[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.

[0010] For example, what is needed are therapeutic compositions which combine cancer T-cell and immunotherapies with potent anti-cancer agents.

SUMMARY

[0011] This disclosure refers to methods 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. Synergistic pharmaceutical therapies of this invention include use of potent, direct anti-tumor agents along with cancer immunotherapeutic agents. Cancer immunotherapeutics may include checkpoint inhibitor agents and protein immunotherapeutic agents. Methods, agents and uses of this invention may combine strategies for cancer immunotherapy with strategies for direct anti-tumor attack for treating various cancers.

[0012] In some examples, methods and therapeutic strategies of this invention can increase efficacy, as well as reduce toxic side effects and adverse health effects in cancer treatment.

[0013] In further examples, methods and therapeutic strategies of this invention can improve guidance of the therapy using appropriate biomarkers to select synergistic effects of the compositions.

[0014] Exemplary synergistic pharmaceutical therapies include compositions of various combinations of active agents including agents for inhibiting or suppressing expression of TGF-β2, checkpoint inhibitor agents, and interleukin immunotherapeutic agents. One or more biomarkers can be used to select subjects who benefit from the method, agent or use, including IRF5 and ITGAM. The compositions can be used in combination with chemotherapy and other standard-of-care therapies.

[0015] Examples of this invention include the following:

[0016] An antisense agent for inhibiting or suppressing expression of TGF-β2 in combination with a checkpoint inhibitor agent for use in treating or ameliorating the symptoms of cancer in a human subject or animal.

group, a 2'-OMe ribose group, a 2'-MOE methoxyethyl ribose group, a 2'-4' constrained methoxyethyl bicyclic ribose group, a 2'-4' constrained ethyl bicyclic ribose group, an LNA ribose group, a 2'-F ribose group, or a 5-methylcytodine base.

[0095] The kit above, wherein the antisense agent is conjugated to a polyethylene glycol, a lipid, or a triantenarry N-acteyl-galactosamine.

[0096] The kit above, wherein each agent comprises a carrier of sterile water for injection, saline, isotonic saline, or a combination thereof, which may be the same or different for each agent.

[0097] The kit above, wherein the agents are substantially free of excipients.

[0098] The kit above, wherein the agents are stable in a carrier substantially free of excipients for at least 14 days at 37°C.

[0098a] In one aspect, the present disclosure refers to use of an antisense agent for inhibiting or suppressing expression of TGF-β2 in the manufacture of a medicament for use in combination therapy for treating or ameliorating the symptoms of a cancer in a human subject or animal, wherein the medicament is to be administered in combination with a checkpoint inhibitor agent.

[0098b] In another aspect, the present disclosure refers to a medical product kit for treating or ameliorating the symptoms of cancer, the kit comprising: a therapeutically effective amount of an antisense agent for inhibiting or suppressing expression of TGF-β2; and a therapeutically effective amount of a checkpoint inhibitor agent.

BRIEF DESCRIPTION OF THE DRAWINGS

[0099] FIG. 1 shows Kaplan-Meier overall survival charts for a study of such clinical effects. FIG. 1 shows that for use of a PD-1 inhibitor, improved survival was indicated for high IL2 (left panel), which can be provided with an IL-2 immunotherapy agent, and low TGF-β2 (right panel), which can be provided with an antisense TGF-β2 inhibitor. These clinical data showed basis for a therapeutic combination of an antisense TGF-β2 inhibitor, a PD-1 checkpoint inhibitor, and an IL-2 immunotherapy agent for improved overall survival of melanoma patients.

[00100] FIG. 2 shows that for use of a PD-1 inhibitor, improved survival is highly indicated for a higher ratio of IL-2/ TGF-β2 (left panel), which can be provided with an IL-2 immunotherapy agent and an antisense TGF-β2 inhibitor. These clinical data showed basis for a therapeutic combination of an antisense TGF-β2 inhibitor, a PD-1

checkpoint inhibitor, and an IL-2 immunotherapy agent for improved overall survival of melanoma patients.

[00101] FIG. 3 shows data for biomarker use of ITGAM. FIG. 3 (upper left, right panels) show Kaplan-Meier overall survival charts for this study of melanoma patients. FIG. 3 (lower left, right panels) show Kaplan-Meier overall survival charts for this study of pancreatic cancer patients (PDAC) through the lens of TGF-β2 expression.

