions.

[0013] Exemplary synergistic pharmaceutical therapies include a combination of active agents including an agent for inhibiting or suppressing expression of TGF- β 2, an immune checkpoint inhibitor agent, and an interleukin immunotherapeutic agent.

[0014] In further aspects, one or more biomarkers can be used to select subjects who benefit from the compositions and therapeutic methods, including IRF5 and ITGAM.

[0015] In certain embodiments, the compositions and therapeutic methods can be applied in combination with chemotherapy and other standard of care therapies for cancer.

[0016] Embodiments of this invention include the following:

[0017] A method for treating or ameliorating the symptoms of cancer in a subject in need, the method comprising: administering a composition comprising an agent for inhibiting or suppressing expression of TGF- β 2; administering an immune checkpoint inhibitor; and administering an interleukin immunotherapeutic agent to the subject.

[0018] An agent for inhibiting or suppressing expression of TGF- β 2 in combination with an immune checkpoint inhibitor and an interleukin immunotherapeutic active agent for use in treating or ameliorating the symptoms of cancer.

[0019] A composition comprising an agent for inhibiting or suppressing expression of TGF- β 2 and a pharmaceutically acceptable carrier for use in the preparation of a medicament or for treating or ameliorating the symptoms of a cancer in a subject in combination with an immune checkpoint inhibitor and an interleukin immunotherapeutic active agent.

[0020] The method, agent or composition above, wherein the cancer is a solid cancer, a pancreatic cancer, melanoma, lung cancer, breast cancer, multiple myeloma, or colorectal cancer.

[0021] The method, agent or composition above, wherein the agent for inhibiting or suppressing expression of TGF- β 2, the immune checkpoint inhibitor, and the interleukin immunotherapeutic active agent are administered concurrently, simultaneously, sequentially, or separately in time.

[0022] The method, agent or composition above, wherein the composition and agents are administered by infusion or injection.

[0023] The method, agent or composition above, wherein the agent for inhibiting or suppressing expression of TGF- β 2 is selected from Table 1 or Table 2, and chemically-modified variants thereof, LNA variants thereof, gapmer variants thereof, and any combination or pooling thereof.

[0024] The method, agent or composition above, wherein the agent for inhibiting or suppressing expression of TGF- β 2 is CGGCATGTCTATTTTGT SEQ ID NO:1 or C*G*G*C*A*T*G*T*C*T*A*T*T*T*T*G*T*A SEQ ID NO:137.

[0025] The method, agent or composition above, wherein the agent or composition comprises a carrier of sterile water for injection, saline, isotonic saline, or a combination thereof.

[0026] The method, agent or composition above, wherein the agent or composition is substantially free of excipients.

[0027] The method, agent or composition above, wherein the composition is stable for at least 14 days in carrier at 37°C.

[0028] The method, agent or composition above, wherein the immune checkpoint inhibitor is an inhibitor of PD-1, CTLA-4, or PD-L1.

[0029] The method, agent or composition above, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, cemiplimab, spartalizumab, atezolizumab, avelumab, or durvalumab.

[0030] The method, agent or composition above, wherein the interleukin immunotherapeutic active agent is a natural IL-2, a high dose IL-2, a recombinant IL-2, or aldesleukin.

[0031] The method, agent or composition above, wherein the subject upon the administration or use has a reduced TGF- β 2 expression.

[0032] The method, agent or composition above, comprising selecting the subjects who benefit from the method, agent or use using one or more biomarkers.

[0033] The method, agent or composition above, wherein the one or more biomarkers are a level of IRF5, a level of ITGAM, or a combination thereof.

[0034] The method, agent or composition above, wherein the one or more biomarkers is ITGAM and the subject is selected when expression of ITGAM is at a level above that found in a healthy patient.

[0035] The method, agent or composition above, wherein the subject after the administration or use has an increased level of IRF5.

[0036] The method, agent or composition above, wherein the subject after the administration or use has an increased level of ITGAM.

[0037] The method, agent or composition above, comprising administering a therapeutically sufficient amount of a pharmaceutical composition comprising an expression product of IRF5 or ITGAM to the subject.

[0038] The method, agent or composition above, wherein the expression product is an mRNA, a polypeptide, a protein, or fragment thereof, or combination thereof.

[0039] The method, agent or composition above, wherein the administration or use decreases mortality rate at month 6, 12, 18, 24, 30, or 36.

[0040] The method, agent or composition above, wherein the administration or use increases survival rate at month 6, 12, 18, 24, 30, or 36.

[0041] The method, agent or composition above, wherein the administration or use of the composition is combined with a standard of care treatment for cancer, wherein the standard of care treatment comprises chemotherapy or radiation therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] FIG. 1 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in melanoma patients (KM Plotter). FIG. 1 shows that for use of a PD-1 checkpoint inhibitor for selected patients with high ITGAM, improved survival was shown for low TGF- β 2 (logrank P=0.0039).

[0043] FIG. 2 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in pancreatic cancer (KM Plotter). FIG. 2 shows that for use of IL-2 for selected patients with high ITGAM, improved survival was shown for high IL-2 (logrank P=0.034).

[0044] FIG. 3 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in melanoma (KM Plotter). FIG. 3 shows that for use of a PD-1 checkpoint inhibitor for selected patients with high IRF5, improved survival was shown for low TGF- β 2 (logrank P=0.00053).

[0045] FIG. 4 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in cancer patients (KM Plotter). The study included multiple kinds of tumors. FIG. 4 shows that for use of a PD-1 checkpoint inhibitor, improved survival was shown for high ratio of IL-2 to TGF- β 2 (IL-2/TGF- β 2, logrank P=0.0031).

[0046] FIG. 5 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in melanoma (KM Plotter). FIG. 5 shows that for use of a PD-1 checkpoint inhibitor, improved survival was shown for high ratio of IL-2 to TGF- β 2 (IL-2/TGF- β 2, logrank P=3.7e-06).

[0047] FIG. 6 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in melanoma patients (KM Plotter). FIG. 6 shows that for use of a CKIs, improved survival was shown for high IL-2 (logrank P=0.0015).

[0048] FIG. 7 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in melanoma patients (KM Plotter). FIG. 7 shows that for use of a CKIs, improved survival was shown for low TGF- β 2 (logrank P=0.006).

