SUMMARY

[0008] In one aspect, the present invention refers to use of an attenuated strain of Salmonella typhi Ty21a comprising at least one copy of a DNA molecule comprising a eukaryotic expression cassette encoding a VEGF receptor protein, in the manufacture of a medicament for the treatment of cancer, wherein further at least one checkpoint inhibitor, particularly selected from an antibody against PD-1, PD-L1 and CTLA4 is to be administered. In another aspect, the present invention refers to use of an attenuated strain of Salmonella typhi Ty21a comprising at least one copy of a DNA molecule comprising a eukaryotic expression cassette encoding a VEGF receptor protein, and at least one further attenuated strain of Salmonella comprising at least one copy of a further DNA molecule comprising a further expression cassette encoding a tumor antigen or a tumor stroma antigen in the manufacture of a pharmaceutical composition for the treatment of cancer, wherein further at least one checkpoint inhibitor is to be administered. In one example, there is described an attenuated strain of Salmonella comprising at least one copy of a DNA molecule comprising an expression cassette encoding a VEGF receptor protein, for use in the treatment of cancer, wherein the treatment further comprises the administration of a further anti-cancer agent.

[0009] The attenuated strain of Salmonella encoding a VEGF receptor protein was surprisingly found to strongly increase the efficacy of cancer therapies which are based on the employment of the patient's immune system, such as treatment with cancer vaccines encoding tumor antigens or tumor stroma antigens, treatment with engineered T-cells that are designed to target tumor cells, treatment with bispecific antibodies designed to mediate the attachment of immune cells to tumor cells, and treatment with checkpoint inhibitors which aim at preventing the tumor induced inhibition of T-cell proliferation.

[0010] Surprisingly, it was observed that the administration of the attenuated strain of Salmonella encoding a VEGF receptor protein leads to a significantly increased infiltration of the tumor by $CD8^+$ and $CD4^+$ T-cells. Furthermore, the administration of the attenuated strain of Salmonella encoding a VEGF receptor protein may lead to an increase in the number of activated $CD8^+$ and $CD4^+$ T-cells and/or to a reduction in the

number of immunosuppressive lymphoid cells such as Treg cells. Without wishing to be bound by theory, it is believed that the administration of the attenuated strain of Salmonella encoding a VEGF receptor protein improves the efficacy of cancer immunotherapies by enhancing the engagement of T-cells in the eradication of the tumor. The combination of the attenuated strain of Salmonella encoding a VEGF receptor protein with other anti-cancer agents, such as engineered T-cells, checkpoint inhibitors, bispecific antibodies and DNA vaccines encoding tumor antigens or tumor stroma antigens was shown to have synergistic effects on tumor specific T-cell responses and overall survival.

[0011] In particular examples, the treatment further comprises the administration of at least one DNA vaccine encoding a tumor antigen or a tumor stroma antigen, of at least one checkpoint inhibitor, of at least one engineered T-cell, of at least one bispecific antibody exhibiting binding specificity for one T-cell surface protein and for a tumor antigen or a tumor stroma antigen, or of any combination thereof.

[0012] In particular examples, the at least one DNA vaccine encoding a tumor antigen or a tumor stroma antigen is selected from at least one further attenuated strain of Salmonella comprising at least one copy of a further DNA molecule comprising a further expression cassette encoding a tumor antigen or a tumor stroma antigen.

[0013] In particular examplesthe at least one checkpoint inhibitor is selected from an antibody against PD-1, PD-L1 and CTLA4.

[0014] In particular examples, the attenuated strain of Salmonella and the at least one further attenuated strain of Salmonella are of the species Salmonella enterica.

[0015] In particular examples, the attenuated strain of Salmonella and the at least one further attenuated strain of Salmonella are Salmonella typhi Ty21a.

[0016] In particular examples, the expression cassette and the further expression cassette are a eukaryotic expression cassette, particularly comprising a CMV promoter.

[0017] In particular examples, the VEGF receptor protein is selected from the group consisting of human VEGFR-2 having the amino acid sequence as found in SEQ ID NO 1 and a protein that shares at least about 80% sequence identity therewith.