[00102] FIG. 4 shows data for biomarker use of ITGAM. FIG. 4 (left, right panels) show Kaplan-Meier overall survival charts for this study of pancreatic cancer patients (PDAC) through the lens of IL-2 expression.

WHAT IS CLAIMED IS:

1. Use of an antisense agent for inhibiting or suppressing expression of TGF-β2 in the manufacture of a medicament for use in combination therapy for treating or ameliorating the symptoms of a cancer in a human subject or animal, wherein the medicament is to be administered in combination with a checkpoint inhibitor agent.

2. The use of claim 1, wherein the medicament is to be administered in combination with an interleukin immunotherapeutic agent.

3. The use of claim 2, wherein the agent for inhibiting or suppressing expression of TGF-β2 and the checkpoint inhibitor are to be administered concurrently, simultaneously, sequentially, or separately in time.

4. The use of claim 2, wherein the agent for inhibiting or suppressing expression of TGF-β2, the checkpoint inhibitor, and the interleukin immunotherapeutic agent are to be administered separately or in combined formulation by injection or infusion.

5. The use of claim 1 or 2, wherein the cancer is a pancreatic cancer, a melanoma, a skin cancer, a lung cancer, a breast cancer, a prostate cancer, a colorectal cancer, a kidney cancer, a stomach cancer, an ovarian cancer, a cervical cancer, a liver cancer, or a multiple myeloma.

6. The use of claim 1 or 2, wherein the agent for inhibiting or suppressing expression of TGF-β2 is a TGF-β2-specific antisense oligonucleotide complementary to a TGF-β2 transcript and 15-30 nucleotides in length.

7. The use of claim 1 or 2, wherein the agent for inhibiting or suppressing expression of TGF-β2 is a TGF-β2-specific antisense oligonucleotides complementary to a TGF-β2 pre-RNA, pre-mRNA or mRNA and 18-21 nucleotides in length.

8. The use of claim 1 or 2, wherein the agent for inhibiting or suppressing expression of TGF-β2 is one or more TGF-β2-specific antisense oligonucleotides as shown below (Table 1), complementary to a TGF-β2 transcript:

SEQ ID NO:	Ref.	ANTISENSE SEQUENCE
8	--	CGGCATGTCT ATTTTGTA
1	--	GTTCGTTTAG AGAACAGATC
2	--	TAAAGTTCGT TTAGAGAACA G
3	--	AGCCCTGTAT ACGAC

SEQ ID NO:	Ref.	ANTISENSE SEQUENCE
4	--	GTAGGTAAAA ACCTAATAT
5	--	CGTTTAGAGA ACAGATCTAC
6	--	CATTGTAGAT GTCAAAAGCC
7	--	CTCCCTCATG GTGGCAGTTG A
9	178-195	TTTGTTCCTG GATGACTC
10	179-196	GTTTGTTCCT GGATGACT
11	180-197	AGTTTGTTCC TGGATGAC
12	181-198	CAGTTTGTTC CTGGATGA
13	182-199	TCAGTTTGTT CCTGGATG
14	183-200	CTCAGTTTGT TCCTGGAT
15	716-733	TGTGTGTGTG TGCGTGTG
16	717-734	GTGTGTGTGT GTGCGTGT
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 CTGAACTC
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 TGTAGATG
42	1574-1591	CCTGGTGCTG TTGTAGAT
43	1575-1592	CCCTGGTGCT GTTGTAGA
44	1576-1593	TCCCTGGTGC TGTTGTAG

SEQ ID NO:	Ref.	ANTISENSE SEQUENCE
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
57	2000-2017	GTTCCTGTCT TTATGGTG
58	2001-2018	GGTTCCTGTC TTTATGGT
59	2002-2019	AGGTTCCTGT CTTTATGG
60	2003-2020	CAGGTTCCTG TCTTTATG
61	2004-2021	CCAGGTTCCT 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	CCGGTTGGTC TGTTGTGA
68	2522-2539	GAGAATGGTT AGAGGTTC
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

SEQ ID NO:	Ref.	ANTISENSE SEQUENCE
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 GGGAAGGG
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 TTTTTCCC
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

SEQ ID NO:	Ref.	ANTISENSE SEQUENCE
125	5627-5644	CTATCCAAGT TATGGGGC
126	5759-5776	ATTGGAGGAA ATAGGGTG
127	5783-5800	GTCTTGTAGG TAGCAGCC
128	5784-5801	GGTCTTGTAG 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 TCTTGTAG

and chemically-modified variants thereof, LNA variants thereof, gapmer variants thereof, and any combination or pooling thereof.