[0049] FIG. 8 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in cancer patients (KM Plotter). FIG. 8 shows that for use of a PD-1 checkpoint inhibitor, improved survival was shown for high IL-2 (logrank P=0.007).

[0050] FIG. 9 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in cancer patients (KM Plotter). FIG. 9 shows that for use of a PD-1 checkpoint inhibitor, improved survival was shown for low TGF- β 2 (logrank P=0.0028).

[0051] FIG. 10 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in melanoma patients (KM Plotter). The study included multiple kinds of tumors. FIG. 10 shows that for use of CKIs, improved survival was shown for high ratio of IL-2 to TGF- β 2 (IL-2/TGF- β 2, logrank P=3.7e-06).

[0052] FIG. 11 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in cancer patients (KM Plotter). FIG. 11 shows that for use of a PD-1 checkpoint inhibitor, improved survival was shown for high ratio of IL-2 to TGF- β 2 (IL-2/TGF- β 2, logrank P=2.2e-08).

DETAILED DESCRIPTION OF THE DISCLOSURE

[0053] This invention relates to methods, compositions and uses thereof for treating or ameliorating the symptoms of cancer in a human or animal subject with pharmaceutical compositions designed to promote anti-tumor effects over a range of different cancers.

[0054] Exemplary synergistic pharmaceutical therapies include compositions of various combinations of active agents including agents for inhibiting or suppressing expression of TGF- β 2, checkpoint inhibitor agents, and interleukin immunotherapeutic agents.

[0055] In some embodiments, one or more biomarkers can be used to select subjects who benefit from the method, agent or use, including IRF5 and/or ITGAM. The compositions can be used in combination with chemotherapy and other standard of care therapies.

[0056] A patient or subject in need of cancer therapy as described herein may be a human or animal subject.

[0057] As used herein, the term agent can refer to one or more active compounds, a combination of active compounds, or a composition containing one or more active compounds and a carrier, and/or a solvent, and/or any number of excipients. In some embodiments, the composition may be a pharmaceutical composition. In certain embodiments, the composition may be a pharmaceutical composition containing a therapeutically effective amount of one or more active compounds. Formulations of active agents can be determined by those skilled in the art. Some examples of

excipients are given in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa. 1975, and Liberman, H. A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980. Methods for determining a therapeutically effective amount of a compound are known in the art.

Anti-cancer methods and compositions

[0058] Embodiments of this invention include combinations of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a checkpoint inhibitor.

[0059] In certain embodiments, this invention provides therapeutic combinations of one or more TGF- β 2-specific antisense agents, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor.

[0060] In some aspects, therapeutic combinations of this invention can be used in treating or ameliorating symptoms of cancer.

[0061] In further aspects, therapeutic combinations of this invention can be used in treating or ameliorating symptoms of solid tumors.

[0062] In certain aspects, therapeutic combinations of this invention can be used in treating or ameliorating symptoms of a solid cancer, breast cancer, pancreatic cancer, melanoma, lung cancer, multiple myeloma, and colorectal cancer.

[0063] In certain aspects, therapeutic combinations of this invention can provide cytotoxicity effects which provide anti-tumor effects. For example, tumor growth may be delayed.

[0064] Embodiments of this invention provide therapeutic combinations exhibiting profound effects on survival of cancer patients. In some embodiments, tumoral levels of TGF- β 2, IL-2, or the ratio IL-2/TGF- β 2 may greatly impact overall survival of patients treated with immune checkpoint inhibitors.

[0065] In certain embodiments, the checkpoint inhibitors can be PD-1 checkpoint inhibitors.

[0066] In some aspects, embodiments of this invention can result in a surprisingly increased IL2/TGF- β 2 ratio and improved survival for patients treated with a checkpoint inhibitor. The increase in the IL2/TGF- β 2 ratio and improved survival can result from the therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2

immunotherapy agent, and a checkpoint inhibitor. In certain embodiments, the checkpoint inhibitor can be a PD-1 checkpoint inhibitor.

[0067] Embodiments of this invention contemplate using the therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a checkpoint inhibitor as a strong driver of survival for cancer patients.

[0068] In additional embodiments, overall survival for multiple kinds of cancer and tumors can be more than doubled for therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a checkpoint inhibitor as described herein.

[0069] In further embodiments, the number of long-term survivors for multiple kinds of cancer and tumors can be increased with a therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a checkpoint inhibitor as described herein.

[0070] Embodiments of this invention can demonstrate a synergy via a surprising increase in anti-cancer potency when a TGF- β 2-specific antisense agent is used in combination with a checkpoint inhibitor and an interleukin immunotherapeutic agent in treating or ameliorating the symptoms of cancer.

[0071] In certain embodiments, a surprising, synergistic anti-cancer potency can be observed for treating or ameliorating the symptoms of cancer using the combination of an antisense agent for inhibiting or suppressing expression of TGF- β 2, a PD-1 checkpoint inhibitor, and an IL-2 immunotherapeutic agent. This combination can be particularly effective for patients with high levels of tumor associated monocytes and/or tumor associated macrophages.

[0072] Without wishing to be bound by theory, combination of an agent for inhibiting or suppressing expression of TGF- β 2 with a PD-1 checkpoint inhibitor and an IL-2 immunotherapeutic agent exhibited synergy against high levels of tumor associated monocytes and macrophages. This synergistic effect can be strongest in the combination with PD-1 checkpoint inhibitors. Because PD-1 is present in M2-type tumor associated macrophages, and antisense agents for inhibiting or suppressing expression of TGF- β 2 have effects in repolarizing M2 and promoting anti-tumor effects, these two agents appear to be operable synergistically against the same target when used in combination with IL-2.

[0073] Without wishing to be bound by theory, TGF- β 2 has a central role in programming M1-type tumor associated macrophages, which can exhibit anti-tumor effects. It has been demonstrated by the inventors herein that inhibiting or suppressing TGF- β 2 with antisense agents has anti-tumor effects. The antisense agents may have the effect of re-programming to promote M1-type tumor associated macrophages. Such re-programming may have the effect of actively reducing and/or eliminating cancer tumors, especially when combined with an agent that is hampered by high TGF- β 2.

[0074] This invention includes methods for treating or ameliorating the symptoms of cancer in a human or animal subject in need, by administering a therapeutically sufficient amount of a composition comprising an agent for inhibiting or suppressing expression of TGF- β 2 to the subject, administering a therapeutically sufficient amount of a composition comprising a checkpoint inhibitor to the subject, and administering a therapeutically sufficient amount of a composition comprising an interleukin immunotherapeutic agent to the subject.

