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(54) Title: BACTERIAL BIOMARKER

(57) Abstract: The invention relates to bacterial compositions useful in the treatment of cancer. In particular, the compositions can be used as a co-therapy with an immune checkpoint therapy. The invention also relates to methods for identifying a subject that will respond to therapy with an immune checkpoint inhibitor comprising determining the abundance of bacteria in a biological sample from said subject.

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Bacterial biomarker

Introduction

Immune suppression and evasion by malignant cancer cells is known as one of the hallmarks of cancer. A number of co-inhibitory receptors and their ligands, known as immune checkpoints, contribute to this process. Immune checkpoint inhibitor cancer immunotherapies have been transformational in cancer management in that they can lead to long-term remission and they can be effective across many cancers. Among these checkpoints are programmed cell death 1 (PD-1), PD-L1 and CTLA-4. The introduction of PD-1 inhibitors into clinical practice has had a revolutionary effect on cancer treatment, but consistent responses and favourable long-term outcomes are only observed in a fraction of patients. The majority of patients do not respond to therapy. The highest proportion is for melanoma (reaching 40%), but it is much lower for the other cancers. Moreover, a significant number of patients develop immune-related adverse events and have to stop therapy.

Accordingly, there is a need for (a) biomarkers to predict response to immune checkpoint inhibitors and (b) approaches to increase the proportion of cancer patients that respond to therapy.

PD-1 (UniProt Accession No. Q15116, GenBank Accession No. U6486) protein is encoded by the PDCD1 gene and expressed as a 55kDa type I transmembrane protein (Agata 1996 Int Immunol 8(5):765-72). PD-1 is an immunoglobulin superfamily member (Ishida 1992 EMBO 11(11):3887-95) and it is an inhibitory member of the extended CD28/CTLA-4 family of T cell regulators. Other members of this family include CD28, CTLA-4, ICOS and BTLA. PD-1 exists as a monomer, lacking the unpaired cysteine residue characteristic of other CD28 family members (Zhang 2004 Immunity 20:337-47). Its cytoplasmic domain contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM) that are phosphorylated during signal transduction (Riley 2009 Immunol Rev 229(1):114-25).

PD-1 is expressed on B cells, T cells, and monocytes (Agata 1996). The role of PD-1 in maintaining immunologic self-tolerance was demonstrated in PDCD1-/- mice, which develop autoimmune disorders (Nishimura 1999 Immunity 11:141-51, Nishimura 2001 Science 291(5502):319-22). The PD-1 pathway therefore regulates antigen responses, balancing autoimmunity and tolerance.

There are two ligands for PD-1 that mediate its regulatory function. PD-L1 (B7-H1) is normally expressed on dendritic cells, macrophages, resting B cells, bone marrow-derived mast cells and T cells as well as non-hematopoietic cell lineages (reviewed in Francisco 2010 Immunol Rev 236:219-42). PD-L2 (B7-DC) is largely expressed on dendritic cells and macrophages (Tseng 2001 J Exp Med

193(7):839-45). Ligand expression is influenced by local mediators and can be upregulated by inflammatory cytokines.

PD-1 is known as an immunoinhibitory protein that negatively regulates TCR signals. The interaction between PD-1 and PD-L1 can act as an immune checkpoint, which can lead to, e.g., a decrease in tumour infiltrating lymphocytes, a decrease in T- cell receptor mediated proliferation, and/or immune evasion by cancerous cells. Immune suppression can be reversed by inhibiting the local interaction of PD-1 with PD-L1 or PD-L2; the effect is additive when the interaction of PD-1 with both PD-L1 and PD-L2 is blocked.

The PD-1 pathway can be exploited in cancer or infection, whereby tumours or viruses can evade effective immune recognition and T cells demonstrate an 'exhausted' phenotype.

Disruption of the PD-1:PD-L1 interaction enhances T cell activity. Inhibitory anti-PD-1 monoclonal antibodies demonstrate blockade of the interaction between PD-1 and its ligands (Wang 2014 Cancer Immunol Res 2(9):846-56). T cell function in vitro can be enhanced by PD-1 blockade, as demonstrated by improved proliferation and cytokine responses in mixed lymphocyte reactions of T cells and dendritic cells. Cytotoxic T Lymphocytes (CTLs) derived from melanoma patients have also been shown to be enhanced by PD-1 blockade in vitro using the antibody nivolumab, and can become resistant to suppression by regulatory T cells (Wang 2009 Int Immunol 21(9):1065-1077). This antibody has been shown to be efficacious in melanoma and in non-small-cell lung carcinoma (NSCLC) patients. Another PD-1 blocking antibody, pembrolizumab, demonstrates responses in NSCLC patients refractory to CTLA-4 blockade. Nivolumab and pembrolizumab both functionally block the interaction of human PD-1 with its ligands.

The gut microbiome of cancer patients is a major driver of response to immune checkpoint therapy.

Previous studies have analysed clinical datasets to identify gut microbiota associated with treatment efficacy (Frankel *Neoplasia* (2017) 19:848; Gopalakrishnan *Science* (2018) 359:97; Matson *Science* (2018) 359:104; Routy *Science* (2018) 359:91). However, the major challenge in the field has been that the microbiome signatures identified in the independent studies are very different. The published studies vary in response criteria and cancer indication, but also factors that are known to impact microbiome analysis such as sample collection, storage and processing and geographical location. Therefore, it has been difficult to understand what the true signature is amongst the inter-study noise.

Thus, there is a need to provide efficacious treatments of cancer as well as biomarkers that are predictive for response to treatment and the present invention is aimed at addressing this need.

Summary of the invention

The invention is based on the finding that the gut microbiome in subjects that respond to treatment with an immune checkpoint inhibitor is different to the gut microbiome in subjects that do not respond to treatment with the immune checkpoint inhibitor, and that the gut microbiome may therefore be employed either as a diagnostic for immune checkpoint inhibitor treatment or as the source of a therapy.

The invention is therefore aimed at a number of aspects, including, but not limited to the following:

- A composition comprising certain bacteria as defined herein which have been identified in patients who respond to treatment with an immune checkpoint inhibitor and which can be used as a treatment of disease, including treatment of cancer, an infectious disease or use as a vaccine adjuvant;
- A co-therapy comprising a composition having certain bacteria as defined herein and an immune checkpoint inhibitor treatment and
- Provision of certain bacteria as defined herein as a diagnostic for immune checkpoint inhibitor treatment to identify patients that benefit from immune checkpoint inhibitor treatment and also to identify patients which may receive bacterial or other therapy, e.g. before administration of the checkpoint inhibitor therapy.

These aspects as well as other related aspects of the invention and embodiments are further described herein.

The inventors have identified a microbiome biomarker signature associated with and highly predictive of response to treatment with an immune checkpoint inhibitor. This is of great significance in the field, providing the basis for the following: a predictive biomarker for checkpoint inhibitor therapy; a live bacterial therapeutic (LBT) therapy; a live bacterial therapeutic co-therapy with an immune checkpoint inhibitor, for example anti- PD-1, anti- PD-L1 or anti-CTLA-4 drugs for the treatment of cancer, to increase the proportion of patients responding to checkpoint inhibitors. In particular, the inventors have identified a number of bacterial species present in the gut microbiome that exhibit modulated abundance indicative of a response to treatment with an immune checkpoint inhibitor. Detecting modulated abundance of these bacteria may therefore be employed to discriminate responders to checkpoint inhibitor therapy from non-responders. In addition, the administration of such live bacteria as a medicine is predicted to convert patients not responding to checkpoint inhibitors to responders.

The bacteria identified and described herein may be employed individually to determine response and/or provide treatment, or combinations of the bacteria may be provided to increase the

discriminatory power of the diagnostic method and provide non-invasive methods of diagnosis for response versus non-response as well as methods of treatment.

The inventors have identified specific gut bacteria associated with checkpoint inhibitor response. The invention thus provides gut bacteria that can be used to modulate the microbiome to improve the therapeutic response to immune checkpoint inhibitors patients, for example cancer patients. Studies in the present disclosure used a cohort of patients with melanoma undergoing therapy with anti-PD-1 drugs or combination therapy with anti-PD-1 plus anti-CTLA-4 drugs. Gut microbiome samples taken prior to immune checkpoint therapy were characterized in these patients via metagenomic whole genome shotgun sequencing. Significant differences were observed in the composition of the gut microbiome in responders versus non-responders to immune checkpoint blockade therapy (e.g., to PD-1-based therapy), with an increase or decrease in abundance of specific bacteria in the gut microbiome of responders versus non-responders pre-treatment. In particular, the bacteria as described herein were found to be more abundant in responders. Therefore, these bacteria and subsets thereof find use in a composition which can be employed for the treatment of disease, including cancer, either alone or in combination with an immune checkpoint inhibitor treatment. Furthermore, these bacteria can be used as biomarkers, i.e. as a diagnostic to distinguish responders to checkpoint inhibitor, e.g. PD-1 inhibitor, therapy from non-responders for immune checkpoint inhibitor treatment.

The present studies show that patients with a "favourable" gut microbiome (with modulated, e.g. high relative abundance of one or more of bacteria as described herein) have enhanced anti-tumour immune responses. In contrast, patients with an "unfavourable" gut microbiome (with low relative abundance of the species B1-B15 as defined herein) have impaired anti-tumour immune responses. These findings highlight the potential for parallel modulation of the gut microbiome to significantly enhance checkpoint blockade treatment efficacy. Based on these findings, methods of disease management, e.g. cancer treatment and diagnosis are provided herein. Also provided herein are methods to use the compositions described herein as predictive biomarker compositions to identify patients who will have a favourable response to immune checkpoint blockade. Moreover, the compositions described herein have immunostimulatory properties. Therefore, treatment of disease is not limited to cancer, but the compositions provides treatment of other diseases, e.g. diseases that benefit form immunostimulatory treatment, e.g. non-cancer immunotherapies.

In a first aspect, the invention thus relates to a composition comprising one or more bacterial isolate, in particular a bacterial population, belonging to one or more bacterial species selected from Table 1. Thus, the invention relates to a composition comprising a bacterium selected from one or more bacteria selected from Table 1. Specifically, the invention thus relates to a composition comprising one or more bacterial isolate having a 16SrDNA selected from SEQ ID. Nos 1 to 15.

The composition may comprise or consists of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 isolated bacteria. These are different bacteria selected from different species, that is bacteria having a 16SrDNA of selected from SEQ ID. Nos 1 to 29 or a sequence having at least 95%, 97%, 98% 98.7%, 99% or 100% sequence identity with a nucleic acid sequence selected from SEQ ID. Nos 1 to 29, e.g. selected from SEQ ID. Nos 1 to 15 or a sequence having at least 95%, 97%, 98% 98.7% or 99% sequence identity with a nucleic acid sequence selected from SEQ ID. Nos 1 to 15.

In one embodiment, the composition thus comprises or consists of isolated bacteria selected from at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 bacterial species wherein the bacteria comprise a 16S rDNA sequence selected from SEQ ID. Nos 1 to 29, e.g. 1 to 15, or a sequence having at least 95%, 97%, 98% 98.7%, 99% or 100% sequence identity with a nucleic acid sequence selected from SEQ ID. Nos 1 to 15.

In one embodiment, the composition comprises or consists of isolated bacteria selected from at least two species wherein the bacteria from the first species comprise a 16S rDNA sequence having least 95%, 97%, 98%, 98.7%, 99% or 100% sequence identity with a nucleic acid sequence according to SEQ ID NO: 1, and the bacteria from the second species comprise a 16S rDNA sequence having at least 95%, 97%, 98%, 98.7%, 99% or 100% sequence identity with a nucleic acid sequence according to SEQ ID NO: 2.

In one embodiment, the composition comprises or consists of isolated bacteria selected from at least 9 species wherein the bacteria comprise a 16S rDNA sequence selected from SEQ ID. Nos 1 to 29, e.g. 1 to 15 or a sequence having at least 95%, 97%, 98%, 98.7%, 99% or 100% sequence identity with a sequence selected from SEQ ID. Nos 1 to 29, e.g. 1 to 15. In one embodiment, the 9 species include bacteria comprising a 16S rDNA sequence according to SEQ ID NO: 1, or a sequence having at least 95%, 97%, 98% 98.7%, 99% or 100% sequence identity thereto and bacteria comprising a 16S rDNA sequence according to SEQ ID NO: 2 or a sequence having at least 95%, 97%, 98%, 98.7%, 99% or 100% sequence identity thereto.

In another aspect, the invention relates to a pharmaceutical composition as described herein, a pharmaceutical carrier and optionally an immune checkpoint inhibitor.

In another aspect, the invention relates to a composition as described herein for use in the treatment of disease, such as particular cancer or an infectious disease. The composition can also be used as a vaccine adjuvant. This is used to enhance vaccine response and administration may be together with the vaccine.

In another aspect, the invention relates to a composition as described herein in increasing efficacy of an anti-cancer treatment with an immune checkpoint inhibitor.

In another aspect, the invention relates to a method for treating cancer comprising modulating the level / abundance of one or more bacteria selected from those of Table 1 in a subject.

In another aspect, the invention relates to a kit comprising a composition as described herein and optionally an anti-cancer treatment that includes an immune checkpoint inhibitor.

In another aspect, the invention relates to a method for identifying a subject that will respond to therapy with an immune checkpoint inhibitor comprising determining the abundance of one or more

bacteria selected from those of Table 1 in a biological sample from said subject that comprises gut intestinal flora wherein an increase in the abundance of one or more of bacteria selected from those of Table 1 is indicative that the subject will respond to therapy with an immune checkpoint inhibitor.

In another aspect, the invention relates to a use of a bacterium selected from one or more bacteria selected from those of Table 1 in identifying a patient that will respond to therapy with an immune checkpoint inhibitor.

In another aspect, the invention relates to a kit comprising;

a sealable container configured to receive a biological sample;

polynucleotide primers for amplifying a 16S rDNA polynucleotide sequence from at least one gut associated bacterium to form an amplified 16S rDNA polynucleotide sequence, wherein the amplified 16S rDNA sequence has at least 95%, 97%, 98%, 98.7%, 99% or 100% sequence identity to a polynucleotide sequence selected from SEQ ID NOs 1 to SEQ ID NO 29; e.g. 1 to 15, a detecting reagent to detect the amplified 16S rDNA sequence; and instructions for use.