9. The use of claim 8, wherein the TGF-β2-specific antisense oligonucleotides have no more than one or two mismatches as compared to a target human TGF-β2.

10. The use of claim 8, wherein the TGF-β2-specific antisense oligonucleotides reduce a TGF-β2 transcript level by at least 60%, or at least 70%, or at least 80%, or at least 90%.

11. The use of claim 8, wherein the TGF-β2-specific antisense oligonucleotides reduce any TGF-β1 transcript level and any TGF-β3 transcript level by less than 10%, or less than 5%, or less than 1%.

12. The use of claim 8, wherein the TGF-β2-specific antisense oligonucleotides have one or more nucleotides chemically modified as a phosphorothioate internucleoside linkage, a methoxypropylphosphonate internucleoside linkage, an aminophosphoro linkage to a morpholino group, a 2'-OMe ribose group, a 2'-MOE methoxyethyl ribose group, a 2'-4' constrained methoxyethyl bicyclic ribose group, a 2'-4' constrained ethyl bicyclic ribose group, an LNA ribose group, a 2'-F ribose group, or a 5-methylcytodine base.

13. The use of claim 8, wherein the antisense agent is conjugated to a polyethylene glycol, a lipid, or a triantenarry N-acteyl-galactosamine.

14. The use of claim 1 or 2, wherein each agent comprises a carrier of sterile water for injection, saline, isotonic saline, or a combination thereof, which may be the same or different for each agent.

15. The use of claim 1 or 2, wherein the agents are substantially free of excipients.

16. The use of claim 1 or 2, wherein the agents are stable in a carrier substantially free of excipients for at least 14 days at 37°C.

17. The use of claim 1 or 2, wherein the checkpoint inhibitor agent is an inhibitor of PD-1.

18. The use of claim 1 or 2, wherein the checkpoint inhibitor agent is pembrolizumab, nivolumab, cemiplimab, spartalizumab, atezolizumab, avelumab, or durvalumab.

19. The use of claim 1 or 2, wherein the interleukin immunotherapeutic agent is aldesleukin, a natural IL-2, a high dose IL-2, or a recombinant IL-2.

20. The use of claim 1 or 2, wherein subjects who benefit from the medicament based on levels of one or more biomarkers TGF-β2, IL-2, CD19, IRF5, ITGAM, and a combination thereof are to be selected.

21. The use of claim 20, wherein the one or more biomarkers is IRF5 and the subject is to be selected when expression of IRF5 is at a level above the median.

22. The use of claim 20, wherein the one or more biomarkers is ITGAM and the subject is to be selected when expression of ITGAM is at a level above the median.

23. The use of claim 1 or 2, wherein the subject after administration of the medicament has a decreased level of TGF-β2 as compared to before administration of the medicament.

24. The use of claim 1 or 2, wherein the subject after administration of the medicament has an increased level of IRF5 as compared to before administration of the medicament.

25. The use of claim 1 or 2, wherein the subject after administration of the medicament has a decreased level of ITGAM as compared to before administration of the medicament.

26. The use of claim 1 or 2, wherein a therapeutically effective amount of an expression product of IRF5 or ITGAM is to be administered to the subject.

27. The use of claim 26, wherein the expression product is an mRNA, polypeptide, protein, or fragment thereof, or combination thereof.

28. The use of claim 1 or 2, wherein the medicament decreases mortality rate at month 6, 12, 18, 24, 30, or 36.

29. The use of claim 1 or 2, wherein the medicament increases overall survival rate at month 6, 12, 18, 24, 30, or 36.

30. The use of claim 1 or 2, wherein the medicament is to be administered in combination with any one or more medicaments comprising a targeted cancer drug, a cancer growth blocker, an EGFR inhibitor, and combinations thereof.