[0075] Further embodiments of this invention include methods for treating or ameliorating the symptoms of cancer in a human or animal subject in need by the steps of administering a therapeutically sufficient amount of a composition comprising an agent for inhibiting or suppressing expression of TGF- β 2 to the subject, administering a therapeutically sufficient amount of a composition comprising an immune checkpoint inhibitor to the subject, and administering a therapeutically sufficient amount of a composition comprising an interleukin immunotherapeutic agent to the subject.

[0076] In additional aspects, this invention includes uses of a composition comprising an agent for inhibiting or suppressing expression of TGF- β 2 in the preparation of a medicament for treating or ameliorating the symptoms of a cancer in a human subject or animal in combination with a checkpoint inhibitor and an interleukin immunotherapeutic agent.

[0077] In additional aspects, an agent for inhibiting or suppressing expression of TGF- β 2, an immune checkpoint inhibitor, and an interleukin immunotherapeutic agent can be administered concurrently, simultaneously, sequentially, or separately in time.

Human TGF- β 2-specific phosphorothioate antisense oligodeoxynucleotide

[0078] An antisense oligonucleotide (ASO) can be a single-stranded deoxyribonucleotide, which may be complementary to an mRNA target. The antisense therapy may downregulate a molecular target, which may be achieved by induction of RNase H endonuclease activity that cleaves the RNA-DNA heteroduplex with a significant reduction of the target gene translation. Other ASO mechanisms can include inhibition of 5' cap formation, alteration of splicing process such as splice-switching, and steric hindrance of ribosomal activity.

[0079] Antisense therapeutic strategies can utilize single-stranded DNA oligonucleotides that inhibit protein production by mediating the catalytic degradation of a target mRNA, or by binding to sites on mRNA needed for translation. Antisense oligonucleotides can be designed to target the viral RNA genome or viral transcripts. Antisense oligonucleotides can provide an approach for identifying potential targets, and therefore represent potential therapeutics.

[0080] Antisense oligonucleotides can be small synthetic pieces of single-stranded DNA that may be 15–30 nucleotides in length. An ASO may specifically bind to a complementary DNA/RNA sequence by Watson–Crick hybridization and once bound to the target RNA, inhibit the translational processes either by inducing cleavage mechanisms or by inhibiting mRNA maturation. An ASO may selectively inhibit gene expression with specificity. Chemical modifications of DNA or RNA can be used to increase stability.

[0081] For example, modifications can be introduced in the phosphodiester bond, the sugar ring, and the backbone. ASO antiviral agents may block translational processes either by (i) ribonuclease H (RNase H) or RNase P mediated cleavage of mRNA or (ii) by sterically (non- bonding) blocking enzymes that are involved in the target gene translation. Human TGF- β 2-specific phosphorothioate antisense oligodeoxynucleotide (OT-101; AP 12009; Trabedersen), hereafter referred to as OT-101 or AP 12009, is intended to reduce the level of TGF- β 2 protein in malignant gliomas, and thereby delay the progression of disease.

[0082] Antisense oligodeoxynucleotides are short strings of DNA that are designed to downregulate gene expression by interfering with the translation of a specific encoded protein at the mRNA level. OT-101 is a synthetic 18-mer phosphorothioate

oligodeoxynucleotide (S-ODN) where all 3'-5' linkages are modified to phosphorothioates. The molecular formula is $C_{177}H_{208}N_{60}Na_{17}O_{94}P_{17}S_{17}$ and the molecular weight 6,143 g/mol. OT-101 can be designed to be complementary to a specific sequence of human TGF- β 2 mRNA following expression of the gene.

[0083] OT-101 can be supplied as a lyophilized powder in 50 mL glass vials in three different quantities. Each vial is identified by the name of the investigational product, trial number, dosing group, mode of application, quantity of OT-101 contained (in mg), total volume after dissolving (in mL) and resulting concentration (in μ M), name of sponsor, name of manufacturer, batch number, vial number, storage temperature, and expiry date. The study medication can be provided in closed units, packaged separately for each concentration. The packages may contain the appropriate vial(s) and all necessary components of the application system (i.e., syringes, tube, and filter). OT-101 lyophilized powder can be dissolved in isotonic (0.9%) aqueous sodium chloride prior to use.

[0084] Examples of agents of this disclosure for inhibiting or suppressing expression of TGF- β 2 include TGF- β 2-specific antisense oligonucleotides given in SEQ ID NOs:1-136 in Table 1.

Table 1: TGF- β 2-specific antisense oligonucleotides

SEQ ID NO:	Ref.	ANTISENSE SEQUENCE
1	1676-1693	CGGCATGTCT ATTTTGTA (OT-101)
2	--	TAAAGTTCGT TTAGAGAACA G
3	--	AGCCCTGTAT ACGAC
4	--	GTAGGTAAAA ACCTAATAT
5	--	CGTTTAGAGA ACAGATCTAC
6	--	CATTGTAGAT GTCAAAAGCC
7	--	CTCCCTCATG GTGGCAGTTG A
8	--	GTTCGTTTAG AGAACAGATC
9	178-195	TTTGTTCTCTG GATGACTC
10	179-196	GTTTGTTCTT GGATGACT
11	180-197	AGTTTGTTCC TGGATGAC
12	181-198	CAGTTTGTTT CTGGATGA
13	182-199	TCAGTTTGTT CCTGGATG
14	183-200	CTCAGTTTGT TCCTGGAT
15	716-733	TGTGTGTGTG TGCGTGTG
16	717-734	GTGTGTGTGT GTGCGTGT