In another aspect, the invention relates to a food product or a vaccine co-therapy to boost vaccine response comprising the composition as described herein.

In another aspect, the invention relates to a method for identifying a faecal donor, e.g. for treatment of cancer, comprising assessing a faecal sample of a subject for the presence of one or more bacteria selected from Table 1 and identifying the faecal donor based on the presence and/or abundance of one or more bacteria selected from Table 1.

In another aspect, the invention relates to a use of one or more bacteria selected from Table 1 in a method for identifying a donor for FMT therapy, e.g. for treatment of cancer.

In another aspect, the invention relates to a method for treating a faecal transplant prior to administration to a subject comprising supplementing the faecal transplant with one or more isolated bacteria selected from Table 1.

In another aspect, the invention relates to a method for screening/ identifying a faecal donor comprising assessing a faecal sample of a subject for the presence of one or more bacteria associated with response to cancer; and identifying the faecal donor based on the presence and/or abundance of one or more bacteria.

Description of Figures

Figure 1. A) All 147 patients from the four melanoma studies were divided into responders and non-responders according to clinical outcome. The probability of not responding to immunotherapy was predicted from the baseline faecal sample based on machine learning predictions that used the abundance of bacteria in a defined signature. A cut off of 0.5 was used to determine the accuracy of the prediction. Accuracy was 91.16%. x axis is group. B) As A except each study is considered separately. frankel accuracy 84.62%; gajewski accuracy 89.74%; melresist accuracy 93.18%; wargo accuracy 100%. X axis is group. C) Receiver Operating Characteristic (ROC) curve of the combined melanoma dataset showing False Positive Rate as a function of True Positive Rate based on machine

learning predictions based the same microbiome signature. AUC= 0.98. X axis is 1-specificity, y axis is sensitivity. D) As C but each study is considered separately. Random forest out-of-bag error was used to prevent overoptimistic performance and improve generalizability. AUC frankel 0.958; AUC gajewski 0.978; AUC melresist 0.983; AUC wargo 1. X axis is 1-specificity, y axis is sensitivity.

Figure 2. A) All 147 patients from the four melanoma studies were divided into responders and non-responders according to clinical outcome. The probability of not responding to immunotherapy was predicted from the baseline faecal sample based on machine learning predictions that used the abundance of the 15 bacteria in consortium 1. A cut off of 0.5 was used to determine the accuracy of the prediction. Accuracy was 77.55%. B) As A except each study is considered separately. frankel accuracy 79.49%; gajewski accuracy 66.67%; melresist accuracy 81.82%; wargo accuracy 84%. C) Receiver Operating Characteristic (ROC) curve of the combined melanoma dataset showing False Positive Rate as a function of True Positive Rate based on machine learning predictions based on consortium 1. AUC= 0.8. D) As C but each study is considered separately. Random forest out-of-bag error was used to prevent overoptimistic performance and improve generalizability. AUC frankel 0.867; AUC gajewski 0.725; AUC melresist 0.879; AUC wargo 0.773.

Figure 3. A) All 147 patients from the four melanoma studies were divided into responders and non-responders according to clinical outcome. The probability of not responding to immunotherapy was predicted from the baseline faecal sample based on machine learning predictions that used the abundance of the 9 bacteria in consortium 2. A cut off of 0.5 was used to determine the accuracy of the prediction. Accuracy was 74.15%. B) As A except each study is considered separately. frankel accuracy 76.92%; gajewski accuracy 69.23%; melresist accuracy 75%; wargo accuracy 76%. C) Receiver Operating Characteristic (ROC) curve of the combined melanoma dataset showing False Positive Rate as a function of True Positive Rate based on machine learning predictions based on consortium 2. AUC= 0.75. D) As C but each study is considered separately. Random forest out-of-bag error was used to prevent overoptimistic performance and improve generalizability. AUC frankel 0.831; AUC gajewski 0.676; AUC melresist 0.788; AUC wargo 0.734.

Figure 4. A) All 147 patients from the four melanoma studies were divided into responders and non-responders according to clinical outcome. The probability of not responding to immunotherapy was predicted from the baseline faecal sample based on machine learning predictions that used the abundance of the 12 bacteria in consortium 3. A cut off of 0.5 was used to determine the accuracy of the prediction. Accuracy was 74.15%. B) As A except each study is considered separately. frankel accuracy 76.92%; gajewski accuracy 66.67%; melresist accuracy 81.82%; wargo accuracy 68%. C) Receiver Operating Characteristic (ROC) curve of the combined melanoma dataset showing False Positive Rate as a function of True Positive Rate based on machine learning predictions based on consortium 3. AUC= 0.773. D) As C but each study is considered separately. Random forest out-of-bag error was used to prevent overoptimistic performance and improve generalizability. AUC frankel 0.844; AUC gajewski 0.685; AUC melresist 0.862; AUC wargo 0.76.

Figure 5. A) All 147 patients from the four melanoma studies were divided into responders and non-responders according to clinical outcome. The probability of not responding to immunotherapy was

predicted from the baseline faecal sample based on machine learning predictions that used the abundance of the 9 bacteria in consortium 4. A cut off of 0.5 was used to determine the accuracy of the prediction. Accuracy was 71.43%. B) As A except each study is considered separately. frankel accuracy 76.92%; gajewski accuracy 64.1%; melresist accuracy 77.27%; wargo accuracy 64%. C) Receiver Operating Characteristic (ROC) curve of the combined melanoma dataset showing False Positive Rate as a function of True Positive Rate based on machine learning predictions based on consortium 4. AUC= 0.737. D) As C but each study is considered separately. Random forest out-of-bag error was used to prevent overoptimistic performance and improve generalizability. AUC frankel 0.781; AUC gajewski 0.667; AUC melresist 0.791; AUC wargo 0.708.

Figure 6. A) All 147 patients from the four melanoma studies were divided into responders and non-responders according to clinical outcome. The probability of not responding to immunotherapy was predicted from the baseline faecal sample based on machine learning predictions that used the abundance of the 9 bacteria in consortium 5. A cut off of 0.5 was used to determine the accuracy of the prediction. Accuracy was 68.71%. B) As A except each study is considered separately. frankel accuracy 71.79%; gajewski accuracy 58.97%; melresist accuracy 70.45%; wargo accuracy 76%. C) Receiver Operating Characteristic (ROC) curve of the combined melanoma dataset showing False Positive Rate as a function of True Positive Rate based on machine learning predictions based on consortium 3. AUC= 0.69. D) As C but each study is considered separately. Random forest out-of-bag error was used to prevent overoptimistic performance and improve generalizability. AUC frankel 0.75; AUC gajewski 0.596; AUC melresist 0.766; AUC wargo 0.675.

Figure 7. A) All 147 patients from the four melanoma studies were divided into responders and non-responders according to clinical outcome. The probability of not responding to immunotherapy was predicted from the baseline faecal sample based on machine learning predictions that used the abundance of the 9 bacteria in consortium 6. A cut off of 0.5 was used to determine the accuracy of the prediction. Accuracy was 69.39%. B) As A except each study is considered separately. frankel accuracy 69.23%; gajewski accuracy 58.97%; melresist accuracy 77.27%; wargo accuracy 72%. C) Receiver Operating Characteristic (ROC) curve of the combined melanoma dataset showing False Positive Rate as a function of True Positive Rate based on machine learning predictions based on consortium 3. AUC= 0.71. D) As C but each study is considered separately. Random forest out-of-bag error was used to prevent overoptimistic performance and improve generalizability. AUC frankel 0.767; AUC gajewski 0.577; AUC melresist 0.81; AUC wargo 0.708.

Figure 8. Receiver Operating Characteristic (ROC) curve of a NSCLC cohort showing False Positive Rate as a function of True Positive Rate based on machine learning predictions based on consortium 1. NSCLC dataset is metagenomic sequence from Routy and Zitvogel et al (2018 Science 359:91-97) classified using the Microbiotica high-precision platform. Random forest out-of-bag error was used to prevent overoptimistic performance and improve generalizability. AUC= 0.744.

Figure 9. Isolated bacteria induce dendritic cell maturation and cytokine production. The ability of the isolated bacteria to activate dendritic cells (DCs) was determined by co-culturing with human monocyte-derived Dendritic cells in anaerobic conditions at multiplicity of infection (MOI) of

approximately 10:1. Subsequent maturation of the dendritic cells was determined by expression levels of the maturation markers CD86 (A) and CD83 (B) as determined by flow cytometry. Data is displayed as fold change of mean fluorescence intensity (MFI), compared to the LPS control to normalise across different donors and experiments. The DC expression of CD86 (C) and CD83 (D) following treatment with consortium 5 (MOI of approximately 10:1) was similarly determined by flow cytometry. The MFIs of those markers following stimulation with consortium 5 are displayed in white bars. The DC expression of CD86 (E) and CD83 (F) following treatment with consortia 6, 7, 8 and 9 was determined by flow cytometry. The MFIs of those markers are displayed in white bars. (G) IL-12 and IL-10 production by DCs following treatment with isolated bacteria alone (MOI of approximately 10:1) or as consortia 5 and 6 (MOI of approximately 10:1) were determined by ELISA. MOI 10 N= 5 (5 donors in 5 independent experiments). Data are displayed as ratio of IL-12 to IL-10. LPS (10 ng/ml), Poly I:C (20 µg/ml) and *Salmonella typhimurium* (MOI of approximately 10:1) are strong inducers of DC activation and used as positive controls for all assays (grey bars). Unstimulated or immature (Imm) DCs are shown for comparison (grey bars). Results are the mean ± SEM of 5 (A and B), 2 (C, D, E and F) and 3 (G) independent experiments.

Figure 10. Dendritic cells treated with isolated bacteria activate Cytotoxic CD8+ T Lymphocytes (CTLs). Following co-culture with isolated bacteria or control stimuli as in Figure 9, human monocyte-derived DCs were washed and co-cultured with purified allogenic CD8+ T cells for 6 days. CTL activation was determined by analysing the expression of Granzyme B (A), IFN-γ (B) and Perforin (C) using intracellular staining and flow cytometry. Data is displayed as fold change of the percentage of positive cells, compared to the LPS control to normalise across different donors and experiments. LPS (10 ng/ml), Poly I:C (20 µg/ml) and *Salmonella typhimurium* (MOI of approximately 10:1) are strong inducers of DC activation and used as positive controls for all assays (grey bars). Unstimulated or immature (Imm) DCs are shown for comparison (grey bars). Results are the mean ± SEM of 7 donors in 4 independent experiments.

Figure 11. Dendritic cells treated with consortia 6, 7, 8 and 9 activate Cytotoxic CD8+ T Lymphocytes (CTLs). Following co-culture with consortia 6, 7, 8 and 9 or control stimuli as in Figure 9, human monocyte-derived DCs were washed and co-cultured with purified allogenic CD8+ T cells for 6 days. CTL activation was determined by analysing the expression of Granzyme B (A), IFN-γ (B) and Perforin (C) using intracellular staining and flow cytometry. Data is displayed as the percentage of positive cells. LPS (10 ng/ml), Poly I:C (20 µg/ml) and *Salmonella typhimurium* (MOI of approximately 10:1) are strong inducers of DC activation and used as positive controls for all assays (grey bars). Unstimulated or immature (Imm) DCs are shown for comparison (grey bars). Results are the mean ± SEM of a duplicate of a single representative experiment.

Figure 12. Dendritic cells treated with consortium 5 activate Cytotoxic CD8+ T Lymphocytes (CTLs). Following co-culture with consortium 5 or control stimuli as in Figure 11, human monocyte-derived DCs were washed and co-cultured with purified allogenic CD8+ T cells for 6 days. CTL activation was determined by analysing the expression of Granzyme B (A), IFN-γ (B) and Perforin (C) using intracellular staining and flow cytometry. Data is displayed as the percentage of positive cells. LPS (10

ng/ml), Poly I:C (20 µg/ml) and *Salmonella typhimurium* (MOI of approximately 10:1) are strong inducers of DC activation and used as positive controls for all assays (grey bars). Unstimulated or immature (Imm) DCs are shown for comparison (grey bars). Results are the mean ± SEM of a duplicate of a single representative experiment.

Figure 13. Isolated bacteria and consortia 6, 7, 8 and 9 endow the induced CTLs with tumor killing capacity. CD8+ T cells primed by bacteria/consortia-treated DCs (as in Figures 10 and 11) were assessed for their capacity to kill SKOV-3 cells. Cytolysis is determined by measuring the decreasing electric impedance of the SKOV-3 cells. Data is displayed as the percentage of cytolysis of SKOV-3 cells, 72 hours following co-culture with CD8+ T cells. LPS (10 ng/ml), Poly I:C (20 µg/ml) and *Salmonella typhimurium* (MOI of approximately 10:1) are strong inducers of DC activation and used as positive controls for all assays (grey bars). Unstimulated or immature (Imm) DCs are shown for comparison (grey bars). Results are the mean ± SEM of 3 independent experiments.

Figure 14. Consortium 5 and *Blautia sp.* endow the induced CTLs with tumor killing capacity. CD8+ T cells primed by consortium 5- or *Blautia sp.*-treated DCs (as in Figure 12) were assessed for their capacity to kill SKOV-3 cells. Cytolysis is determined by measuring the decreasing electric impedance of the SKOV-3 cells. Data is displayed as the percentage of cytolysis of SKOV-3 cells, 72 hours following co-culture with CD8+ T cells. LPS (10 ng/ml), Poly I:C (20 µg/ml) and *Salmonella typhimurium* (MOI of approximately 10:1) are strong inducers of DC activation and used as positive controls for all assays (grey bars). Unstimulated or immature (Imm) DCs are shown for comparison (grey bars). Results are the mean ± SEM of a duplicate of a single representative experiment.

Figure 15. Isolated bacteria possess a variable capacity to induce IFN-α production by plasmacytoid dendritic cells. IFN-α production by plasmacytoid dendritic cells (pDCs) was determined by ELISA following an overnight incubation with heat-killed bacteria (moimoi of approximately 10:1). 10 ng/ml IL-3 and 10 µg/ml CpG (grey bars) were taken along as a negative and positive controls, respectively. Results are the mean ± SEM of 3 donors in 2 independent experiments.