[0018] In particular examples, human VEGFR-2 has the amino acid sequence as found in SEQ ID NO 1.

[0019] In particular examples, the DNA molecule and the further DNA molecule comprise the kanamycin antibiotic resistance gene, the pMB1 ori, and a CMV promoter.

[0020] In particular examples, the DNA molecule and the further DNA molecule comprise the DNA sequence as found in SEQ ID NO 2.

[0021] In particular examples, the tumor antigen encoded by said further attenuated strain of Salmonella is selected from the group consisting of human Wilms' Tumor Protein (WT1) having the amino acid sequence as found in SEQ ID NO 3 and a protein that shares at least about 80% sequence identity therewith, human Mesothelin (MSLN) having the amino acid sequence as found in SEQ ID NO 4 and a protein that shares at least about 80% sequence identity therewith, human CEA having the amino acid sequence as found in SEQ ID NO 5 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 6 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 7 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 8 and a protein that shares at least about 80% sequence identity therewith, and the tumor stroma antigen encoded by said further attenuated strain of Salmonella is selected from the group consisting of human fibroblast activation protein (FAP).

[0022] In particular examples, human Wilms' Tumor Protein (WT1) has the amino acid sequence as found in SEQ ID NO 3, human Mesothelin (MSLN) has the amino acid sequence as found in SEQ ID NO 4, human CEA has the amino acid sequence as found

in SEQ ID NO 5, and CMV pp65 has the amino acid sequence as found in SEQ ID NO 6, SEQ ID NO 7 or SEQ ID NO 8.

[0023] In particular examples, the attenuated strain of Salmonella is administered simultaneously with or prior to said further anti-cancer agent, i.e. simultaneously with or prior to said at least one DNA vaccine encoding a tumor antigen or a tumor stroma antigen, said at least one checkpoint inhibitor, said at least one engineered T-cell and/or said at least one bispecific antibody.

[0024] In particular examples, the treatment is accompanied by chemotherapy, radiotherapy or biological cancer therapy. Particularly, the attenuated strain of Salmonella is administered before or during the chemotherapy or the radiotherapy treatment cycle or the biological cancer therapy. In other particular examples, the attenuated strain of Salmonella is administered before and during the chemotherapy or the radiotherapy treatment cycle or the biological cancer therapy.

[0025] In particular examples, the attenuated strain of Salmonella and the at least one further attenuated strain of Salmonella are administered orally.

[0026] In particular examples, the cancer is selected from colorectal cancer, pancreatic cancer, lung cancer, ovarian cancer, mesothelioma, acute myeloid leukemia, chronic myeloid leukemia, glioblastoma, gastric cancer, hepatocellular cancer, renal cell cancer, prostate cancer, and cervical cancer.

[0027] In particular examples, the single dose of the attenuated strain of Salmonella and the at least one further 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).

[0028] In particular examples, the treatment is individualized cancer immunotherapy comprising the step of assessing the expression pattern of and/or the pre-immune response against said tumor antigen in a patient.

[0029] In a further examples, there is described a pharmaceutical composition comprising an attenuated strain of Salmonella comprising at least one copy of a DNA molecule comprising an expression cassette encoding a VEGF receptor protein, wherein the pharmaceutical composition further comprises at least one further attenuated strain of Salmonella comprising at least one copy of a further DNA molecule comprising a further expression cassette encoding a tumor antigen or a tumor stroma antigen.

[0030] In particular examples, the attenuated strain of Salmonella and the at least one further attenuated strain of Salmonella is Salmonella typhi Ty21a.

[0031] In particular examples, the expression cassette and the further expression cassette are a eukaryotic expression cassette, particularly comprising a CMV promoter.

[0032] In particular examples, the VEGF receptor protein is selected from the group consisting of human VEGFR-2 having the amino acid sequence as found in SEQ ID NO 1 and a protein that shares at least about 80% sequence identity therewith.

[0033] In particular examples, human VEGFR-2 has the amino acid sequence as found in SEQ ID NO 1.

[0034] In particular examples, the pharmaceutical composition is for use as a medicament, particularly for use in the treatment of cancer.