SEQ ID NO:	Ref.	ANTISENSE SEQUENCE
17	718-735	TGTGTGTGTG TGTGCGTG
18	719-736	GTGTGTGTGT GTGTGCGT
19	720-737	TGTGTGTGTG TGTGTGCG
20	721-738	GTGTGTGTGT GTGTGTGC
21	722-739	TGTGTGTGTG TGTGTGTG
22	723-740	GTGTGTGTGT GTGTGTGT
23	724-741	TGTGTGTGTG TGTGTGTG
24	725-742	GTGTGTGTGT GTGTGTGT
25	726-743	TGTGTGTGTG TGTGTGTG
26	727-744	GTGTGTGTGT GTGTGTGT
27	728-745	TGTGTGTGTG TGTGTGTG
28	729-746	GTGTGTGTGT GTGTGTGT
29	730-747	CGTGTGTGTG TGTGTGTG
30	731-748	GCGTGTGTGT GTGTGTGT
31	732-749	TGCGTGTGTG TGTGTGTG
32	733-750	GTGCGTGTGT GTGTGTGT
33	971-988	AGTGGCGGAT CTGAAC TC
34	972-989	GAGTGGCGGA TCTGAACT
35	1430-1447	GAGTGTGCTG CAGGTAGA
36	1432-1449	TCGAGTGTGC TGCAGGTA
37	1433-1450	ATCGAGTGTG CTGCAGGT
38	1570-1587	GTGCTGTTGT AGATGGAA
39	1571-1588	GGTGCTGTTG TAGATGGA
40	1572-1589	TGGTGCTGTT GTAGATGG
41	1573-1590	CTGGTGCTGT TG TAGATG
42	1574-1591	CCTGGTGCTG TTGTAGAT
43	1575-1592	CCCTGGTGCT GTTGTAGA
44	1576-1593	TCCCTGGTGC TGTGTAG
45	1577-1594	GTCCCTGGTG CTGTTGTA
46	1578-1595	AGTCCCTGGT GCTGTTGT
47	1895-1912	CTGGGTTGGA GATGTTAA
48	1896-1913	GCTGGGTTGG AGATGTTA
49	1897-1914	CGCTGGGTTG GAGATGTT
50	1900-1917	TAGCGCTGGG TTGGAGAT
51	1903-1920	ATGTAGCGCT GGGTTGGA
52	1945-1962	AGCCATTCGC CTTCTGCT
53	1994-2011	GTCTTTATGG TGAAGCCA
54	1995-2012	TGTCTTTATG GTGAAGCC
55	1996-2013	CTGTCTTTAT GGTGAAGC
56	1997-2014	CCTGTCTTTA TGGTGAAG

SEQ ID NO:	Ref.	ANTISENSE SEQUENCE
57	2000-2017	GTTCCTGTCT TTATGGTG
58	2001-2018	GGTTCCTGTC TTTATGGT
59	2002-2019	AGGTTTCCTGT CTTTATGG
60	2003-2020	CAGGTTTCCTG TCTTTATG
61	2004-2021	CCAGGTTTCCT GTCTTTAT
62	2183-2200	GGTCTTCCCA CTGTTTTT
63	2194-2211	AGGAGATGTG GGGTCTTC
64	2195-2212	CAGGAGATGT GGGGTCTT
65	2241-2258	GGTTGGTCTG TTGTGACT
66	2242-2259	CGGTTGGTCT GTTGTGAC
67	2243-2260	CCGTTGGTCT TGTGTGA
68	2522-2539	GAGAATGGTT AGAGGTTT
69	2525-2542	GTAGAGAATG GTTAGAGG
70	2542-2559	GGTGTTTTGC CAATGTAG
71	2543-2560	GGGTGTTTTG CCAATGTA
72	2544-2561	TGGGTGTTTT GCCAATGT
73	2666-2683	CATCATCGTT GTCGTCGT
74	2979-2996	GAACGGTACG TACAGCAA
75	2980-2997	GGAACGGTAC GTACAGCA
76	2981-2998	AGGAACGGTA CGTACAGC
77	2982-2999	TAGGAACGGT ACGTACAG
78	2983-3000	ATAGGAACGG TACGTACA
79	2984-3001	GATAGGAACG GTACGTAC
80	2985-3002	GGATAGGAAC GGTACGTA
81	2986-3003	GGGATAGGAA CGGTACGT
82	3029-3046	GGGTGCCTAT TGCATAGC
83	3030-3047	AGGGTGCCTA TTGCATAG
84	3031-3048	AAGGGTGCCT ATTGCATA
85	3032-3049	GAAGGGTGCC TATTGCAT
86	3033-3050	GGAAGGGTGC CTATTGCA
87	3035-3052	TGGGAAGGGT GCCTATTG
88	3036-3053	ATGGGAAGGG TGCCTATT
89	3037-3054	AATGGGAAGG GTGCCTAT
90	3038-3055	GAATGGGAAG GGTGCCTA
91	3039-3056	AGAATGGGAA GGGTGCCT
92	3040-3057	AAGAATGGGA AGGGTGCC
93	3041-3058	TAAGAATGGG AAGGGTGC
94	3042-3059	GTAAGAATGG GAAGGGTG
95	3043-3060	AGTAAGAATG GGAAGGGT
96	3044-3061	GAGTAAGAAT GGAAGGG

SEQ ID NO:	Ref.	ANTISENSE SEQUENCE
97	3259-3276	CAGACTTTCT CGGTCATA
98	3260-3277	GCAGACTTTC TCGGTCAT
99	3261-3278	TGCAGACTTT CTCGGTCA
100	3262-3279	ATGCAGACTT TCTCGGTC
101	3263-3280	AATGCAGACT TTCTCGGT
102	3264-3281	TAATGCAGAC TTTCTCGG
103	4281-4298	GACCTGGACT TTTTTCCT
104	4282-4299	TGACCTGGAC TTTTTTCC
105	4283-4300	CTGACCTGGA CTTTTTTC
106	4284-4301	GCTGACCTGG ACTTTTTT
107	4467-4484	CTGCAATGAT GTGGCAAA
108	4468-4485	TCTGCAATGA TGTGGCAA
109	4469-4486	TTCTGCAATG ATGTGGCA
110	4470-4487	CTTCTGCAAT GATGTGGC
111	5062-5079	GCTGCCCACT TGCATACT
112	5569-5586	GTTGGCAGAA CATAGAAC
113	5570-5587	CGTTGGCAGA ACATAGAA
114	5571-5588	GCGTTGGCAG AACATAGA
115	5616-5633	ATGGGGCTAC AGGGGATA
116	5617-5634	TATGGGGCTA CAGGGGAT
117	5618-5635	TTATGGGGCT ACAGGGGA
118	5620-5637	AGTTATGGGG CTACAGGG
119	5621-5638	AAGTTATGGG GCTACAGG
120	5622-5639	CAAGTTATGG GGCTACAG
121	5623-5640	CCAAGTTATG GGGCTACA
122	5624-5641	TCCAAGTTAT GGGGCTAC
123	5625-5642	ATCCAAGTTA TGGGGCTA
124	5626-5643	TATCCAAGTT ATGGGGCT
125	5627-5644	CTATCCAAGT TATGGGGC
126	5759-5776	ATTGGAGGAA ATAGGGTG
127	5783-5800	GTCTTGTTAGG TAGCAGCC
128	5784-5801	GGTCTTGTTAG GTAGCAGC
129	5785-5802	TGGTCTTGTA GGTAGCAG
130	5786-5803	CTGGTCTTGT AGGTAGCA
131	5787-5804	TCTGGTCTTG TAGGTAGC
132	5788-5805	GTCTGGTCTT GTAGGTAG
133	5789-5806	AGTCTGGTCT TGTAGGTA
134	5790-5807	GAGTCTGGTC TTGTAGGT
135	5791-5808	GGAGTCTGGT CTTGTAGG
136	5792-5809	AGGAGTCTGG TCTTGTTAG