Figure 16. *In vivo* efficacy in a murine cancer model Prior to tumour implantation, SPF C57BL/6N female mice were administered antibiotics in drinking water (kanamycin (0.4mg/ml), colistin (850U/ml), metronidazole (0.215mg/ml), vancomycin (0.045mg/ml), gentamycin (0.035mg/ml)) for 7 days (day -9 to day -2). Subsequently, mice were reconstituted with human donor stool from a melanoma patient (20mg) by oral gavage on day -1. $5x10^5$ MCA-205 murine fibrosarcoma cells were implanted subcutaneously in the flank on day 0. Consortium 5 (n=8) and consortium 6 (n=8) were administered by oral gavage twice weekly from day -1 for 3 weeks (approximate total of $1x10^9$ CFU/dose) and compared to animals treated with vehicle controls. Anti-PD-1 antibody (RMP1-14) was administered (10mg/kg intraperitoneal) thrice weekly for 2 weeks from day 6. Plots show tumour growth, as measured by volume, over time in response to vehicle control, anti-PD1 and Consortium 5 (A) or consortium 6 (B). Data are mean ± SEM tumour size, and representatives of at least three (A) and two (B) experiments.

Figure 17. A) All 147 patients from the four melanoma studies were divided into responders and non-responders according to clinical outcome. The probability of not responding to immunotherapy was

predicted from the baseline faecal sample based on machine learning predictions that used the abundance of the 9 bacteria in consortium 10. A cut off of 0.5 was used to determine the accuracy of the prediction. Accuracy was 75.51%. B) As A except each study is considered separately. frankel accuracy 74.36%; gajewski accuracy 71.79%; melresist accuracy 77.27%; wargo accuracy. C) Receiver Operating Characteristic (ROC) curve of the combined melanoma dataset showing False Positive Rate as a function of True Positive Rate based on machine learning predictions based on consortium 3. AUC= 0.81. D) As C but each study is considered separately. Random forest out-of-bag error was used to prevent overoptimistic performance and improve generalizability. AUC frankel 0.85; AUC gajewski 0.725; AUC melresist 0.826; AUC wargo 0.805.

In the figures, Con stands for consortium. Consortia are shown in Table 3.

Detailed description

The present invention will now be further described. In the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

Generally, nomenclatures used in connection with, and techniques of microbiology, cell and tissue culture, pathology, molecular biology, immunooncology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Green and Sambrook et al., Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012).

The nomenclatures used in connection with, and the laboratory procedures and techniques of analytical chemistry, microbiology, bioinformatics and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art.

The invention relates to bacterial compositions each comprising or consisting of one or more bacterial isolate from one or more species as disclosed herein, e.g., a consortium of defined bacterial isolates. The compositions have immunostimulatory properties and are thus therapeutic compositions useful in the treatment of disease. In some embodiments, the compositions are mixtures of bacterial isolates selected from more than one species as identified in Table 1.

The compositions are not faecal microbiota transplants (FMT) and do not contain faecal material, but contain defined mixtures of bacterial isolates free of faecal material. Therefore, preparations that contain a defined bacterial mixture are generally accepted to be a safer treatment than FMT. An advantage of the present composition is that it comprises only fully defined and characterised bacteria and no undefined or unwanted components, which may be present in donor stools, thereby allowing the therapeutic composition to be standardised and increasing safety of the composition.

FMT relies on a stool sample from a human donor which is administered directly to the recipient, e.g. via colonoscopy, without bacteria present in the stool sample being isolated prior to the administration of the FMT to the recipient. While FMT is widely used, there are some disadvantages associated with FMT. The composition of the FMT material is very donor dependant and therefore is inconsistent. Despite screening of donors, it is difficult to determine the bacterial load of the samples. Donors also have to be screened for pathogens and to assess the risk of colonization with drug-resistant bacteria. In certain aspects described below, the invention also relates to augmenting FMT therapy with one or more bacterial isolate from one or more species as disclosed herein and methods for screening / identifying a faecal donor.

The compositions as described herein include isolated bacteria. The term "isolated" refers to bacteria that are isolated from the natural environment. The isolated bacteria, e.g. isolated bacterial strains, are substantially free of other cellular material, chemicals and/or faecal material. Thus, as used herein, the term “isolated” bacteria refers to bacteria that have been separated from one or more undesired component, such as another bacterium or bacterial strain, one or more component of a growth medium, and/or one or more component of a sample, such as a faecal sample. In some embodiments, the bacteria are substantially isolated from a source such that other components of the source are not detected. As used herein, the term “species” refers to a taxonomic entity as conventionally defined by genomic sequence and/or phenotypic characteristics. A “strain” is a particular instance of a species that has been isolated and purified according to conventional microbiological techniques. It will be understood that the terms bacteria and bacterial isolates refer to a plurality of bacteria, that is a bacterial population.

In one embodiment, the bacteria of the composition are metabolically inactive prior to administration. For example, the bacteria are lyophilised. In one embodiment, the composition includes vegetative bacterial cells and does not include bacterial spores. In one embodiment, the composition includes vegetative bacterial cells and/or bacterial spores. In one embodiment, the composition includes vegetative bacterial cells and does not include bacterial spores or is substantially devoid of spores. In one embodiment, the composition includes fewer than about 0.5%, 1%, 2%, 3%, 4% or 5% spores.

The composition is preferably a live bacterial therapeutic, bacteriotherapy or a live biotherapeutic product. As described herein, a live bacterial product (also referred to as a bacterial composition, live

bacterial consortium, mixture of bacteria or bacterial consortium) comprises one or more bacterial strain from one or more bacterial species as described herein. The term live bacterial therapy is interchangeably used with bacteriotherapy herein and defines a therapy using live bacteria to restore health or alleviate disease / disease symptoms or increase response to a therapy.

The bacterial compositions of the invention provide an immunostimulatory effect. In some embodiments, the bacterial composition induces or stimulates an immunotherapeutic effect, for example an anti-cancer effect (e.g., inhibition or cytotoxicity of cancer cells), when administered to the subject. In some embodiments, the bacterial composition induces or stimulates an immune response that provides an anti-cancer or other beneficial therapeutic effect when administered to the subject as further explained herein.

As described herein, the composition may comprise one or more bacterial species selected from those listed in Table 1. The ability of the specific bacteria or the combination of bacterial species of the live bacterial product to induce a beneficial effect, i.e. an immunostimulatory effect, such as an anti-cancer effect, can be assessed using any of method known in the art, e.g., in vitro assays for example using cell culture, or in vivo studies. Suitable assays are shown in the examples.

In some embodiments, the anticancer live bacterial product induces a specific immune cell population (e.g., CD8+ T-cells, Th17, Th1 cells). The abundance of a specific population of cells (e.g., CD8+ T-cells, Th17, Th1 cells) can be assessed by any method known in the art, for example by detecting a cellular marker indicative of the cell type, assessing a direct or indirect activity of the cell type, and/or by measuring the production of one or more cytokines produced by the specific cell type. In some embodiments, the anti-cancer live bacterial product induces CD8+ T-cells (or “CD8+ T cells”). As will be appreciated by one of ordinary skill in the art, a combination of bacterial species and/or multiple strains from one or more species as described herein may be selected and combined to produce an anti-cancer live bacterial product that induces CD8+ T-cells.

In one embodiment, the isolated bacteria, e.g. isolated bacterial strains from the species listed herein, can be viable bacteria that are capable of colonising the gastrointestinal gut of a subject when administered to said subject.

The inventors have shown that by combining bacteria from different species, a therapeutic composition can be provided which finds use as a co-therapy with a checkpoint inhibitor. In a first aspect, the invention relates to a composition comprising isolated bacteria, e.g. a bacterial strain, selected from one or more of the bacterial species B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14 and/or B15 as shown in Table 1 or subsets thereof. The invention thus relates to a composition comprising one or more bacterial isolate, e.g. bacterial population, having a 16SrDNA

selected from SEQ ID. Nos 1 to 19, e.g. 1 to 15. The invention thus relates to a composition comprising or consisting of bacterial isolates of one or more of the species as shown in Table 1.

Table 1 below lists the 15 different bacterial species from which the isolated bacteria present in the composition are selected. Reference to exemplary 16S rDNA sequence characterising each species is also provided in Table 1. The terms 16S rDNA sequence or 16S rDNA as used herein refer to DNA nucleic acid sequences, i.e. a nucleic acid molecule, which encodes 16S rRNA nucleic acid sequence i.e. a nucleic acid molecule. Nucleic acid sequences referenced below are listed in Table 2. Also, as explained further below, the bacteria of the composition and of other aspects as described herein may have a 16S rDNA sequence with certain sequence identity to the SEQ ID Nos. as listed below.

Table 1 Bacterial species of the composition and biomarker signature

No	**Taxonomy**	**16S rDNA sequence - sequence identifier**	**Possible alternative taxonomy: name and/or closely related species based on closely related bacteria identified from public databases**
B1	Eisenbergiella sp.	SEQ ID No. 1; SEQ ID No. 21	Eisenbergiella tayi
B2	Butyricicoccus sp.	SEQ ID No. 2; SEQ ID No. 17; SEQ ID No. 22	Butyricicoccus pullicaecorum, bacterium NLAE-zl-H41, bacterium NLAE-zl-H55, bacterium NLAE-zl-H60
B3	Clostridiales sp.	SEQ ID No. 3	n/a
B4	Alistipes obesi	SEQ ID No. 4; SEQ ID No. 16	n/a
B5	Alistipes indistinctus	SEQ ID No. 5	n/a
B6	Gordonibacter urolithinfaciens	SEQ ID No. 6; SEQ ID No. 18; SEQ ID No. 23	n/a
B7	Faecalitalea sp.	SEQ ID No. 7; SEQ ID No. 24	Longicatena caecimuris
B8	Blautia sp.	SEQ ID No. 8; SEQ ID No. 25	Blautia producta, Blautia coccoides, Blautia marasmi, Blautia stercoris
B9	Barnesiella intestinihominis	SEQ ID No. 9; SEQ ID No. 26	n/a
B10	Alistipes timonensis	SEQ ID No. 10	n/a
B11	Blautia sp.	SEQ ID No. 11; SEQ ID No. 19; SEQ ID	n/a

		No. 27	
B12	Lachnospira sp.	SEQ ID No. 12; SEQ ID No. 20; SEQ ID No. 28	Lactobacillus rogosae
B13	Ruminococcus callidus	SEQ ID No. 13	n/a
B14	Roseburia faecis	SEQ ID No. 14; SEQ ID No. 29	n/a
B15	Faecalibacterium prausnitzii	SEQ ID No. 15	n/a

The aspects and embodiments of the invention described herein are defined by reference to the species name and/or SEQ ID NO. as shown in Table 1. In some cases, different exemplary sequences are provided in Table 1 for the same species, e.g. corresponding to different exemplary strains which belong to the same species. Where multiple sequences are provided for a species, these sequences share a high sequence identity, e.g. the different strains defined by SEQ ID No. 2 and SEQ ID No. 17 have at least 99% sequence identity, SEQ ID No. 4 and SEQ ID No. 16 have at least 99% sequence identity, SEQ ID No. 6 and SEQ ID No. 18 have at least 99% sequence identity and SEQ ID No. 12 and SEQ ID No. 20 have at least 99% sequence identity.

In the aspects and embodiments described herein, for each of B1 to B15, any of the sequences defined above (SEQ ID. Nos 1 to 29) can be used. Thus, where multiple sequences are provided for a single species, any of these sequences can be used.

It will be appreciated that the inventors provide compositions with certain bacterial species that have immunostimulatory effects, e.g. anti-cancer effects. It will also be appreciated that for each species, different strains can be used, i.e. strains identified above or other strains that belong to the same species. It should be appreciated that closely related bacterial strains (e.g., as defined by 16S rDNA sequences) having similar or the same biological properties can also be included. In some embodiments, bacterial strains provided herein can be replaced with bacterial strains with similar or the same biological properties.

In some embodiments, the anticancer / live bacterial composition comprises one or more bacterial strain of one or more of the 15 recited species shown in Table 1. In some embodiments, the anticancer / live bacterial composition comprises one or more bacterial strain of more than one of the 15 recited species; e.g. 2, 3, 4, 5, 6, 7, 8, 9,10, 11, 12, 13, 14 or 15 species.

In one embodiment, the composition comprises or consists of 15 isolated bacteria, e.g. bacteria from each of the 15 bacterial species listed in Table 1, for example with reference to the 16S rDNA

sequences as shown in the Table or a sequence with certain percentage identity thereto as explained below or with reference to the species name as shown above.

The invention also relates to compositions that comprise or consist of bacteria selected from a subset of the bacterial species listed in Table 1; e.g. compositions that comprise or consist of different bacteria selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 of the bacterial species listed in Table 1, with reference to the 16S rDNA sequences as shown in the Table 1 or a sequence with certain percentage identity thereto as explained below or with reference to the species name as shown above. All combinations are envisaged.

Thus, in one embodiment, the composition comprises or consists of at least one isolated bacterial population belonging to one or more of the species in Table 1. For example, the composition comprises or consists of bacteria selected from 2, 3, 6, 9 or 12 bacterial species listed in Table 1. These may be selected from the consortia shown in Table 3, for example consortia 2, 4, 5, 6 and 10. The bacteria may be defined by reference to their 16S rDNA as shown in the sequence identifiers Table 1. Thus, different bacteria selected from those listed in Table 1 can be combined in a single composition.

For example, the composition comprises or consists of isolated bacteria selected from at least 2, e.g. up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, up to 10, up to 11, up to 12, up to 13, up to 14 or up to 15 species shown in Table 1, for example with reference to the sequences as shown in the Table. For example, the composition comprises or consists of isolated bacteria from 9 bacterial species listed in Table 1. In one example, the composition comprises or consists of isolated bacteria from 9 species as shown in Table 3, i.e. consortia 2, 4, 5, 6 and 10. The bacteria may be defined by reference to their 16S rDNA as shown in the sequence identifiers in Table 1.