CLAIMS

1. Use of an attenuated strain of Salmonella typhi Ty21a comprising at least one copy of a DNA molecule comprising a eukaryotic expression cassette encoding a VEGF receptor protein, in the manufacture of a medicament for the treatment of cancer, wherein further at least one checkpoint inhibitor, particularly selected from an antibody against PD-1, PD-L1 and CTLA4 is to be administered.

2. The use of an attenuated strain of Salmonella according to any one of the preceding claims, wherein the VEGF receptor protein is selected from the group consisting of human VEGFR-2 having the amino acid sequence as found in SEQ ID NO 1 and a protein that shares at least about 80% sequence identity therewith, particularly wherein human VEGFR-2 has the amino acid sequence as found in SEQ ID NO 1.

3. The use of an attenuated strain of Salmonella according to any one of the preceding claims, 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 of an attenuated strain of Salmonella according to any one of the preceding claims, wherein further at least one further attenuated strain of Salmonella typhi Ty21a comprising at least one copy of a further DNA molecule comprising a further eukaryotic expression cassette encoding a tumor antigen or a tumor stroma antigen is to be administered.

5. The use of an attenuated strain of Salmonella according to any one of the preceding claims, wherein said tumor antigen encoded by said further attenuated strain of Salmonella is selected from the group consisting of human Wilms' Tumor Protein (WT1) having the amino acid sequence as found in SEQ ID NO 3 and a protein that shares at least about 80% sequence identity therewith, human Mesothelin (MSLN) having the amino acid sequence as found in SEQ ID NO 4 and a protein that shares at least about 80% sequence identity therewith, human CEA having the amino acid sequence as found in SEQ ID NO 5 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 6 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 7 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 8 and a protein that shares at least about 80% sequence identity therewith, particularly wherein human Wilms' Tumor Protein (WT1) has the amino acid sequence as found in SEQ ID NO 3, human Mesothelin (MSLN) has the amino acid sequence as found in SEQ ID NO 4, human CEA has the amino acid sequence as found in SEQ ID NO 5, and CMV pp65 has the amino acid sequence as found in SEQ ID NO 6, SEQ ID NO 7 or SEQ ID NO 8, and wherein said tumor stroma antigen encoded by said further attenuated strain of Salmonella is selected from the group consisting of human fibroblast activation protein (FAP).

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

7. The use of an attenuated strain of Salmonella according to any one of the preceding claims, wherein the treatment is accompanied by chemotherapy, radiotherapy or biological cancer therapy, particularly wherein the attenuated strain of Salmonella is to be administered before or during 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.

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

9. The use of an attenuated strain of Salmonella according to any one of the preceding claims, wherein the cancer is selected from colorectal cancer, pancreatic cancer, lung cancer, ovarian cancer, mesothelioma, acute myeloid leukemia, chronic myeloid leukemia, glioblastoma, gastric cancer, hepatocellular cancer, renal cell cancer, prostate cancer, and cervical cancer.

10. The use of an attenuated strain of Salmonella according to of any one of the preceding claims, wherein the single dose of the attenuated strain of Salmonella and the at least one further 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).

11. The use of an attenuated strain of Salmonella according to any one of the preceding claims, wherein the treatment is individualized cancer immunotherapy comprising the step of assessing the expression pattern of and/or the pre-immune response against said tumor antigen in a patient.

12. Use of an attenuated strain of Salmonella typhi Ty21a comprising at least one copy of a DNA molecule comprising a eukaryotic expression cassette encoding a VEGF receptor protein, and at least one further attenuated strain of Salmonella comprising at least one copy of a further DNA molecule comprising a further expression cassette encoding a tumor antigen or a tumor stroma antigen in the manufacture of a pharmaceutical composition for the treatment of cancer, wherein further at least one checkpoint inhibitor is to be administered.

13. The use of an attenuated strain of Salmonella according to claim 12, wherein the VEGF receptor protein is selected from the group consisting of human VEGFR-2 having the amino acid sequence as found in SEQ ID NO 1 and a protein that shares at least about 80% sequence identity therewith, particularly wherein human VEGFR-2 has the amino acid sequence as found in SEQ ID NO 1.