[0085] The sequences of Table 1 can be chemically-modified to provide active variants thereof, LNA variants thereof, as well as gapmer variants thereof, as known in the art. The sequences of Table 1 can be used in any combination as active agents, such as pooling combinations.

[0086] It is understood that additional antisense oligonucleotides of this disclosure can be constructed based on the TGF-β2 gene sequence.

[0087] In some embodiments, an agent of antisense sequences can be gapmers formed by adding 1 to 5 protected ribo-nucleotides on each flank of the phosphorothioate deoxy-nucleotide sequences in Table 1. For example, the ribo-nucleotides can be protected with 2'-OMe, 2'-OEt, or 2'-O-MOE substituents, or with LNA, cMOE, or cEt bridges, as well as phosphorothioate linkages.

[0088] In some embodiments, an agent of antisense sequences can be a n-M-n RNA(2'-OMe)*-DNA*-RNA(2'-OMe)* gapmer, where n is from 3-7 and M is from 6-12. In certain embodiments, the gapmer can be a 3-10-3 or 5-10-5 LNA*-DNA*-LNA* or cEt*-DNA*-cEt* gapmer (* designates phosphorothioate linkages).

[0089] Examples of agents of this disclosure for inhibiting or suppressing expression of TGF-β2 include TGF-β2-specific phosphorothioate antisense oligonucleotides given in SEQ ID NOs:137-144 in Table 2, based on the sequences in Table 1.

Table 2: TGF-β2-specific phosphorothioate antisense oligonucleotides

SEQ ID NO:	Ref.	ANTISENSE SEQUENCE
137	1676-1693	C*G*G*C*A*T*G*T*C*T*A*T*T*T*T*G*T*A (OT-101)
138	--	G*T*T*C*G*T*T*T*A*G*A*G*A*A*C*A*G*A*T*C
139	--	T*A*A*A*G*T*T*C*G*T*T*T*A*G*A*G*A*A*C*A*G
140	--	A*G*C*C*C*T*G*T*A*T*A*C*G*A*C
141	--	G*T*A*G*G*T*A*A*A*A*A*C*C*T*A*A*T*A*T
142	--	C*G*T*T*T*A*G*A*G*A*A*C*A*G*A*T*C*T*A*C
143	--	C*A*T*T*G*T*A*G*A*T*G*T*C*A*A*A*A*G*C*C
144	--	C*T*C*C*C*T*C*A*T*G*G*T*G*G*C*A*G*T*T*G*A

[0090] Embodiments of this invention further include pharmaceutical compositions for inhibiting or suppressing expression of TGF-β, or for treating or ameliorating the symptoms of cancer in a human or animal. The pharmaceutical compositions may

contain a TGF-β inhibitor, artemisinin, pharmaceutically acceptable salts forms, esters, polymorphs or stereoisomers thereof, and any combination thereof, as well as a carrier. The TGF-β inhibitor may be selected from TGF-β2-specific antisense oligonucleotides. The carrier may be sterile water for injection, saline, isotonic saline, or a combination thereof.

[0091] Importantly, a composition of this disclosure may be substantially free of excipients. Compositions of this invention which are substantially free of excipients have been found to be surprisingly stable in a carrier. In some embodiments, the composition may be stable for at least 14 days, or at least 21 days, or at least 28 days in a carrier at 37°C.

[0092] In additional embodiments, a pharmaceutical composition for infusion may contain less than 1% by weight of excipients, or less than 0.5% by weight of excipients, or less than 0.1% by weight of excipients.

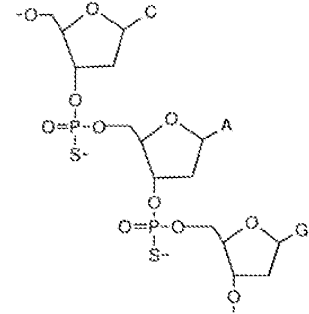
OT-101 antisense oligonucleotide

[0093] The API trabedersen/OT-101 is a synthetic 18-mer S-ODN consisting of the bases adenine (A), thymine (T), guanine (G), and cytosine (C), with all 3'-5' linkages modified to phosphorothioates. The thioate modification can make the drug more resistant to degradation, resulting in an increased stability *in vitro* and *in vivo*. Its molecular structure (nucleotide sequence) can be designed to be complementary to a specific sequence of human transforming growth factor-beta 2 (TGF-β2) mRNA. This sequence can be selected among related molecules for its superior chemical and structural properties, biological activity, and specificity to achieve the best antisense effects *in vitro* and *in vivo*.

[0094] The chemical structure, exemplary of the phosphorothioate moieties (C-A-G), and the physical characteristics of trabedersen are shown in Table 3.