31. The use of claim 1 or 2, wherein the medicament is to be administered in combination with any one or more medicaments selected from bevacizumab, everolimus, belzutifan, dabrafenib, trametinib, and combinations thereof.

32. The use of claim 1 or 2, wherein the medicament is to be administered in combination with any one or more medicaments which are cancer growth blockers selected from an angiogenesis inhibitor, a histone deacetylase inhibitor, a hedgehog blocker, an mTOR inhibitor, a p53 inhibitor, a PARP inhibitor, a proteasome inhibitor, a tyrosine kinase inhibitor, and combinations thereof.

33. The use of claim 1 or 2, wherein the medicament is to be administered in combination with any one or more medicaments which are EGFR inhibitors selected from erlotinib, gefitinib, afatinib, osimertinib, dacomitininb, and combinations thereof.

34. The use of claim 1 or 2, wherein the medicament is to be administered in combination with a chemotherapy medicament.

35. The use of claim 1 or 2, wherein the medicament is to be administered in combination with radiation therapy or electric field therapy.

36. A medical product kit for treating or ameliorating the symptoms of cancer, the kit comprising:

a therapeutically effective amount of an antisense agent for inhibiting or suppressing expression of TGF-β2; and

a therapeutically effective amount of a checkpoint inhibitor agent.

37. The medical product kit of claim 36, comprising a therapeutically effective amount of an interleukin immunotherapeutic agent.

38. The medical product kit of claim 36, wherein the cancer is a pancreatic cancer, a melanoma, a skin cancer, a lung cancer, a breast cancer, a prostate cancer, a colorectal cancer, a kidney cancer, a stomach cancer, an ovarian cancer, a cervical cancer, a liver cancer, a thymus cancer, or a multiple myeloma.

39. The medical product kit of claim 36, wherein the agent for inhibiting or suppressing expression of TGF-β2 is a TGF-β2-specific antisense oligonucleotide complementary to a TGF-β2 transcript and 15-30 nucleotides in length.

40. The medical product kit of claim 36, wherein the agent for inhibiting or suppressing expression of TGF-β2 is a TGF-β2-specific antisense oligonucleotides complementary to a TGF-β2 pre-RNA, pre-mRNA or mRNA and 18-21 nucleotides in length.

41. The medical product kit of claim 36, wherein the agent for inhibiting or suppressing expression of TGF-β2 is one or more TGF-β2-specific antisense oligonucleotides as shown in Table 1, complementary to a TGF-β2 transcript.

42. The medical product kit of claim 36, wherein the TGF-β2-specific antisense oligonucleotides have no more than one or two mismatches as compared to a target human TGF-β2.

43. The medical product kit of claim 36, wherein the TGF-β2-specific antisense oligonucleotides reduce a TGF-β2 transcript level by at least 60%, or at least 70%, or at least 80%, or at least 90%.

44. The medical product kit of claim 36, wherein the TGF-β2-specific antisense oligonucleotides reduce any TGF-β1 transcript level and any TGF-β3 transcript level by less than 10%, or less than 5%, or less than 1%.

45. The medical product kit of claim 36, wherein the TGF-β2-specific antisense oligonucleotides have one or more nucleotides chemically modified as a phosphorothioate internucleoside linkage, a methoxypropylphosphonate internucleoside linkage, an aminophosphoro linkage to a morpholino group, a 2'-OMe ribose group, a 2'-MOE methoxyethyl ribose group, a 2'-4' constrained methoxyethyl bicyclic ribose group, a 2'-4' constrained ethyl bicyclic ribose group, an LNA ribose group, a 2'-F ribose group, or a 5-methylcytodine base.

46. The medical product kit of claim 36, wherein the antisense agent is conjugated to a polyethylene glycol, a lipid, or a triantenarry N-acteyl-galactosamine.

47. The medical product kit of claim 36, wherein each agent comprises a carrier of sterile water for injection, saline, isotonic saline, or a combination thereof, which may be the same or different for each agent.

48. The medical product kit of claim 36, wherein the agents are substantially free of excipients.

49. The medical product kit of claim 36, wherein the agents are stable in a carrier substantially free of excipients for at least 14 days at 37°C.