In one embodiment, the composition comprises or consists of isolated bacteria selected from 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 species listed in Table 1, for example with reference to the sequence IDs as shown in Table 1. In one embodiment, the composition comprises or consists of isolated bacteria selected from the consortia in Table 3. In one embodiment, the composition comprises or consists of isolated bacteria having a 16S rDNA selected from the SEQ ID NOs. as shown in Table 1. The bacteria may be defined by reference to their 16S rDNA as shown in the sequence identifiers in Table 1. Sequences with certain percentage sequence identify as shown herein are also within the scope of the invention.

In one embodiment, the composition comprises isolated bacteria selected from at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14 or at least 15 species listed in table 1, for example with reference to the sequences as shown in table 1, for example with reference to the sequence IDs as shown in Table 1. In one

embodiment, the composition comprises isolated bacteria selected from at least 9 species as shown in Table 1. Sequences with certain percentage sequence identify as shown herein are also within the scope of the invention.

In one embodiment, the composition comprises or consists of isolated bacteria selected from no more than 2, no more than 3, no more than 4, no more than 5, no more than 6, no more than 7, no more than 8, no more than 9, no more than 10, no more than 11, no more than 12, no more than 13, no more than 14 or no more than 15 species listed in Table 1, for example with reference to the sequences as shown in table 1, for example with reference to the sequence IDs as shown in Table 1. Sequences with certain percentage sequence identify as shown herein are also within the scope of the invention.

In one embodiment, the composition comprises or consists of isolated bacteria selected from 2 to 4, 2 to 5, 2 to 6, 2 to 7, 2 to 8, 2 to 9, 2 to 10, 2 to 11, 2 to 12, 2 to 13, 2 to 14 or 2 to 15 species shown in table 1, for example with reference to the sequences as shown in Table 1, for example with reference to the sequence IDs as shown in Table 1. Sequences with certain percentage sequence identify as shown herein are also within the scope of the invention.

In one embodiment, the composition comprises an isolated bacterial mixture comprising or consisting of 2 to 15 bacterial strains having at least 90%, 95%, 97%, 98%, 98.7% or 99% sequence identity to 16s rDNA sequences selected from SEQ ID Nos 1 to 15, e.g. SEQ ID Nos. 16 to 29. Exemplary compositions are set out herein, e.g. in Table 3.

A skilled person would appreciate that that bacterial species selected from Table 1 and for use in the composition and methods of the invention can have the sequence shown in Tables 1 and 2 or a sequence that has certain percentage identity thereto and retains biological activity; i.e. activity against cancer/ efficacy in enhancing the effect of a therapy using an immune checkpoint inhibitor.

In one embodiment, the composition may be as described above, but does not comprise bacteria of any other species, i.e. species not listed in Table 1 or the composition comprise only de minimis or biologically irrelevant amounts of bacteria from another species. By biologically irrelevant is meant bacteria that do not have an effect on the treatment of cancer. Thus, in one embodiment, the composition consists of the recited bacteria.

In one embodiment, the composition does not comprise other bacterial species that fall within a genus listed in Table 1.

In one embodiment, the composition may comprise other bacterial species that fall within a genus listed in Table 1, but does not comprise bacterial species of a genus not listed in Table 1. In one embodiment, the composition may comprise other bacterial species that fall within a different genus.

Methods of determining sequence identity are known in the art. It is known that clades, operational taxonomic units (OTUs), species, and strains are, in some embodiments, identified by their 16S rDNA sequence. The relatedness can be determined by the percent identity and this can be determined using methods known in the art.

Bacterial species and strains used in a composition as described herein can be identified based on the 16S nucleic acid sequence (full length or part thereof, such as V regions). The 16S ribosomal DNA gene codes for the DNA component of the 30S subunit of the bacterial ribosome. It is widely present in all bacterial species. Different bacterial species have one to multiple copies of the 16S rRNA gene. 16S rRNA gene sequencing is by far one of the most common methods targeting housekeeping genes to study bacterial phylogeny and genus/species classification. Thus, bacteria can be taxonomically classified based on the sequence of the gene encoding the 16S nucleic acid sequence, e.g. ribosomal DNA (rDNA) in the bacterium. This gene sequence is also referred to as the ribosomal DNA sequence (rDNA). The bacterial 16S rDNA is approximately 1500 nucleotides in length and is used in reconstructing the evolutionary relationships and sequence similarity of one bacterial isolate to another using phylogenetic approaches. 16S rDNA sequences are used for phylogenetic reconstruction as they are in general highly conserved, but contain specific hypervariable regions that harbor sufficient nucleotide diversity to differentiate genera and species of most microbes.

Using well known techniques to determine a full 16S rDNA sequence or the sequence of any hypervariable region of the 16S rDNA sequence, genomic DNA is extracted from a bacterial sample, the 16S rDNA (full region or specific hypervariable regions) amplified using polymerase chain reaction (PCR), the PCR products cleaned, and nucleotide sequences delineated to determine the genetic composition of the 16S rDNA gene or subdomain of the gene. If full 16S rDNA sequencing is performed, the sequencing method used may be, but is not limited to, Sanger sequencing. If one or more hypervariable regions are used, such as the V4 region, the sequencing may be, but is not limited to being, performed using the Sanger method or using a next-generation sequencing method, such as an Illumina (sequencing by synthesis) method using barcoded primers allowing for multiplex reactions. The V1-V9 regions of the 16S rDNA refer to the first nine hypervariable regions of the 16S rDNA gene that are often used for genetic typing of bacterial samples. In some embodiments, at least one of V1 to V9 is used to characterise the bacterial isolate.

In some embodiments, bacterial species identified as described herein are identified by sequence identity to 16S rDNA sequences as known in the art and described herein. In some embodiments, the selected species are identified by sequence identity to full length 16S rDNA sequences as shown in

Table 2. In some embodiments, the selected species are identified by sequence identity to a part of the 16S rDNA sequences as shown in Table 2, for example V3 and/ or V4.

As used herein, the term "homology" or “identity” generally refers to the percentage of nucleic acid residues in a sequence that are identical with the residues of the reference sequence with which it is compared, after aligning the sequences and in some embodiments after introducing gaps, if necessary, to achieve the maximum percentage homology, and not considering any conservative substitutions as part of the sequence identity. Thus, the percentage homology between two nucleic acid sequences is equivalent to the percentage identity between the two sequences. Methods and computer programs for the alignment are well known. The percentage identity between two sequences can be determined using well known mathematical algorithms.

In one embodiment, the degree of sequence identity between a query sequence and a reference sequence can be determined with the aid of a commercially available sequence comparison program. This typically involves aligning the two sequences using the default scoring matrix and default gap penalty, identifying the number of exact matches, and dividing the number of exact matches with the length of the reference sequence. Suitable computer programs useful for determining identity include, for example, BLAST (blast.ncbi.nlm.nih.gov).

In the various embodiments as set out herein when reference is made to a SEQ ID NO., sequences that have certain percentage sequence identity to the full length sequence are also within the scope of the invention.

Thus, the full length or partial 16S rDNA of the bacterial species listed in Table 2 with reference to the sequence identifier in Table 1 and which is used in the compositions and methods of the invention has at least 90%, e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.7%, 99%, 99.5% or 100% sequence identity to the corresponding reference 16S rDNA (i.e. SEQ IDs 1 to 29). In some embodiments, the threshold sequence identity is at least 94.5%. In one embodiment, said sequence identity is at least 95%. In one embodiment, said sequence identity is at least 96%. In one embodiment, said sequence identity is at least 97%. In one embodiment, said sequence identity is at least 98%. In one embodiment, said sequence identity is at least 98.7%. In one embodiment, said sequence identity is at least 99%.

In one aspect, the composition therefore comprises two or more bacteria, that is bacterial species, comprising a 16S rDNA sequence selected from SEQ ID NO. 1 to 15 or comprising a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity to a nucleic acid sequence selected from SEQ ID NOs. 1 to 15. Such sequences include SEQ ID. Nos 16 to 29, for example SEQ ID. Nos 16 to 20.

In some embodiments, the threshold sequence identity is 94.5%, 94.6%, 94.7%, 94.8%, 94.9%, 95.0%, 95.1%, 95.2%, 95.3%, 95.4%, 95.5%, 95.6%, 95.7%, 95.8%, 95.9%, 96.0%, 96.1%, 96.2%, 96.3%, 96.4%, 96.5%, 96.6%, 96.7%, 96.8%, 96.9%, 97.0%, 97.1%, 97.2%, 97.3%, 97.4%, 97.5%, 97.6%, 97.7%, 97.8%, 97.9%, 98.0%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9% 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%.99.6%, 99.7%, 99.8%, 99.9% or 100%.

In one embodiment, a bacterium present in the composition belongs to the same species as a bacterium disclosed herein, has at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity to a nucleic acid sequence selected from SEQ ID NOs. 1 to 15 and retains activity against cancer/ efficacy in enhancing the effect of a therapy using an immune checkpoint inhibitor. Such sequences include SEQ ID. Nos 16 to 29, for example SEQ ID. Nos 16 to 20.

In one embodiment, the composition comprises or consists of one or more of the following 15 bacteria having a 16sDNA of the following SEQ ID Nos.:
SEQ ID No. 1 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto,
SEQ ID No. 2 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto,
SEQ ID No. 3 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto,
SEQ ID No. 4 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto,
SEQ ID No. 5 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto,
SEQ ID No. 6 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto,
SEQ ID No. 7 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto,
SEQ ID No. 8 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto,
SEQ ID No. 9 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto,
SEQ ID No. 10 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto,
SEQ ID No. 11 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto,
SEQ ID No. 12 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto,

SEQ ID No. 13 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto,
SEQ ID No. 14 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto,
SEQ ID No. 15 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto.

Examples of the above are SEQ ID Nos. 16-29.

Thus, the composition comprises or consists of a population of bacteria that belong to one or more of the 15 bacterial having a 16sDNA as shown above.

In one embodiment, the composition does not include *Faecalibacterium prausnitzii* (e.g. SEQ ID No. 15). In one embodiment, the composition does not include *Alistipes indistinctus* (e.g. SEQ ID No. 5), *Alistipes obesi* (e.g. SEQ ID No. 4 or 16) and/or *Alistipes timonensis* (e.g. SEQ ID No. 10).

In one embodiment, the composition comprises a consortium as shown in Table 3.

Thus, in one embodiment, the composition comprises or consists of bacteria having SEQ ID No. 1 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 2 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 3 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 4 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 5, or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 6 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 7 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto; bacteria having SEQ ID No. 8 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto and bacteria having SEQ ID No. 9 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto (consortium 2 in Table 3).

in one embodiment, the composition comprises or consists of bacteria having SEQ ID No. 1 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 2 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 3 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 6 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 7, or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 8 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 9 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto; bacteria having SEQ ID No. 11 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto; bacteria having SEQ ID No. 12 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 13 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 14 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto and bacteria having SEQ ID No. 15 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto (consortium 3 in Table 3).

In another embodiment, the composition comprises or consists of bacteria having SEQ ID No. 1 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, SEQ ID No. 2 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 3 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 6 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 7 or a 16S rDNA

sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 8 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 9 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 11 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 14 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto (consortium 4 in Table 3).

In another embodiment, the composition comprises or consists of bacteria having SEQ ID No. 1 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 7 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 8 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 9 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 13 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 16 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 17 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 18 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto and bacteria having SEQ ID No. 20 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto (consortium 5 in Table 3).

In another embodiment, the composition comprises or consists of bacteria having SEQ ID No. 1 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 2 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 7 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 9 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 13 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 16 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g.

97% or 98.7% identity thereto, bacteria having SEQ ID No. 18 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 19 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto and bacteria having SEQ ID No. 20 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto (consortium 6 in Table 3).

In another embodiment, the composition comprises or consists of bacteria having SEQ ID No. 1 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 2 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 7 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 9 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 18 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto and bacteria having SEQ ID No. 19 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto (consortium 7 in Table 3).

In another embodiment, the composition comprises or consists of bacteria having SEQ ID No. 1 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 2 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto and , bacteria having SEQ ID No. 7 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto (consortium 8 in Table 3).

In another embodiment, the composition comprises or consists of bacteria having SEQ ID No. 1 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto and bacteria having SEQ ID No. 2 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto (consortium 9 in Table 3).

Thus, in one embodiment, the composition comprises or consists of bacteria having SEQ ID No. 1 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 2 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 3 or a 16S rDNA sequence having at least 90%

e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 5 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 7, or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 10 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto, bacteria having SEQ ID No. 11 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto; bacteria having SEQ ID No. 13 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto and bacteria having SEQ ID No. 14 or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto or a 16S rDNA sequence having at least 90% e.g. at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%; e.g. 97% or 98.7% identity thereto (consortium 10 in Table 3).

With reference to the percentage identities recited for the embodiments of the compositions above, in one embodiments sequence identity is at least 98.7% or 99%. It will be understood that where Table 1 provides multiple sequences for a single species, any of these sequences can be used according to the above embodiments.

In one example, species used in the composition are identified based on their 16S rDNA sequence (e.g., full-length sequence, or partial sequence). In some cases, strains of bacterial species useful in an invention, e.g., strains of the species disclosed herein, can be obtained from a public biological resource center such as the ATCC (atcc.org), the DSMZ (dsmz.de), or the Riken BioResource Center (en.brc.riken.jp). 16s rDNA sequences useful for identifying species or other aspects of the invention can be obtained from public databases, e.g., the Human Microbiome Project (HMP) web site or GenBank.

A skilled person would appreciate that the compositions may include one or more than one strain of a particular bacterial species as listed in Table 1. For example, the composition of the invention comprises more than one bacterial strain for a species. For example, in some embodiments, the composition of the invention comprises more than one strain from within the same species (e.g. more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40 or 45 strains). In another embodiment, the composition of the invention comprises one bacterial strain for each species.

In one embodiment, the bacteria of the composition are capable of colonising the gastrointestinal tract of a subject. In one embodiment, the bacteria of the composition are capable of sustained engraftment in the gastrointestinal tract of a subject.