Table 3: Chemical and Physical Characteristics of Trabedersen

Chemical and Physical Characteristics of Trabedersen		
	Appearance:	White to off-white solid
	Molecular Formula (<i>sodium salt, anhydrous</i>):	C ₁₇₇ N ₂₀₈ N ₆₀ O ₉₄ P ₁₇ S ₁₇ Na ₁₇
	Molecular Weight (<i>sodium</i>	6143 g/mol

Chemical and Physical Characteristics of Trabedersen		
 Phosphorothioate linkages (C-A-G)	<i>salt, anhydrous</i>):	
	Number of moieties:	18
	Backbone characteristics:	Phosphorothioates
	Polymorphism:	No crystalline morphology in the solid
	Water content:	Up to 15%
	Solubility:	Very soluble in water (> 50 mg/mL) Soluble in methanol (> 8.5 mg/mL) Insoluble in <i>iso</i> -propanol (0.01 mg/mL)
	pH of solution:	6 - 8

[0095] The IMP can be supplied as a sterile lyophilizate for solution for infusion in 50H glass vials (primary container) containing 7.37 mg trabedersen (intratumoral treatment) and in 20R glass vials (primary container) containing 250 mg trabedersen (intravenous treatment), respectively. No excipients may be in the finished drug product. Glass vials are commonly used for parenterals. Sterile rubber stoppers appropriate for lyophilization can seal the glass vial. The stopper may be sealed with a crimping capsule that includes a colored flip-off cap. For clinical use, each vial can be provided within a white-colored folding box to protect the vials from light exposure and damage during transport. Both the glass vials and the folding boxes can be labeled according to local requirements. The primary and secondary containers of the closure system can fulfill international quality standards for the packaging of sterile solid drug products for injections.

Immune checkpoint inhibitor agents

[0096] As referred to herein, checkpoint inhibitors as known in the art are immune checkpoint inhibitor agents. Checkpoint inhibitors are immunotherapy drugs which block checkpoint proteins from binding with their partner proteins. This prevents an “off” signal from being sent, which allows T cells to kill cancer cells. More particularly, checkpoint proteins, such as PD-1 on T cells, keep immune responses in check. Binding of PD-L1 to PD-1 keeps T cells from killing tumor cells. Thus,

blocking the binding of PD-L1 to PD-1 with an immune checkpoint inhibitor may allow the T cells to kill tumor cells. The immune system can be essentially turned back on so that T cells can attack cancer cells.

[0097] In some embodiments, a checkpoint inhibitor of this disclosure may be an inhibitor of PD-1, CTLA-4, or PD-L1.

[0098] In certain embodiments, a checkpoint inhibitor of this disclosure may be an inhibitor of PD-1.

[0099] In certain embodiments, a checkpoint inhibitor of this disclosure may be pembrolizumab.

[00100] In certain embodiments, a checkpoint inhibitor of this disclosure may be pembrolizumab, nivolumab, cemiplimab, spartalizumab, atezolizumab, avelumab, or durvalumab.

[00101] Without wishing to be bound by theory, the PD-1 receptor-ligand interaction can be a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases, SHP-1 and SHP-2, to the immunoreceptor tyrosine-based switch motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 zeta (CD3 ζ), protein kinase C-theta (PKC θ), and zeta-chain-associated protein kinase (ZAP70), which are involved in the CD3 T-cell signaling cascade.

IL-2 immunotherapeutic active agents

[00102] In certain embodiments, an interleukin immunotherapeutic agent of this disclosure may be a natural or synthetic IL-2, a high dose IL-2, a recombinant IL-2, or aldesleukin.

[00103] In certain embodiments, an interleukin immunotherapeutic agent of this disclosure can be a recombinant human IL-2 protein.

[00104] In additional embodiments, an interleukin immunotherapeutic agent of this disclosure can be an IL-2 protein which is modified to reduce toxic or off-target effects, or is attached to a targeting moiety, or is engineered to target particular cells.

[00105] Numbered embodiments of this invention include the following:

[00106] (1) A method for treating or ameliorating the symptoms of cancer in a subject in need, the method comprising: administering a composition comprising an agent for inhibiting or suppressing expression of TGF- β 2; administering an immune checkpoint inhibitor; and administering an interleukin immunotherapeutic agent to the subject.

[00107] (2) An agent for inhibiting or suppressing expression of TGF- β 2 in combination with an immune checkpoint inhibitor and an interleukin immunotherapeutic active agent for use in treating or ameliorating the symptoms of cancer.

[00108] (3) A composition comprising an agent for inhibiting or suppressing expression of TGF- β 2 and a pharmaceutically acceptable carrier for use in the preparation of a medicament or for treating or ameliorating the symptoms of a cancer in a subject in combination with an immune checkpoint inhibitor and an interleukin immunotherapeutic active agent.

[00109] (4) The method, agent or composition of any of embodiments 1-3, wherein the cancer is a solid cancer, a pancreatic cancer, melanoma, lung cancer, breast cancer, multiple myeloma, or colorectal cancer.

[00110] (5) The method, agent or composition of any of embodiments 1-4, wherein the agent for inhibiting or suppressing expression of TGF- β 2, the immune checkpoint inhibitor, and the interleukin immunotherapeutic active agent are administered concurrently, simultaneously, sequentially, or separately in time.

[00111] (6) The method, agent or composition of any of embodiments 1-5, wherein the composition and agents are administered by infusion or injection.

[00112] (7) The method, agent or composition of any of embodiments 1-6, wherein the agent for inhibiting or suppressing expression of TGF- β 2 is selected from Table 1 or Table 2, and chemically-modified variants thereof, LNA variants thereof, gapmer variants thereof, and any combination or pooling thereof.

[00113] (8) The method, agent or composition of any of embodiments 1-7, wherein the agent for inhibiting or suppressing expression of TGF- β 2 is **CGGCATGCTATTTTGTA** SEQ ID NO:1 or **C*G*G*C*A*T*G*T*C*T*A*T*T*T*T*G*T*A** SEQ ID NO:137.

[00114] (9) The method, agent or composition of any of embodiments 1-8, wherein the agent or composition comprises a carrier of sterile water for injection, saline, isotonic saline, or a combination thereof.

