SUMMARY

[0008a] In one aspect, the present disclosure refers to the use of an attenuated strain of Salmonella comprising at least one copy of a DNA molecule comprising a eukaryotic expression cassette encoding Wilms' Tumor Protein (WT1) for the manufacture of a medicament, for the treatment of cancer, wherein the treatment further comprises the administration of at least one checkpoint inhibitor, wherein the checkpoint inhibitor is selected from at least one antibody against PD-1, PD-L1, CTLA-4, IDO, OX-40, GITR, TIM-3, and LAG-3, and wherein the attenuated strain of Salmonella is Salmonella typhi Ty21a.

[0009] In one example, the present disclosure relates to an attenuated strain of Salmonella comprising at least one copy of a DNA molecule comprising an expression cassette encoding Wilms' Tumor Protein (WT1), for use in the treatment of cancer, wherein the treatment further comprises the administration of at least one checkpoint inhibitor.

[0010] The combined treatment with an attenuated strain of Salmonella encoding WT1 and at least one checkpoint inhibitor was surprisingly found to exhibit a strong, and sustained anti-tumor effect. Surprisingly, it was observed that the combined administration of an attenuated strain of Salmonella encoding WT1 with either of the checkpoint inhibitors anti-PD-L1 and anti-CTLA-4 has a synergistic effect on overall survival.

[0011] Without wishing to be bound by theory, it is believed that VXM06 is able to generate WT-1 specific effector T-cells which might become inactivated by the tumor microenvironment. Checkpoint inhibitor monoclonal antibodies target structures on immune cells or tumor cells, respectively, and are therefore reported to counterbalance or prevent T-cell inhibitory effects.

[0012] In particular examples, the at least one checkpoint inhibitor is selected from at least one antibody against PD-1, PD-L1, CTLA-4, IDO, OX-40, GITR, TIM-3, and LAG-3.

[0013] In particular examples the attenuated strain of Salmonella is of the species Salmonella enterica. Particularly, the attenuated strain of Salmonella is Salmonella typhi Ty21a.

[0014] In particular examples, the expression cassette is a eukaryotic expression cassette. Particularly, the expression cassette comprises a CMV promoter.

[0015] In particular examples, WT1 is selected from the group consisting of human WT1 having the amino acid sequence as found in SEQ ID NO 4 and a protein

that shares at least about 80% sequence identity therewith. Particularly, WT1 is truncated, more particularly the zinc finger domain of WT1 is deleted. In particular such examples, WT1 is selected from the group consisting of WT1 having the amino acid sequence as found in SEQ ID NO 1 and a protein that shares at least 80% sequence identity therewith.

[0016] In particular examples, the DNA molecule comprises the kanamycin antibiotic resistance gene, the pMB1 ori, and a CMV promoter. Particularly, the DNA molecule comprises the DNA sequence as found in SEQ ID NO 2.

[0017] In particular examples, the attenuated strain of Salmonella is administered simultaneously with, prior to or after said at least one checkpoint inhibitor.

[0018] In particular examples, the treatment is accompanied by chemotherapy, radiotherapy or biological cancer therapy, particularly wherein the attenuated strain of Salmonella is administered before, during or after the chemotherapy or the radiotherapy treatment cycle or the biological cancer therapy, or before and during the chemotherapy or the radiotherapy treatment cycle or the biological cancer therapy.

[0019] In particular examples, the biological cancer therapy comprises administration of one or more further attenuated strain(s) of Salmonella comprising at least one copy of a DNA molecule comprising an expression cassette encoding a tumor antigen and/or a tumor stroma antigen. In particular such examples, said one or more further attenuated strain(s) of Salmonella is/are Salmonella typhi Ty21a comprising a eukaryotic expression cassette. Particularly, said tumor antigen is selected from the group consisting of Mesothelin (MSLN), CEA, CMV pp65, preferably said tumor antigen is selected from the group (a) Mesothelin (MSLN), particularly MSLN having the amino acid sequence as found in SEQ ID NO 5 and a protein that shares at least about 80% sequence identity therewith, (b) CEA, particularly CEA having the amino acid sequence as found in SEQ ID NO 6 and a protein that shares at least about 80% sequence identity therewith, and (c) CMV pp65, particularly CMV pp65 having the amino acid sequence as found in SEQ ID NO 7 and a protein that shares at least about 80% sequence identity therewith, CMV

pp65 having the amino acid sequence as found in SEQ ID NO 8 and a protein that shares at least about 80% sequence identity therewith, and CMV pp65 having the amino acid sequence as found in SEQ ID NO 9 and a protein that shares at least about 80% sequence identity therewith. Particularly, said tumor stroma antigen is selected from the group consisting of a VEGF receptor protein and human fibroblast activation protein (FAP), wherein preferably the VEGF receptor protein is VEGFR-2 more preferably human VEGFR-2 and even more preferably human VEGFR-2 having the amino acid sequence as found in SEQ ID NO 10 or a protein that shares at least about 80% sequence identity therewith.

[0020] In particular examples, the attenuated strain of Salmonella is administered orally.

[0021] In particular examples, the cancer is selected from leukemia, particularly from acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL), from multiple myeloma, and from solid tumors, particularly from lung cancer, breast cancer, esophageal, colon, colorectal, gastric, cholangioductal, pancreatic cancer, glioblastoma, head and neck cancer, synovial sarcoma, angiosarcoma, osteosarcoma, thyroid cancer, cervical, endometrial, ovarian cancer, neuroblastoma, rhabdomyosarcoma, and prostate cancer.