In one embodiment, the composition has one or more of the following characteristics:

- The composition has an immunostimulatory effect;
- The composition is effective in treating and/or preventing cancer in a subject, tissue or cell, e.g. when used together with a checkpoint inhibitor therapy;
- The composition is effective in treating and/or preventing an infectious disease in a subject, tissue or cell;
- Administration of the composition to a subject, tissue or cell induces an immune response in a subject and/or increases the efficacy according to an anti-cancer therapy that includes an immune checkpoint inhibitor;
- Administration of the composition to a subject, tissue or cell enhances CD8+ response;
- Administration of the composition to a subject, tissue or cell enhances immune checkpoint blockade;
- Administering of the composition maintains or induces responsiveness of a tumour an immune checkpoint;
- Administration of the composition to a subject, tissue or cell increases the number or activity of a cell type of the immune system, e.g. T cells, B cells, dendritic cells, macrophages, neutrophils, NK cells, plasmacytoid dendritic cells and combinations thereof;
- Administration of the composition to a subject, tissue or cell shifts a ratio of immune cells in the subject in favor of a cell type capable of suppressing growth of a tumour e.g. T cells, cytotoxic T lymphocytes, T helper cells, natural killer (NK) cells, natural killer T (NKT) cells, plasmacytoid dendritic cells, anti-tumour macrophages, B cells, dendritic cells, and combinations thereof and/or
- Administration of the composition to a subject, tissue or cell shifts a ratio of immune cells in the subject against a cell type capable of protecting a tumour e.g. myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), tumour associated neutrophils (TANs), M2 macrophages, tumour associated macrophages (TAMs), and a combination thereof;
- Administration of the composition to a subject, tissue or cell increases the abundance / level of bacteria in the subject which discourages cancer/tumour growth, spread, and/or evasion of treatment/immune response;
- Administration of the composition to a subject, tissue or cell increases the abundance of bacteria in the subject tissue or cell which creates an environment or microenvironment (e.g., metabolome) that is conducive to the treatment of cancer and/or inhibits cancer/tumour growth.

The subject may be a human or an animal in an animal model, for example a mouse model. In vitro models can also be used for testing efficacy, e.g. tissue or cell-based models. Suitable models and assays are also shown in the examples.

As used herein, an "immune response" refers to the action of a cell of the immune system (e.g., T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells, neutrophils, etc.) and soluble macromolecules produced by any of these cells or the liver (including antibodies, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a subject of invading pathogens, cells or tissues infected with pathogens, or cancerous or other abnormal cells. This can be measured by assessing suitable markers or cell types.

As used herein, the term "immunotherapy" refers to the treatment or prevention of cancer by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response.

The bacterial isolates can be isolated and cultured as described in WO2013/171515 or WO2017/182796, both incorporated herein by reference. In one embodiment, bacterial strains are cultured and grown individually and then combined in the composition.

A bacterial isolate used in the composition is preferably a non-pathogenic strain. In other words, the bacterium preferably does not cause a disease in a healthy human individual when administered to said individual.

In one embodiment, each bacterium present in the composition is susceptible to treatment with one or more antibiotics. In other words, the bacterium is not resistant to treatment with at least one antibiotic. This allows antibiotic treatment of an individual in the event that one or more of the bacteria included in a therapeutic composition administered to the individual cause disease in the individual, contrary to expectations. Thus, in one embodiment, the bacterium is susceptible to treatment with one or more antibiotics selected from the group consisting of: a beta-lactam, fusidic acid, elfamycin, aminoglycoside, fosfomycin, tunicamycin metronidazole and/or vancomycin. In vitro and in silico methods for screening bacteria for antibiotic resistance are known in the art.

In one embodiment, the isolated bacterium included in the compositions may not comprise one or more genes encoding one or more virulence factors and/or preferably does not produce one or more virulence factors. Virulence factors in this context are properties which enhance the potential of a bacterium to cause disease in an individual. Virulence factors include the production of bacterial toxins, such as endotoxins and exotoxins by a bacterium, as well as the production of hydrolytic enzymes that may contribute to the pathogenicity of the bacterium. Methods for screening bacteria for genes encoding virulence factors are known in the art.

In some embodiments, one or more of the bacterial strains are human-derived bacteria, meaning the one or more bacterial strains were obtained from or identified from a human or a sample therefrom (e.g., a human donor). In some embodiments of the compositions provided herein, all of the bacterial

strains are human-derived bacteria. In some embodiments of the compositions provided herein, the bacterial strains are derived from more than one human donor.

The bacterial strains used in the live bacterial products provided herein generally are isolated from the microbiome of healthy individuals, e.g. from human faeces, but in some cases may not be from healthy individuals. In some embodiments, the live bacterial products include strains originating from a single individual. In some embodiments, the live bacterial products include strains originating from multiple individuals. In some embodiments, the bacterial strains are obtained from multiple individuals, isolated and grown up individually. The bacterial compositions that are grown up individually may subsequently be combined to provide the compositions of the disclosure. It should be appreciated that the origin of the bacterial strains of the live bacterial products provided herein is not limited to the human microbiome from a healthy individual.

Isolation and characterisation can be achieved using standard methods in the art. For example, the V4-V5 region of the 16S rRNA encoding gene can be amplified and sequenced. Sequences can then be aligned and compared to the 16S sequences provided herein for the bacterial isolates. Sequence protocols and alignment software are well known in the art.

In some cases, strains of bacterial species useful in an invention, e.g., strains of the species disclosed herein, can be obtained from a public biological resource centre as described above.

In some embodiments in which the composition of the invention comprises more than one bacterial strain or species as listed herein, the individual bacterial strains or species may be for separate, simultaneous or sequential administration. For example, the composition may comprise bacteria from all or a subset of the species listed in Table 1, or the bacterial strains or species are selected from those listed in Table 1, but may be stored separately and be administered separately, simultaneously or sequentially. In some embodiments, the more than one bacterial strain or species are stored separately, but are mixed together prior to use.

As explained herein, the bacterial compositions of the invention have therapeutic effects when administered to a subject and can be used in the treatment or prevention of cancer. Thus, the compositions as described herein are therapeutic compositions. Thus, the invention also extends to pharmaceutical compositions comprising a composition of bacteria as described herein. This may include further ingredients, for example a vaccine.

In one embodiment, the composition may comprise a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the isolated bacteria present in the therapeutic composition. The precise nature of the pharmaceutically acceptable excipient or other material will

depend on the route of administration, which may be oral or rectal. Many methods for the preparation of therapeutic compositions are known to those skilled in the art.

The bacterial compositions of the invention may comprise a prebiotic, a pharmaceutically acceptable carrier, insoluble fibre, a buffer, an osmotic agent, an anti-foaming agent and/or a preservative. Particular examples of excipients included in the composition are disclosed below.

Prebiotics may provide nutrients for the isolated bacteria present in the bacterial composition to assist their early growth and colonisation after administration to the individual. Any prebiotic known in the art may be used. Non-limiting examples of prebiotics include oligosaccharides, e.g., fructooligosaccharides such as oligofructose and inulin, mannan oligosaccharides and galactooligosaccharides, soluble, oligofructose-enriched inulin and soluble fibre. Insoluble fibre may be included in the therapeutic composition as a carrier, e.g., to provide protection during transit or storage. A buffer may be included in the bacterial composition to promote the viability of the isolated bacteria present. An anti-fungal agent may be included in the bacterial composition as a preservative.

In one embodiment, the therapeutic bacterial compositions may comprise no other active ingredient other than the bacterial isolates as described herein, including no other isolated bacterium, and optionally a prebiotic. Thus, the active ingredient of the therapeutic composition may consist of the group of bacterial isolates as described herein, and optionally a prebiotic.

The bacterial compositions of the invention can be administered to a subject in a variety of ways as described in more detail elsewhere herein, including in the form of a capsule, tablet, gel or liquid.

The bacterial compositions of the invention may be for oral or rectal administration to the subject. Where the composition is for oral administration, the composition may be in the form of a capsule, or a tablet. Where the therapeutic composition is for rectal administration, the therapeutic composition may be in the form of an enema, tablet or capsule. The preparation of suitable capsules, tablets and enemas is well-known in the art. The capsule or tablet may comprise an enteric coating to protect the capsule or tablet from stomach acid. For example, the capsule or tablet may be enteric-coated, pH dependant, slow-release, and/or gastro-resistant. Such capsules and tablets are used, for example, to minimize dissolution of the capsule or tablet in the stomach but allow dissolution in the small intestine. When intended for oral administration, the composition can be in solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

As a solid composition for oral administration, the composition can be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like. Such a solid composition typically contains one or more inert diluents. In addition, one or more of the following can be present:

binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, or gelatin, excipients such as starch, lactose or dextrins, disintegrating agents such as alginic acid, sodium alginate, corn starch and the like; lubricants such as magnesium stearate, glidants such as colloidal silicon dioxide, sweetening agents such as sucrose or saccharin, a flavoring agent such as peppermint, methyl salicylate or orange flavoring; and a coloring agent. When the composition is in the form of a capsule (e. g. a gelatin capsule), it can contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol, cyclodextrin or a fatty oil.

When intended for oral administration, a composition can comprise one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition for administration by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent can also be included.

The bacterial composition may include a pharmaceutically acceptable carrier or vehicle that can be particulate, so that the compositions are, for example, in tablet or powder form. The term "carrier" refers to a diluent, adjuvant or excipient, with which the composition is administered. Such pharmaceutical carriers can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The carriers can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents can be used. In one embodiment, the composition and pharmaceutically acceptable carriers are sterile. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The compositions can take the form of one or more dosage units. In an embodiment, the dose unit comprises at least 1×10^{3}, 1×10^{4}, 1×10^{5}, 1×10^{6}, 1×10^{7}, 1×10^{8}, 1×10^{9}, 1×10^{10}, 1×10^{11}, 1×10^{12}, 1×10^{13} or greater than 1×10^{13} colony forming units (cfu) of vegetative bacterial cells. In an embodiment, the dose unit comprises a pharmaceutically acceptable excipient, an enteric coating or a combination thereof. The bacterial isolates or composition may be provided at a suitable dose.

Treatments or specific processes can be applied to improve the stability or viability of the bacterial isolates in the composition. The bacterial composition can be applied in a dry form or in a wet from. The bacterial composition may be lyophilized. The lyophilized therapeutic composition may comprise one or more stabilisers and/or cryoprotectants. The lyophilized bacterial composition may be reconstituted using a suitable diluent prior to administration to the individual.

Then invention also relates to a pharmaceutical composition comprising one or more bacteria of selected from the bacterial species of Table 1 or comprising a composition as described herein and further comprising an effective amount of an immune checkpoint inhibitor.

Immune checkpoints are regulatory pathways within the immune system that are involved in maintaining immune homeostasis (e.g., self-tolerance, modulating the duration and extent of an immune response) to minimize cellular damage due to aberrant immune responses. Inhibitors of immune checkpoints, herein referred to as "immune checkpoint inhibitors," specifically inhibit immune checkpoints and may have a stimulatory or inhibitory effect on the immune response.

In one embodiment, the immune checkpoint inhibitor is an antibody or fragment thereof, an interfering nucleic acid molecule or another chemical entity.

A number of checkpoint inhibitors are known in the art and a number of treatments have been approved by regulatory authorities, including antibody treatments, whilst others, including treatments with monoclonal antibodies or antibody fragments, such as single domain antibodies, have shown efficacy across a wide range of cancers.

In one embodiment, the immune checkpoint inhibitor inhibits PD-1 activity, i.e. acts as PD-1 antagonist.

"PD-1 antagonist" or "PD-1 inhibitor" means any chemical compound or biological molecule that blocks binding of PD-L1 expressed on a cancer and or immune cell to PD-1 expressed on an immune cell (T cell, B cell or NKT cell) and preferably also blocks binding of PD-L2 expressed on a cancer and or immune cell to the immune-cell expressed PD-1.

In one embodiment, the immune checkpoint inhibitor is a PD-1 inhibitor, PD-L1 inhibitor or PD-L2 inhibitor, e.g. an anti PD-1 antibody or anti PD-L1 or anti PD-L2 antibody. In one embodiment, the immune checkpoint inhibitor is an anti PD-1 antibody. In one embodiment, the immune checkpoint inhibitor is an anti PD-1 or PD-L1 antibody optionally selected from nivolumab (MDX- 1106, MDX-1106-04, ONO-4538, or BMS-936558), pembrolizumab (Trade name KEYTRUDA® formerly Lambrolizumab®, also known as Merck 3745, MK-3475 or SCH-900475), cemiplimab, avelumab, durvalumab, atezolizumab, spartalizumab, camrelizumab, sintilimab, tislelizumab, pidilizumab or toripalimab.

In one embodiment, the immune checkpoint inhibitor is an anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4 inhibitor), i.e. inhibits the activity of CTLA-4. CTLA-4 (CD152) is a B7/CD28 family member that inhibits T cell functions with NCBI Gene ID: 1493. CTLA-4 mAbs or CTLA-4 ligands can

prevent CTLA-4 from binding to its native ligands, thereby blocking the transduction of the T-cell negative regulating signal by CTLA-4 and enhancing the responsiveness of T-cells to various antigens. In this aspect, results from in vivo and in vitro studies are substantially in concert.

The CTLA4 inhibitor can be a CTLA4 antibody, optionally Ipilimumab or Tremelimumab.

In one embodiment, the immune checkpoint inhibitor is an anti-TGIT, anti-LAG3 or anti-TIM3 agent, e.g. and antibody. The checkpoint targets listed herein are not limiting and a skilled person would understand that other checkpoint targets are also within the scope of the invention and may be inhibited.

It should further be appreciated that multiple immune checkpoint inhibitors may be used in the methods, compositions, and kits disclosed herein.

In some embodiments, the cancer immunotherapy agent comprises an anticancer vaccine (also referred to herein as a cancer vaccine). Cancer vaccines generally act to increase an immune response to cancer cells. For example, cancer vaccines include cancer antigen(s) that act to induce or stimulate an immune response against cells bearing the cancer antigen(s). The immune response induced or stimulated can include an antibody (humoral) immune response and/or a T-cell (cell-mediated) immune response.

Unless otherwise specified, the term PD-1 as used herein refers to human PD-1. The terms "Programmed Death 1", "Programmed Cell Death 1", "Protein PD-1", "PD-1", PD1," "PDCD1", "hPD-1" and "hPD-1" are used interchangeably, and include variants, isoforms, species homologs of human PD-1. The term PD-1 antibody or antibody fragment refers to a molecule capable of specifically binding to the human PD-1 antigen and antagonising PD-1 action. Human PD-1 amino acid sequences can be found in NCBI Locus No.: NP_005009. Human PD-L1 and PD-L2 amino acid sequences can be found in NCBI Locus No.: NP_054862 and NP_079515, respectively.