- [00115] (10) The method, agent or composition of any of embodiments 1-9, wherein the agent or composition is substantially free of excipients.
- [00116] (11) The method, agent or composition of any of embodiments 1-10, wherein the composition is stable for at least 14 days in carrier at 37°C.
- [00117] (12) The method, agent or composition of any of embodiments 1-11, wherein the immune checkpoint inhibitor is an inhibitor of PD-1, CTLA-4, or PD-L1.
- [00118] (13) The method, agent or composition of any of embodiments 1-12, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, cemiplimab, spartalizumab, atezolizumab, avelumab, or durvalumab.
- [00119] (14) The method, agent or composition of any of embodiments 1-13, wherein the interleukin immunotherapeutic active agent is a natural IL-2, a high dose IL-2, a recombinant IL-2, or aldesleukin.
- [00120] (15) The method, agent or composition of any of embodiments 1-14, wherein the subject upon the administration or use has a reduced TGF- β 2 expression.
- [00121] (16) The method, agent or composition of any of embodiments 1-15, comprising selecting the subjects who benefit from the method, agent or use using one or more biomarkers.
- [00122] (17) The method, agent or composition of any of embodiments 1-16, wherein the one or more biomarkers are a level of IRF5, a level of ITGAM, or a combination thereof.
- [00123] (18) The method, agent or composition of any of embodiments 1-17, wherein the one or more biomarkers is ITGAM and the subject is selected when expression of ITGAM is at a level above that found in a healthy patient.
- [00124] (19) The method, agent or composition of any of embodiments 1-18, wherein the subject after the administration or use has an increased level of IRF5.
- [00125] (20) The method, agent or composition of any of embodiments 1-19, wherein the subject after the administration or use has an increased level of ITGAM.
- [00126] (21) The method, agent or composition of any of embodiments 1-20, comprising administering a therapeutically sufficient amount of a pharmaceutical composition comprising an expression product of IRF5 or ITGAM to the subject.
- [00127] (22) The method, agent or composition of any of embodiments 1-21, wherein the expression product is an mRNA, a polypeptide, a protein, or fragment thereof, or combination thereof.

[00128] (23) The method, agent or composition of any of embodiments 1-22, wherein the administration or use decreases mortality rate at month 6, 12, 18, 24, 30, or 36.

[00129] (24) The method, agent or composition of any of embodiments 1-23, wherein the administration or use increases survival rate at month 6, 12, 18, 24, 30, or 36.

[00130] (25) The method, agent or composition of any of embodiments 1-24, wherein the administration or use of the composition is combined with a standard of care treatment for cancer, wherein the standard of care treatment comprises chemotherapy or radiation therapy.

[00131] All publications including patents, patent application publications, and non-patent publications referred to in this description, as well as the sequence listing are each expressly incorporated herein by reference in their entirety for all purposes.

[00132] Although the foregoing disclosure has been described in detail by way of example for purposes of clarity of understanding, it will be apparent to the artisan that certain changes and modifications are comprehended by the disclosure and may be practiced without undue experimentation within the scope of the appended claims, which are presented by way of illustration not limitation. This invention includes all such additional embodiments, equivalents, and modifications. This invention includes any combinations or mixtures of the features, materials, elements, or limitations of the various illustrative components, examples, and claimed embodiments.

[00133] The designations of agents, compounds and structures of this disclosure are meant to encompass all possible isomers, stereoisomers, diastereomers, enantiomers, and/or optical isomers that would be understood to exist for the specified structure, including any mixture, racemic or otherwise, thereof.

EXAMPLES

[00134] **Example 1.** This example shows ITGAM is a biomarker for melanoma patients who benefit from a therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor.

[00135] FIG. 1 shows the use of ITGAM as a biomarker for melanoma patients who benefit from a therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor.

[00136] FIG. 1 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in melanoma patients (KM Plotter). FIG. 1 shows that for use of a

PD-1 checkpoint inhibitor for selected patients with high ITGAM, improved survival was shown for low TGF- β 2 (logrank P=0.0039).

[00137] **Example 2.** This example shows ITGAM is a biomarker for pancreatic cancer patients who benefit from a therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor.

[00138] FIG. 2 shows the use of ITGAM as a biomarker for pancreatic cancer patients who benefit from a therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor.

[00139] FIG. 2 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in pancreatic cancer (KM Plotter). FIG. 2 shows that for use of IL-2 for selected patients with high ITGAM, improved survival was shown for high IL-2 (logrank P=0.034).

[00140] **Example 3.** This example shows IRF5 is a biomarker for melanoma patients who benefit from a therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor.

[00141] FIG. 3 shows the use of IRF5 as a biomarker for melanoma patients who benefit from a therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor.

[00142] FIG. 3 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in melanoma (KM Plotter). FIG. 3 shows that for use of a PD-1 checkpoint inhibitor for selected patients with high IRF5, improved survival was shown for low TGF- β 2 (logrank P=0.00053).

[00143] **Example 4.** This example shows that the therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor improves survival in cancer.

[00144] FIG. 4 shows the impact on overall survival in cancer for a therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor.

[00145] FIG. 4 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in cancer patients (KM Plotter). The study included multiple kinds of tumors as follows: bladder (N=73), esophageal adenocarcinoma (N=103), glioblastoma (N=28), hepatocellular carcinoma (N= 22), HNSCC (N=5), melanoma (N=423), NSCLC

(N=21), NSCL (n=22), and urothelial (N=348). FIG. 4 shows that for use of a PD-1 checkpoint inhibitor, improved survival was shown for high ratio of IL-2 to TGF- β 2 (IL-2/TGF- β 2, logrank P=0.0031).

[00146] Referring to FIG. 4, the median overall survival for the high expression cohort, i.e. high IL-2/TGF- β 2, was surprisingly increased to 23 months as compared to 10 months for median overall survival of the low expression cohort, i.e. low IL-2/TGF- β 2.

[00147] This study showed the unexpectedly advantageous impact on overall survival and established a basis for a therapeutic combination in cancer of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor.

[00148] **Example 5.** This example shows that the therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor improves survival in melanoma.

[00149] FIG. 5 shows the impact on overall survival in melanoma for a therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor.

[00150] FIG. 5 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in melanoma (KM Plotter). FIG. 5 shows that for use of a PD-1 checkpoint inhibitor, improved survival was shown for high ratio of IL-2 to TGF- β 2 (IL-2/TGF- β 2, logrank P=3.7e-06).

[00151] Referring to FIG. 5, the median overall survival for the high expression cohort, i.e. high IL-2/TGF- β 2, was surprisingly increased to 31 months as compared to 20 months for median overall survival of the low expression cohort, i.e. low IL-2/TGF- β 2.

[00152] This study showed the unexpectedly advantageous impact on overall survival and established a basis for a therapeutic combination in melanoma of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor.

[00153] **Example 6.** This example substantiates that the therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor improves survival in melanoma.

[00154] FIG. 6 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in melanoma patients (KM Plotter). FIG. 6 shows that for use of an

immune checkpoint inhibitor, improved survival was shown for high IL-2 (logrank $P=0.0015$).