[0022] In particular examples, the single dose of the attenuated strain of Salmonella comprises from about 10^5 to about 10^{11}, particularly from about 10^6 to about 10^{10}, more particularly from about 10^6 to about 10^9, more particularly from about 10^6 to about 10^8, most particularly from about 10^6 to about 10^7 colony forming units (CFU).

[0023] In particular examples, the treatment is individualized cancer immunotherapy comprising the step of assessing the WT1 expression and/or the pre-immune response against WT1 in a patient.

[0024] In a further example, the present disclosure relates to a pharmaceutical composition comprising an attenuated strain of Salmonella comprising at least one copy of a DNA molecule comprising an expression cassette encoding WT1 for use in

the treatment of cancer, wherein the treatment further comprises the administration of at least one checkpoint inhibitor. Particularly, the at least one checkpoint inhibitor is selected from at least one antibody against PD-1, PD-L1, CTLA-4, IDO, OX-40, GITR, TIM-3, and LAG-3.

[0025] In particular examples, the attenuated strain of Salmonella is Salmonella typhi Ty21a, the expression cassette is a eukaryotic expression cassette, and WT1 is selected from the group consisting of human WT1 having the amino acid sequence as found in SEQ ID NO 1 and a protein that shares at least about 80% sequence identity therewith.

[0026] Particularly, the eukaryotic expression cassette comprises a CMV promoter. Particularly, human WT1 has the amino acid sequence as found in SEQ ID NO 1.

DETAILED DESCRIPTION OF THE INVENTION

[0027] The present invention may be understood more readily by reference to the following detailed description of the invention and the examples included therein.

[0028] In one aspect, the present invention relates to an attenuated strain of Salmonella comprising at least one copy of a DNA molecule comprising an expression cassette encoding Wilms' Tumor Protein (WT1) for use in the treatment of cancer, wherein the treatment further comprises the administration of at least one checkpoint inhibitor.

[0029] The zinc finger transcription factor Wilms' tumor protein 1 is encoded by the WT1 gene. It contains four zinc finger motifs at the C-terminus and a proline/glutamine-rich DNA-binding domain at the N-terminus. Multiple transcript variants, resulting from alternative splicing at two coding exons, have been well characterized. WT1 plays an essential role in the development of the urogenital system and is involved in cell proliferation and differentiation. The WT1 gene was isolated as the gene responsible for a childhood renal neoplasm, Wilms' tumor. It is highly expressed in a wide variety of malignancies including several types of hematological malignancies and various solid tumors. In contrast, normal tissue

CLAIMS

1. Use of an attenuated strain of Salmonella comprising at least one copy of a DNA molecule comprising a eukaryotic expression cassette encoding Wilms' Tumor Protein (WT1) for the manufacture of a medicament, for the treatment of cancer, wherein the treatment further comprises the administration of at least one checkpoint inhibitor, wherein the checkpoint inhibitor is selected from at least one antibody against PD-1, PD-L1, CTLA-4, IDO, OX-40, GITR, TIM-3, and LAG-3, and wherein the attenuated strain of Salmonella is Salmonella typhi Ty21a.

2. The use according to claim 1, wherein
   (a) WT1 is selected from the group consisting of human WT1 having the amino acid sequence as found in SEQ ID NO 4 and a protein that shares at least about 80% sequence identity therewith,
   (b) wherein WT1 is truncated, more particularly wherein the zinc finger domain of WT1 is deleted, or
   (c) wherein WT1 is selected from the group consisting of WT1 having the amino acid sequence as found in SEQ ID NO 1 and a protein that shares at least about 80% sequence identity therewith.

3. The use according to claim 1 or 2, wherein the DNA molecule comprises the kanamycin antibiotic resistance gene, the pMB1 ori, and a CMV promoter, particularly wherein the DNA molecule comprises the DNA sequence as found in SEQ ID NO 2.

4. The use according to any one of the preceding claims, wherein the attenuated strain of Salmonella is administered simultaneously with, prior to or after said at least one checkpoint inhibitor.

5. The use according to any one of the preceding claims, wherein the treatment is accompanied by chemotherapy, radiotherapy or biological cancer therapy.

6. The use according to any one of the preceding claims, wherein the attenuated strain of Salmonella is administered orally.

7. The use according to any one of the preceding claims, wherein the cancer is selected from leukemia, particularly from acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL), from multiple myeloma, and from solid tumors, particularly from lung cancer, breast cancer, esophageal, colon, colorectal, gastric, cholangioductal, pancreatic cancer, glioblastoma, head and neck cancer, synovial sarcoma, angiosarcoma, osteosarcoma, thyroid cancer, cervical, endometrial, ovarian cancer, neuroblastoma, rhabdomyosarcoma, and prostate cancer.

8. The use according to any one of the preceding claims, wherein the single dose of the attenuated strain of Salmonella comprises from about 10^5 to about 10^{11} colony forming units (CFU).

9. The use according to any one of the preceding claims, wherein the treatment is individualized cancer immunotherapy comprising the step of assessing the WT1 expression and/or the pre-immune response against WT1 in a patient.