The term "antibody" as used herein broadly refers to any immunoglobulin (Ig) molecule, or antigen binding portion thereof, comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional fragment, mutant, variant, or derivation thereof, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative antibody formats are known in the art. The antibody may be mono or multispecific, e.g. bispecific. The antibody may be administered in combination with another antibody therapy, e.g. another antibody that targets a checkpoint inhibitor or in combination with another anti-cancer therapy, e.g. chemotherapy and targeted therapies, surgery and/ or radiotherapy.

In a full-length antibody, each heavy chain is comprised of a heavy chain variable region or domain (abbreviated herein as HCVR) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region or domain (abbreviated herein as LCVR) and a light chain constant region. The light chain constant region is comprised of one domain, CL.

The heavy chain and light chain variable regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each heavy chain and light chain variable region is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG 1, IgG2, IgG 3, IgG4, IgAl and IgA2) or subclass.

The term antibody as used herein includes antibody fragments, such as F(ab')2, Fab, Fv, scFv, a heavy chain only antibody, single domain antibodies (V_H, V_L, V_{HH}) or an antibody mimetic protein. Various antibody formats have been shown to show efficacy against checkpoint inhibitors, including single domain antibodies (e.g. Yu S et al. Nanobodies targeting immune checkpoint molecules for tumor immunotherapy and immunoimaging, Int J Mol Med. 2021;47(2):444-454).

scFv fragments (~25kDa) consist of the two variable domains, V_H and V_L. Naturally, V_H and V_L domain are non-covalently associated via hydrophobic interaction and tend to dissociate. However, stable fragments can be engineered by linking the domains with a hydrophilic flexible linker to create a single chain Fv (scFv). The smallest antigen binding fragment is the single variable fragment, namely the V_H or V_L domain. Binding to a light chain/heavy chain partner respectively is not required for target binding. Such fragments are used in single domain antibodies. A single domain antibody (~12 to 15 kDa) therefore has either the V_H or V_L domain.

The antibody may be human, humanised or chimeric. A chimeric antibody is a recombinant protein that contains the variable domains including the complementarity determining regions (CDRs) of an antibody derived from one species, preferably a rodent antibody, while the constant domains of the antibody molecule are derived from those of a human antibody.

A humanized antibody is a recombinant protein in which the CDRs from an antibody from one species; e.g., a rodent antibody, are transferred from the heavy and light variable chains of the rodent antibody into human heavy and light variable domains (e.g., framework region sequences). The constant domains of the antibody molecule are derived from those of a human antibody. In certain

embodiments, a limited number of framework region amino acid residues from the parent (rodent) antibody may be substituted into the human antibody framework region sequences.

Checkpoint inhibitors are not limited to antibodies. In one embodiment, the immune checkpoint inhibitor is an interfering nucleic acid molecule, optionally wherein the interfering nucleic acid molecule is an siRNA molecule, an shRNA molecule or an antisense RNA molecule.

In one embodiment, the immune checkpoint inhibitor is a small molecule or PROteolysis TArgeting Chimera (PROTAC), alternative scaffold protein, biologics or other immune checkpoint inhibitor. In one embodiment, the immune checkpoint inhibitor is an interfering nucleic acid molecule. In one embodiment, the interfering nucleic acid molecule is an siRNA molecule, an shRNA molecule or an antisense RNA molecule. In one embodiment, the immune checkpoint inhibitor is a small molecule or a PROteolysis TArgeting Chimera (PROTAC) or other immune checkpoint inhibitor. Examples that small molecules can be used as checkpoint inhibitors is provided by research on sulfamonomethoxine and sulfamethizole. Exemplary small molecule compounds that inhibit PD-L1 are disclosed in US9850225 incorporated herein by reference. A small molecule currently in human clinical trials is a molecule called Ca-170, which inhibits both the PD-L1 pathway and the V-domain Ig suppressor of the T-cell activation (VISTA) pathway.

In one embodiment, the immune checkpoint inhibitor is a peptide inhibitor. An example is the peptide antagonist, (D)PPA-1, which blocks the PD-1/PD-L1 interaction and decreases tumor growth in vivo (Chang H.N et al, Blocking of the PD-1/PD-L1 Interaction by a D-Peptide Antagonist for Cancer Immunotherapy. Angew. Chem. Int. Ed. 2015;54:11760–11764). Another peptide inhibitor is PL120131, shown to act as a competitive inhibitor of PD-L1 (Magiera-Mularz K. et al Bioactive Macrocyclic Inhibitors of the PD-1/PD-L1 Immune Checkpoint. Angew. Chem. Int. Ed. 2017;56:13732–13735) and TPP-1 (Li C., Zhang N et al, Peptide Blocking of PD-1/PD-L1 Interaction for Cancer Immunotherapy. Cancer Immunol. Res. 2018;6:178–188).

In another aspect, there is provided a bacterial composition described herein for use in the treatment of disease, e.g. cancer. In another aspect, there is provided the use of a bacterial composition described herein in the manufacture of a medicament for the treatment or prevention of a disease, e.g. cancer.

In another aspect, there is provided a method for treating or preventing a disease comprising administering a bacterial composition described herein to a subject. In another aspect, there is provided a method for treating or preventing a disease in a subject comprising modulating the level of, e.g. increasing the level / relative abundance of one or more bacterium selected from B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14 and/or B15 as shown in Table 1 or a subset thereof in a subject. In one embodiment, the subset comprises or consists of bacteria selected from 1, 2, 3, 4, 5, 6,

7, 8, 9, 10, 11, 12, 13, 14 or 15 bacterial species shown in Table 1. Modulating the level according to one or more bacterium in the subject enhances an immune response by the subject and/or inhibits immune evasion by the cancer and/or increases efficacy according to an anti-cancer treatment with an immune checkpoint inhibitor. In one embodiment, the method comprises administering a composition as described herein.

As explained below, the level / abundance can be compared to a reference value from a reference subject or population of subjects.

In one embodiment, the disease is cancer. In one embodiment, the cancer is melanoma. "Melanoma" is taken to mean a tumour arising from the melanocytic system of the skin and other organs. Non-limiting examples of melanomas are Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, nodular melanoma, subungual melanoma, Cutaneous melanoma, uveal/intraocular melanoma and superficial spreading melanoma.

The compositions of the present invention are particularly useful for the treatment of cancers that are treatable by checkpoint inhibitors.

In one embodiment, the cancer is associated with cells (e.g., exhausted T cells, B cells, monocytes, etc.) that express abnormally high levels of PD-1. Other cancers include those characterized by elevated expression of PD-1 and/or its ligands PD-L1 and/or PD-L2.

In one embodiment, the cancer is selected from a cancer that has high levels of cancer-associated genetic mutations and/or high levels of expression of tumour antigens. In another embodiment, the cancer is selected from a cancer known to be immunogenic or that is able to become immunogenic upon treatment with other cancer therapies. In a further embodiment the cancer can be selected from a cancer generally treated by non-immunological therapies, such as chemotherapy, in which the patient's immune system is likely to have a role.

The cancer can be selected from a solid or non-solid tumour. For example, in addition to melanoma, the cancer may be selected from another skin cancer or from bone cancer, pancreatic cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, breast cancer, brain cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, kidney cancer, sarcoma of soft tissue, cancer of the urethra, cancer of the bladder, renal cancer, lung cancer, non-small cell lung cancer, thymoma, urothelial carcinoma

leukemia, prostate cancer, mesothelioma, adrenocortical carcinoma, lymphomas, such as such as Hodgkin's disease, non-Hodgkin's, gastric cancer, and multiple myelomas.

In one embodiment, the tumour is a solid tumour. Examples of solid tumours which may be accordingly treated include breast carcinoma, lung carcinoma, colorectal carcinoma, pancreatic carcinoma, glioma and lymphoma. Some examples of such tumours include epidermoid tumours, squamous tumours, such as head and neck tumours, colorectal tumours, prostate tumours, breast tumours, lung tumours, including small cell and non-small cell lung tumours, pancreatic tumours, thyroid tumours, ovarian tumours, and liver tumours. Other examples include Kaposi's sarcoma, CNS, neoplasms, neuroblastomas, capillary hemangioblastomas, meningiomas and cerebral metastases, melanoma, gastrointestinal and renal carcinomas and sarcomas, rhabdomyosarcoma, glioblastoma, preferably glioblastoma multiforme, and leiomyosarcoma. Examples of vascularized skin cancers for which the antagonists of this invention are effective include squamous cell carcinoma, basal cell carcinoma and skin cancers that can be treated by suppressing the growth of malignant keratinocytes, such as human malignant keratinocytes. In one embodiment, the cancer is NSCL.

In one embodiment, the tumour is a non-solid tumour. Examples of non-solid tumours include leukemia, multiple myeloma and lymphoma.

In one aspect, the cancer is identified as a PD-1 and/or PD-L1 positive cancer or a cancer positive for another checkpoint protein. In one aspect, the cancer is locally advanced, unresectable, metastatic, or recurrent cancer.

Preferred cancers whose growth may be inhibited using the agents of the invention include cancers typically responsive to immunotherapy. Non-limiting examples of preferred cancers for treatment include melanoma (e.g., metastatic malignant melanoma), renal cancer (e.g. clear cell carcinoma), prostate cancer (e.g. hormone refractory prostate adenocarcinoma), breast cancer, colon cancer and lung cancer (e.g. non-small cell lung cancer).

As used herein, "treat", "treating" or "treatment" means inhibiting or relieving a disease or disorder. For example, treatment can include a postponement of development of the symptoms associated with a disease or disorder, and/or a reduction in the severity of such symptoms that will, or are expected, to develop with said disease. The terms include ameliorating existing symptoms, preventing additional symptoms, and ameliorating or preventing the underlying causes of such symptoms. Thus, the terms denote that a beneficial result is being conferred on at least some of the mammals, e.g., human patients, being treated. Many medical treatments are effective for some, but not all, patients that undergo the treatment.

The term "subject" or "patient" refers to an animal, e.g. a human, which is the object of treatment, observation, or diagnosis. By way of example only, a subject includes, but is not limited to, a mammal, including, but not limited to, a human or a non-human mammal, such as a non-human primate, murine, bovine, equine, canine, ovine, or feline. In one embodiment, the subject is a cancer patient that has received prior anti-cancer treatment or is receiving anti-cancer treatment. In one embodiment, the anti-cancer treatment is treatment with an immune checkpoint inhibitor. Exemplary immune checkpoint inhibitors are described herein.

The term “anti-cancer therapy” refers to any therapeutic regimen that aims to reduce or eliminate cancer, slow the progression of cancer, prevent or reduce the risk of cancer metastasis, and/or reduce or prevent any one or more symptoms associated with cancer. The anti-cancer therapies described herein involve administering anti-cancer therapies to a subject, e.g., a subject having cancer or at risk of having cancer.

Administration according to the method and uses above includes oral administration or rectal administration.

In one embodiment, the subject has received prior anti-cancer therapy with an immune checkpoint inhibitor. In one embodiment, an anti-cancer therapy comprising an immune checkpoint inhibitor is administered to the subject. This can be administered at the same time as the composition of the invention, either as part of the same medicament or as a second medicament. It can also be administered prior or after the administration of the composition of the invention. Other treatment schedules are also within the scope of the invention.

In one embodiment, the immune checkpoint inhibitor is administered before, after or at the same time as the bacterial composition. In one embodiment, checkpoint therapy is initiated, and then supplemented with treatment using the bacterial composition described herein if no response is seen after 3-6 months.

In one embodiment, the immune checkpoint inhibitor is administered after the bacterial composition. In one embodiment the immune checkpoint inhibitor is administered by injection / infusion. In one embodiment the injection is an intravenous, intramuscular, intratumoural or subcutaneous injection.

Checkpoint inhibitors that can be used in accordance with the treatment aspects are defined above. For example, the immune checkpoint inhibitor inhibits PD-1, CTLA-4 or PD-L1 activity. In one embodiment the immune checkpoint inhibitor is an anti PD-1, CTLA-4 or PD-L1 antibody. In one embodiment, the anti PD-1, CTLA-4 or PD-L1 antibody is selected from nivolumab, pembrolizumab, cemiplimab, avelumab, durvalumab, atezolizumab, Spartalizumab, Camrelizumab, Sintilimab, Tislelizumab, Pidilizumab or Toripalimab, Ipilimumab or Tremelimumab.

The amount of the antibody that is effective/active in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition and can be determined by standard clinical techniques. In addition, in vitro or in vivo assays can optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of the disease shall be taken into account.

Typically, the amount is at least about 0.01% of an anti-PD-1, CTL-4 or PD-L1 antibody by weight of the composition. When intended for oral administration, this amount can be varied to range from about 0.1 % to about 80% by weight of the composition. Oral compositions can comprise from about 4% to about 50% of the antibody by weight of the composition.

Antibody compositions can be prepared so that a parenteral dosage unit contains from about 0.01 % to about 2% by weight of the antibody.

For administration by injection, the composition can comprise from about typically about 0.1 mg/kg to about 250 mg/kg of the animal's body weight, preferably, between about 0.1 mg/kg and about 20 mg/kg of the animal's body weight, and more preferably about 1 mg/kg to about 10 mg/kg of the animal's body weight. In one embodiment, the composition is administered at a dose of about 1 to 30 mg/kg, e.g., about 5 to 25 mg/kg, about 10 to 20 mg/kg, about 1 to 5 mg/kg, or about 3 mg/kg. The dosing schedule can vary from e.g., once a week to once every 2, 3, or 4 weeks.

In one embodiment, the immune checkpoint inhibitor is an interfering nucleic acid molecule. In one embodiment, the interfering nucleic acid molecule is an siRNA molecule, an shRNA molecule or an antisense RNA molecule. In one embodiment, the immune checkpoint inhibitor is a small molecule or a PROteolysis TArgeting Chimera (PROTAC) or other immune checkpoint inhibitor, for example as described above.