[00155] Example 7. This example substantiates that the therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor improves survival in melanoma.

[00156] FIG. 7 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in melanoma patients (KM Plotter). FIG. 7 shows that for use of an immune checkpoint inhibitor, improved survival was shown for low TGF- β 2 (logrank $P=0.006$).

[00157] Example 8. This example substantiates that the therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor improves survival in cancer.

[00158] FIG. 8 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in cancer patients (KM Plotter). FIG. 8 shows that for use of a PD-1 checkpoint inhibitor, improved survival was shown for high IL-2 (logrank $P=0.007$).

[00159] Example 9. This example substantiates that the therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor improves survival in cancer.

[00160] FIG. 9 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in cancer patients (KM Plotter). FIG. 9 shows that for use of a PD-1 checkpoint inhibitor, improved survival was shown for low TGF- β 2 (logrank $P=0.0028$).

[00161] Example 10. This example shows that the therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor improves survival in cancer.

[00162] FIG. 10 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in melanoma patients (KM Plotter). The study included multiple kinds of tumors. FIG. 10 shows that for use of an immune checkpoint inhibitor, improved survival was shown for high ratio of IL-2 to TGF- β 2 (IL-2/TGF- β 2, logrank $P=3.7e-06$). Thus, the therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor improves survival in cancer.

[00163] Example 11. This example shows that the therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor improves survival in cancer.

[00164] FIG. 11 shows a Kaplan-Meier overall survival chart obtained in a study of clinical outcomes in cancer patients (KM Plotter). FIG. 11 shows that for use of a PD-1 checkpoint inhibitor, improved survival was shown for high ratio of IL-2 to TGF- β 2 (IL-2/TGF- β 2, logrank $P=2.2e-08$). Thus, the therapeutic combination of a TGF- β 2-specific antisense agent, an IL-2 immunotherapy agent, and a PD-1 checkpoint inhibitor improves survival in cancer.

WHAT IS CLAIMED IS:

1. A method for treating or ameliorating the symptoms of cancer in a subject in need, the method comprising:
 - administering a composition comprising an agent for inhibiting or suppressing expression of TGF- β 2;
 - administering an immune checkpoint inhibitor; and
 - administering an interleukin immunotherapeutic agent to the subject.
2. An agent for inhibiting or suppressing expression of TGF- β 2 in combination with an immune checkpoint inhibitor and an interleukin immunotherapeutic active agent for use in treating or ameliorating the symptoms of cancer.
3. A composition comprising an agent for inhibiting or suppressing expression of TGF- β 2 and a pharmaceutically acceptable carrier for use in the preparation of a medicament or for treating or ameliorating the symptoms of a cancer in a subject in combination with an immune checkpoint inhibitor and an interleukin immunotherapeutic active agent.
4. The method, agent or composition of any of claims 1-3, wherein the cancer is a solid cancer, a pancreatic cancer, melanoma, lung cancer, breast cancer, multiple myeloma, or colorectal cancer.
5. The method, agent or composition of any of claims 1-3, wherein the agent for inhibiting or suppressing expression of TGF- β 2, the immune checkpoint inhibitor, and the interleukin immunotherapeutic active agent are administered concurrently, simultaneously, sequentially, or separately in time.
6. The method, agent or composition of any of claims 1-3, wherein the composition and agents are administered by infusion or injection.
7. The method, agent or composition of any of claims 1-3, wherein the agent for inhibiting or suppressing expression of TGF- β 2 is selected from Table 1 or Table 2, and chemically-modified variants thereof, LNA variants thereof, gapmer variants thereof, and any combination or pooling thereof.
8. The method, agent or composition of any of claims 1-3, wherein the agent for inhibiting or suppressing expression of TGF- β 2 is CGGCATGCTCTATTTTGTA SEQ ID NO:1 or C*G*G*C*A*T*G*T*C*T*A*T*T*T*T*G*T*A SEQ ID NO:137.

9. The method, agent or composition of any of claims 1-3, wherein the agent or composition comprises a carrier of sterile water for injection, saline, isotonic saline, or a combination thereof.
10. The method, agent or composition of any of claims 1-3, wherein the agent or composition is substantially free of excipients.
11. The method, agent or composition of any of claims 1-3, wherein the composition is stable for at least 14 days in carrier at 37°C.
12. The method, agent or composition of any of claims 1-3, wherein the immune checkpoint inhibitor is an inhibitor of PD-1, CTLA-4, or PD-L1.
13. The method, agent or composition of any of claims 1-3, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, cemiplimab, spartalizumab, atezolizumab, avelumab, or durvalumab.
14. The method, agent or composition of any of claims 1-3, wherein the interleukin immunotherapeutic active agent is a natural IL-2, a high dose IL-2, a recombinant IL-2, or aldesleukin.
15. The method, agent or composition of any of claims 1-3, wherein the subject upon the administration or use has a reduced TGF- β 2 expression.
16. The method, agent or composition of any of claims 1-3, comprising selecting the subjects who benefit from the method, agent or use using one or more biomarkers.
17. The method, agent or composition of claim 16, wherein the one or more biomarkers are a level of IRF5, a level of ITGAM, or a combination thereof.
18. The method, agent or composition of claim 16, wherein the one or more biomarkers is ITGAM and the subject is selected when expression of ITGAM is at a level above that found in a healthy patient.
19. The method, agent or composition of any of claims 1-3, wherein the subject after the administration or use has an increased level of IRF5.
20. The method, agent or composition of any of claims 1-3, wherein the subject after the administration or use has an increased level of ITGAM.

21. The method, agent or composition of any of claims 1-3, comprising administering a therapeutically sufficient amount of a pharmaceutical composition comprising an expression product of IRF5 or ITGAM to the subject.
22. The method, agent or composition of claim 21, wherein the expression product is an mRNA, a polypeptide, a protein, or fragment thereof, or combination thereof.
23. The method, agent or composition of any of claims 1-3, wherein the administration or use decreases mortality rate at month 6, 12, 18, 24, 30, or 36.
24. The method, agent or composition of any of claims 1-3, wherein the administration or use increases survival rate at month 6, 12, 18, 24, 30, or 36.
25. The method, agent or composition of any of claims 1-3, wherein the administration or use of the composition is combined with a standard of care treatment for cancer, wherein the standard of care treatment comprises chemotherapy or radiation therapy.