In one embodiment, the method and uses further comprise administration of an anti-cancer therapy, e.g. a second anti-cancer therapeutic in addition to an immune checkpoint inhibitor. The anti-cancer therapy may include a therapeutic agent or radiation therapy and includes gene therapy, viral therapy, RNA therapy bone marrow transplantation, nanotherapy, targeted anti-cancer therapies or oncolytic drugs or a combination thereof. Examples of other therapeutic agents include other checkpoint inhibitors, antineoplastic agents, immunogenic agents, attenuated cancerous cells, tumour antigens, antigen presenting cells such as dendritic cells pulsed with tumour-derived antigen or nucleic acids, immune stimulating cytokines (e.g., IL-2, IFNa2, GM-CSF), targeted small molecules and biological

molecules (such as components of signal transduction pathways, e.g. modulators of tyrosine kinases and inhibitors of receptor tyrosine kinases, and agents that bind to tumour- specific antigens, including EGFR antagonists), an anti-inflammatory agent, a cytotoxic agent, a radiotoxic agent, or an immunosuppressive agent and cells transfected with a gene encoding an immune stimulating cytokine (e.g., GM-CSF), chemotherapy. In one embodiment, the composition is used in combination with surgery. In one embodiment, the composition is used in combination with a stem-cell transplant therapy comprising a peripheral blood transplant, a bone marrow transplant, a cord blood transplant, or a skin-derived stem cell transplant.

In one embodiment, the composition is used in combination with adoptive cell transfer (ACT). In general, adoptive cell transfer therapy involves harvesting cells from a subject, specifically producing or expanding a specific cell population, optionally activating the cells, and administering the expanded cells to the subject. In some embodiments, the desired cells are immune cells capable of killing or eliminating cancer cells.

In some embodiments, the adoptive cell transfer therapy uses engineered T-cell receptors or chimeric antigen receptors, which may be referred to as CAR-T therapy. CAR-T cells include T-cells taken from a subject that are genetically engineered to express chimeric antigen receptors (CARs) on the cell surface. The CAR-T cell receptors are designed to recognize a specific antigen on cancer cells (e.g., a cancer antigen). After the CAR-T cells are infused into the subject, the CAR-T cells recognize and kill cancer cells that express the specific antigen on their surfaces. In some embodiments, the CAR-T cells are autologous cells, meaning the T cells were harvested and re-administered to the same subject. In some embodiments, the CAR-T cells are CD8+ T cells. In some embodiments, the CAR-T cells are allogeneic cells, meaning the T cells were harvested from one subject (e.g., the donor) and administered to a different subject (e.g., the recipient).

Examples of cancer antigens that may be targeted by CAR-T cells are known in the art, and selection of a cancer antigen for targeting will depend on factors such as the cancer that is being targeted.

In some embodiments, the anticancer therapy involves administering one or more costimulatory agents. In some embodiments, the costimulatory agent is a molecule that targets one or more costimulatory molecules, thereby modulating the immune response. In some embodiments, the costimulatory agent enhances an anticancer immune response, for example, by preventing the downregulation of an immune response. A costimulatory agent may be administered alone in a cancer therapy or in combination with one or more cancer therapies to enhance the therapeutic effect of the cancer therapy. In some embodiments, the costimulatory agent is an antibody that targets CD-28, OX-40, 4-1BB, or CD40.

In one embodiment of the present invention, the composition is administered concurrently with a chemotherapeutic agent and/or with radiation therapy. In another specific embodiment, the chemotherapeutic agent and/or radiation therapy is administered prior or subsequent to administration of the composition of the present invention, preferably at least an hour, five hours, 12 hours, a day, a week, a month, more preferably several months (e. g. up to three months), prior or subsequent to administration of composition of the present invention.

As used herein, a chemotherapy agent refers to a molecule (e.g., drug) that specifically or preferentially kills cancer cells or prevents the proliferation of cancer cells. Chemotherapy agents can generally be categorized based on the molecular target of the chemotherapy agent, the mechanism of action, and/or the structure of the agent. In some embodiments, the chemotherapy agent is an alkylating agent, a plant alkaloid, an antitumor antibiotic, an antimetabolite, a topoisomerase inhibitor, or other antineoplastic agent.

In one embodiment, the chemotherapeutic agent is selected from the group consisting of: alkylating agents, alkyl sulfonates, aziridines, an ethylenimine, a methylamelamine, an acetogenin, a camptothecin bryostatin, cally statin, CC-1065, a cryptophycin, dolastatin, duocarmycin, eleutherobin, pancratistatin, a sarcodictyin, spongistatin, a nitrogen mustard, a nitrosurea, an antibiotic, a dynemicin; a bisphosphonate, an esperamicin, neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores, an aclacinomysin, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, a mitomycin, mycophenolic acid, nogalamycin, an olivomycin, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin an anti-metabolite, a folic acid analogue, a purine analog, a pyrimidine analog, an androgen, an anti-adrenal, a folic acid replenishes aceglatone, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatrexate, demecolcine, diaziquone, elformithine, elliptinium acetate, an epothilone, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, a maytansinoid, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2- ethylhydrazide, procarbazine, PSK polysaccharide complex, razoxane, rhizoxin, sizofuran, spirogermanium, tenuazonic acid, triaziquone, 2,2',2"-trichlorotriethylamine, a trichothecene, urethan, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, arabinoside ("Ara-C"), cyclophosphamide, thiotepa, a taxoid, ABRAXANE Cremophor-free, an albumin-engineered nanoparticle formulation of paclitaxel and TAXOTERE doxetaxel, chlorambucil, GEMZAR gemcitabine, 6-thioguanine, mercaptopurine, methotrexate, a platinum analog, vinblastine, platinum, etoposide (VP-16), ifosfamide, mitoxantrone, vincristine, NAVELBINE, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, xeloda, ibandronate, irinotecan (Camptosar, CPT-11), topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO), a retinoid, capecitabine, combretastatin, leucovorin (LV), oxaliplatin,

Binimetinib (Mektovi), Encorafenib (Braftovi), lapatinib (TYKERB), an inhibitor of PKC-a, an inhibitor of Raf, an inhibitor of H-Ras, an inhibitor of EGFR, an inhibitor of VEGF-A, pharmaceutically acceptable salt, acid or derivative thereof, and combinations thereof.

In some embodiments, the composition of the invention may be administered with two or more (e.g., 2, 3, 4, 5, or more) therapeutic agents.

In one embodiment, administration is together with an agent involved in T-cell activation, a tumour microenvironment modifier (TME) or a tumour-specific target.

In one embodiment, the method and uses further comprise administering an antibiotic to the subject.

In yet another aspect, the invention provides a method of modulating an immune response in a subject comprising administering to the subject a composition of the invention.

In some embodiments, the individual has cancer that is resistant (has been demonstrated to be resistant) to one or more anti-cancer therapies. In some embodiments, resistance to anti-cancer therapy includes recurrence of cancer or refractory cancer. Recurrence may refer to the reappearance of cancer, in the original site or a new site, after treatment. In some embodiments, resistance to anti-cancer therapy includes progression of the cancer during treatment with the anti-cancer therapy. In some embodiments, the cancer is at early stage or at late stage.

The composition of the invention has immunostimulatory properties. Therefore, use of the composition is not limited to the treatment of cancer. Thus, due to the immunostimulatory properties, the composition finds use in the treatment of any disease which requires immunostimulation, e.g. non-cancer immunotherapies. Immunotherapy is collectively defined as a therapeutic approach that targets or manipulates the immune system. Ultimately, immunotherapy aims to harness the host's adaptive and innate immune response to effectuate long-lived elimination of diseased cells and can be categorized broadly into passive (including adoptive and antibody-based) and active (including vaccine therapy and allergen-specific) approaches. Passive-mediated immunotherapy involves the administration of ex vivo-generated immune elements (antibodies, immune cells) to patients and does not stimulate the host immune response, while active immunotherapy induces the patient's immune response and results in the development of specific immune effectors (antibodies and T cells). Immunotherapy offers a possible modality to improve the ability to prevent or treat infectious diseases (Naran et al, Front Microbiol. 2018; 9: 3158). Thus, in some embodiments, the disease is an infectious disease.

The recent success of PD-1 and PD-L1 blockade in cancer therapy illustrates the important role of the PD-1/PD-L1 pathway in the regulation of antitumor immune responses. However, signaling regulated

by the PD-1/PD-L pathway is also associated with substantial inflammatory effects that can resemble those in autoimmune responses, chronic infection, and sepsis, consistent with the role of this pathway in balancing protective immunity and immunopathology, as well as in homeostasis and tolerance (Quin et al, Front Immunol. 2019; 10: 2298; Rao et al, Int. J. Infect. Dis. 2017; 56: 223). Thus, in another aspect, the invention provides a composition as described herein; e.g. comprising one or more of B1 to B15 as in table 1, e.g. a composition with one or more bacterial isolate having a 16SrDNA having a sequence selected from SEQ ID Nos. 1 to 15 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto, e.g. SEQ ID Nos. 16-29, for use in the treatment of an infectious disease. Also provided is a method for the treatment of an infectious disease comprising administering a composition of the invention to a subject. Also covered is a composition as described herein for use in the manufacture of a medicament for the treatment of an infectious disease.

An infectious disease may be a viral, fungal and bacterial infection. The infectious disease may be a chronic infectious disease. Non-limiting examples include human immunodeficiency virus (HIV), hepatitis B (HBV), hepatitis C (HCV), JC (John Cunningham) virus/ progressive multifocal leukoencephalopathy and tuberculosis.

Treatment of infections with the composition of the invention can be as co-therapy with an immunotherapy, for example an immune checkpoint inhibitor, other anti-viralS or anti-infectives.

In another aspect, the invention provides a composition as described herein, e.g. comprising one or more of B1 to B15 as in table 1, e.g. a composition with one or more bacterial isolate having a 16S rDNA having a sequence selected from SEQ ID Nos. 1 to 15 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto, for use as a vaccine adjuvant. Also provided is a method for increasing vaccine efficacy comprising administering a composition as described herein, e.g. comprising one or more of B1 to B15 as in Table 1, e.g. a composition with one or more bacterial isolate having a 16S rDNA having a sequence selected from SEQ ID Nos. 1 to 15 or a sequence with at least 97%, 98%, 98.7%, 99% or 100% sequence identity thereto, e.g. SEQ ID Nos. 16-29, to a subject. Said subject may receive a vaccine before, after or concurrently with administration of the bacterial composition.

Administration may be in a "therapeutically effective amount", this being sufficient to show benefit to the individual. Such benefit may be at least amelioration of at least one symptom. Thus “treatment” of a specified disease refers to amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated, the particular patient being treated, the clinical condition of the individual patient, the site of delivery of the composition, the type of therapeutic composition, the method of administration, the scheduling of administration and other factors known to medical practitioners. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other

medical doctors and may depend on the severity of the symptoms and/or progression of a disease being treated. A therapeutically effective amount or suitable dose of a therapeutic composition of the invention can be determined by comparing its in vitro activity and in vivo activity in an animal model. Methods for extrapolation of effective dosages in mice and other test animals to humans are known. The precise dose will depend upon a number of factors, including whether the therapeutic composition is for prevention or for treatment.

In one embodiment of the methods which require administration of the composition, the method includes the further step of detecting the presence of one or more of the bacterial strain that has been administered in the subject subsequent to administration. Methods for detection include for example detecting a 16S nucleic acid sequence as defined herein of at least one administered bacterial isolate in said subject.

The composition of the present invention may be prepared by a method comprising culturing the two or more isolated bacteria present in the composition in a suitable medium or media. Media and conditions suitable for culturing the bacteria to be included in the therapeutic composition of the present invention are described in detail elsewhere herein. For example, a method of preparing a therapeutic composition according to the present invention may comprise the steps of:
(i) culturing a first isolated bacterium;
(ii) culturing a second and optionally a further isolated bacterium; and
(iii) mixing the bacteria obtained in (i) and (ii) to prepare the therapeutic composition.

The isolated bacteria to be included in the composition may be cultured in separate steps. In other words, a separate culture of each bacterium to be included in the therapeutic composition is preferably prepared. This allows the growth of each bacterium to be evaluated and the amount of each bacterium to be included in the pharmaceutical composition to be controlled as desired. The bacteria cultured in steps (i) and (ii) preferably have distinct 16S nucleic acid sequences, that is 16S nucleic acid sequences that share less than 99%, 98%, 97%, 96% or 95% sequence identity.

The above method may include steps of culturing each isolated bacterium which is to be included in the composition.

The method may optionally comprise one or more further steps in which the bacteria are mixed with one or more additional ingredients, such as a pharmaceutically acceptable excipient, prebiotic, carrier, insoluble fibre, buffer, osmotic agent, antifoaming agent, and/or preservative. In addition, or alternatively, the method may comprise suspending the bacteria obtained in (i) and optionally (ii) in a chemostat medium, or saline, e.g. 0.9% saline. The bacteria obtained in (i) and optionally (ii) may be provided under a reduced atmosphere, such as N2, CO2, H2, or a mixture thereof, e.g. N2:CO2:H2. The gases may be present in appropriate ratios for the preservation of the bacteria present in the

therapeutic composition. For example, the reduced atmosphere may comprise 80% N2, 10% CO2 and 10% H2. In addition, or alternatively, the method may comprise a step of lyophilising the bacteria obtained in (i) and optionally (ii), optionally in the presence of a stabiliser and/or cryprotectant. The method may also comprise a step of preparing a capsule, tablet, or enema comprising the bacteria obtained in (i) and optionally (ii). The capsule or tablet may be enteric-coated, pH dependant, slow-release, and/or gastro-resistant.

The composition of the invention may also be provided in the form of a food supplement, beverage or other food stuff. The invention thus also relates to a food product or a vaccine comprising a composition of the invention.

Also provided is an immunogenic composition comprising fragments of bacteria selected from the those listed in Table 1, for use as an adjuvant to an anti-PD-1/PD-L1/PD-L2 antibody- based therapy administered to a cancer patient.

Biomarker

The invention provides microbiome biomarkers that are predictive of tumor response to therapy in a cancer patient with an immune checkpoint inhibitor. In particular, the invention provides a microbiome biomarker signature that is predictive of tumor response therapy with an immune checkpoint inhibitor. As used herein, a microbiome biomarker signature is a composite biomarker signature that comprises bacteria from at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 bacterial species as shown in Table 1 which each have increased abundance in subjects that are responsive to therapy with an immune checkpoint inhibitor. In one embodiment, the signature comprises bacteria from at least 9, 10, 11, 12, 13, 14 or 15 bacterial species selected from Table 1 which each have increased abundance in a population of subjects that are responsive to therapy with an immune checkpoint inhibitor. The biomarker signature is described in more detail below.

Another aspect provides a method of treating cancer in a subject comprising administering a therapeutically effective amount of an immune checkpoint inhibitor to said subject, wherein the subject has been determined to have a favorable microbial profile in the gut microbiome. A favorable microbial profile is characterised by the presence of the biomarkers/ biomarker signature described herein.

Another aspect provides a method of treating cancer in a subject, wherein the subject has been determined to have an unfavorable microbial profile in the gut microbiome. A unfavorable microbial profile is characterised by the absence of the biomarkers / biomarker signature described herein. The method may further comprise administration of an anti-cancer therapy that is not an immune checkpoint inhibitor therapy. In another embodiment, the method comprises administration of a

therapeutic bacterial composition described herein and co-therapy with an immune checkpoint inhibitor therapy, e.g. a PD-1 inhibitor.

Thus, the invention also relates to a method for identifying a subject that will respond to therapy with an immune checkpoint inhibitor, e.g. PD-1, comprising determining the abundance of one or more of the bacteria identified as B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14 and/or B15 in Table 1 in a biological sample from said subject that comprises gut (i.e. intestinal) flora wherein an increase in the abundance of one or more of B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14 and/or B15, e.g. one or more of B1, B2, B3, B4, B5, B6, B7, B8 and/or B9, is indicative that the subject will respond to therapy with an immune checkpoint inhibitor, e.g. PD-1. B1 to B15 are listed in Table 1 and this includes references to sequence identifiers to define the bacteria. Corresponding sequences are listed in Table 2. In one embodiment, the subject is a patient that has been diagnosed with a cancer, e.g. melanoma.

In particular, the invention also relates to a method for predicting a response to an immune checkpoint inhibitor therapy in a subject having cancer / a method for identifying a subject that will respond to therapy with an immune checkpoint inhibitor, the method comprising:
a) determining the abundance of one or more of the bacteria selected from B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14 and/or B15 in Table 1 in a biological sample obtained from the subject and
b) comparing the abundance to a reference level from cancer patients that do not respond to therapy with an immune checkpoint inhibitor; or comparing the abundance to a reference level from cancer patients that respond to therapy with an immune checkpoint inhibitor;
wherein if the reference level is from cancer patients that do not respond to therapy with an immune checkpoint inhibitor, then an increase in the abundance of one or more of B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14 and/or B15 compared to the reference level, is indicative that the subject will respond to therapy with an immune checkpoint inhibitor or
wherein if the reference level is from patients that do respond to therapy with an immune checkpoint inhibitor, then the same, substantially the same or an increase in the abundance of one or more of B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14 and/or B15, is indicative that the subject will respond to therapy with an immune checkpoint inhibitor.

An additional step may include identifying the subject that will respond to therapy.

In particular, the invention also relates to a method for predicting a response to an immune checkpoint inhibitor therapy in a subject having cancer / a method for identifying a subject that will respond to therapy with an immune checkpoint inhibitor, the method comprising:

a) determining the abundance of one or more of the bacteria selected from B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14 and/or B15 in Table 1 in a biological sample obtained from the subject and;
b) comparing the abundance to a reference level from cancer patients or healthy subjects and
c) applying random forest analysis. In this embodiment, the reference level is from cancer patients, that is a pool of cancer patients. These may include responders and non-responders.

An additional step may include identifying the subject that will respond to therapy or prediction a response.

Thus, the abundance of bacteria from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 different species selected from B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14 and B15 in Table 1 is determined. Respective sequences characterising the species are provided as SEQ IDs 1 to 15. As explained elsewhere, SEQ IDs 16-29 can also be used. In some embodiments, the abundance of bacteria selected from at least 9, 10, 11, 12, 13, 14 or 15 different species identified as B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14 and B15 in Table 1 is determined. Thus, the abundance of bacteria having sequences IDs selected from at least 9 of the following SEQ IDs is determined: SEQ ID NO. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 or sequences with at least 97%, 98%, 98.7% or 99% sequence identity thereto, such as, for example SEQ IDs 16 to 29.

Also provided is a method for predicting relapse in a patient who is treated or who has been treated for a cancer, comprising assessing, in faeces samples from said patient obtained e.g. at different time-points, the presence / relative abundance of one or more bacteria selected from B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14 and/or B15.

When abundance is determined, an abundance score is obtained for each of the bacteria, i.e. bacterial species, and measured. According to the method, an increase in the abundance, i.e. the abundance score, of one or more of the bacteria listed in Table 1 is indicative that the subject will respond to therapy with an immune checkpoint inhibitor. An increase refers to an increase of abundance, i.e. the abundance score, compared to a reference value. Therefore, the method may also comprise comparing the abundance one or more of the bacteria listed in Table 1 to one or more reference value. For example, the abundance of one or more of the bacteria listed in Table 1 can be compared to a reference value for one or more of the bacteria listed in Table 1. Alternatively, the arithmetic mean of the abundance of one or more of the bacteria listed in Table 1 can be compared to a single reference value which is the reference arithmetic mean of the abundance of one or more of the bacteria listed in Table 1. In one embodiment, the method determines the abundance of at least 9, 10, 11, 12, 13, 14 or 15 different bacteria selected from B1 to B15, thus determining a microbiome biomarker signature, i.e. a microbiome biomarker signature score, that is based on the composite signature.

In one embodiment of the methods, the abundance of all of the bacteria listed in Table 1 is determined. In another embodiment, the abundance of a subset the bacteria listed in Table 1 is determined. For example, the subset comprises or consists of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 different bacteria selected from Table 1. In one embodiment, the subset comprises or consists of 9 bacteria, e.g. *Eisenbergiella sp., Butyricicoccus sp., Clostridiales sp., Alistipes obesi, Alistipes indistinctus, Gordonibacter urolithinfaciens, Faecalitalea sp., Blautia sp. (B8),* and *Barnesiella intestinihominis*. In one embodiment, the subset comprises or consists of 9 or 12 bacteria, e.g. one of the bacterial consortia in Table 3. In one embodiment, the biomarker does not comprise an Alistipes species.

The reference value may be a predetermined value from a reference sample. For example, the reference value can be the average abundance of each of the bacteria or their composite signature, respectively, in a pool of reference subjects.

For example, the reference value is a predetermined value, e.g. a predetermined threshold value. Such a value can be predetermined from a reference sample. A predetermined threshold value relating to abundance of one or more bacteria of B1 to B15 refers to the abundance of the bacteria in the sample as a proportion of the total microbiota in the sample, for example a stool sample, above or below which the sample is scored as being positive for the signature and thus responsive to therapy with an immune checkpoint inhibitor. For example, if the abundance score for the test sample is at or above a predetermined threshold, then the sample is considered to be positive for the signature and the subject is responsive to therapy with an immune checkpoint inhibitor.

For example, abundance scores of the tested bacteria levels in a sample pool are stored on a computer, or on computer-readable media, to be used as reference levels in comparisons to the abundance of the tested bacteria from the test sample when needed. Machine learning algorithms and/or models commonly used in the identification of biomarkers, such as a Cox model, trained using training data comprising information on a plurality of biomarkers in a set of subjects or other models may be used to establish reference values and or to correlate abundance of the bacteria selected from one or more of the bacteria listed in Table 1 in the sample with the subject's responsiveness to treatment with an immune checkpoint inhibitor.

The term "correlating" is used herein to determine or calculate responsiveness to treatment status based on modulated abundance of one or more bacteria should be understood to mean any methods of correlation, e.g. algorithmic methods. The methodology described herein employs a mathematical modelling technique known as Random Forest Classification, but other modelling techniques may be employed. Therefore, in one embodiment, a Random Forest Classification Model or similar model is used to correlate abundance of the bacteria selected from one or more of the bacteria listed in Table 1

in the sample with the subject's responsiveness to treatment with an immune checkpoint inhibitor. Thus, in one embodiment, the methods of the invention may employ a computer program to correlate modulated abundance of the bacteria with immune checkpoint inhibitor treatment response.

Alternatively, the reference value is not predetermined, but it is established as part of a single experiment. Thus, the abundance of the one or more tested bacteria in the test sample may be compared to the abundance of the one or more tested bacteria in the pool of samples, where abundance of the tested bacteria from the test sample and abundance of the tested bacteria from the pool are determined during the course of a single experiment.

In the various embodiments, the reference sample/sample pool may be a population of cancer patients that have been shown to be responsive or non-responsive to therapy with an immune checkpoint inhibitor. In other embodiments, the reference sample/sample pool may be a population of cancer patients that have not yet received therapy with a checkpoint inhibitor.

In one embodiment, the reference sample used to establish a reference value may be from non-responders to immune checkpoint inhibitor therapy. If the test sample shows an increased abundance of the one or more bacteria selected from B1 to B15 compared to the reference sample, then the test subject is likely to respond to therapy with a checkpoint immune inhibitor. The increase may be at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more.

In one embodiment, the reference sample used to establish a reference value may be from responders to immune checkpoint inhibitor therapy. If the test sample shows the same, substantially the same or an increase in the abundance of the one or more bacteria selected from B1 to B15 than the reference sample, then the test subject is likely to respond to therapy with a checkpoint immune inhibitor.

As a skilled person would understand, a reference value or reference gene signature score as used herein means the score for a bacterial abundance signature that has been determined to divide at least the majority of responders from at least the majority of non-responders in a reference population of subjects.

As used herein, a "good responder to a treatment", also called a "responder" or "responsive" patient or in other words a patient who "benefits from" this treatment, refers to a patient who is affected with a cancer and who shows or will show a clinically significant relief in the cancer after receiving this treatment. Conversely, a "bad responder" or "non-responder" is one who does not or will not show a clinically significant relief in the cancer after receiving this treatment. The response to treatment may be assessed according to the standards recognized in the art, such as immune- related response criteria (irRC), WHO or RECIST criteria.

A signature biomarker described herein is useful to identify cancer patients who are most likely to achieve a clinical benefit from treatment with an immune checkpoint inhibitor. This utility supports the use of these biomarkers in a variety of research and commercial applications, including but not limited to, clinical trials of PD-1 antagonists in which patients are selected on the basis of their microbiome gene signature score, diagnostic methods and products for determining a patient's microbiome gene signature score or for classifying a patient as positive or negative for a microbiome signature biomarker, personalized treatment methods which involve tailoring a patient's drug therapy based on the patient's microbiome signature score, as well as pharmaceutical compositions and drug products comprising a PD-1 antagonist for use in treating patients who test positive for a microbiome signature biomarker.

A skilled person would also understand that the utility of any of the applications claimed herein does not require that 100% of the patients who test positive for a biomarker of the invention achieve an anti-tumor response to an immune checkpoint inhibitor; nor does it require a diagnostic method or kit to have a specific degree of specificity or sensitivity in determining the presence or absence of a biomarker in every subject, nor does it require that a diagnostic method claimed herein be 100% accurate in predicting for every subject whether the subject is likely to have a beneficial response to a PD-1 antagonist. Thus, the inventors herein intend that the terms "determine", "determining" and "predicting" should not be interpreted as requiring a definite or certain result; instead these terms should be construed as meaning either that a claimed method provides an accurate result for at least the majority of subjects or that the result or prediction for any given subject is more likely to be correct than incorrect. Preferably, the accuracy of the result provided by a diagnostic method of the invention is one that a skilled artisan or regulatory authority would consider suitable for the particular application in which the method is used.

As used herein, the sample is a biological sample from the gut, i.e. one that comprises gut intestinal flora. This refers to a sample obtained from the gut of a subject, for example a faecal sample. Methods of isolating bacteria from a faecal sample are known. In some cases, the microbiome sample is obtained by mucosal biopsy. A test sample is sample obtained from a subject that is being assessed.

In one embodiment of the method, the abundance is relative abundance. As used herein, the term "relative abundance" as applied to a bacterium in a sample should be understood to mean the abundance of the bacterium in the sample as a proportion of the total microbiota in the sample or a reference sample. In one embodiment, the relative abundance is the abundance of the bacterium in the sample as a proportion of the total microbiota in the sample.

In one embodiment, the modulated abundance is a difference in relative abundance of the bacterium in the sample compared with the relative abundance in the same sample from a reference subject.

In one embodiment, the abundance of the bacterium in the sample as a proportion of the total microbiota in the sample is measured to determine the relative abundance of the bacterium. Then, in such embodiments, the relative abundance of the bacterium in the sample is compared with the relative abundance in the same sample from a reference individual (also referred to herein as the "reference relative abundance"). A difference in relative abundance of the bacterium in the sample, e.g. an increase, compared to the reference relative abundance is a modulated relative abundance. Detection of modulated abundance can also be performed in an absolute manner by comparing sample abundance values with absolute reference values.

Any suitable method of detecting bacterial presence / abundance may be employed, including, for example, agar plate quantification assays, fluorimetric sample quantification, PCR methods, 16S rRNA/rDNA gene amplicon sequencing, Shotgun metagenomic sequencing and dye-based metabolite depletion or metabolite production assays. The PCR technique used can quantitatively measure starting amounts of DNA, cDNA, or RNA. Examples of PCR-based techniques according to the invention include techniques such as, but not limited to, quantitative PCR (Q-PCR), reverse-transcriptase polymerase chain reaction (RT-PCR), quantitative reverse-transcriptase PCR (QRT-PCR), rolling circle amplification (RCA) or digital PCR. These techniques are well known and easily available and do not need a precise description. In a particular embodiment, the determination of the copy number of the bacterial genes of the invention is performed by quantitative PCR.

In one embodiment, the sample is analysed using a nucleic acid amplification reaction. Analysing may include detecting family, order-, class- and/or genus-specific 16S rRNA/rDNA or other sequences in the bacterial genome. In one embodiment, full length 16S rDNA may be detected. In one embodiment, partial 16S rDNA may be detected, for example one of the V regions. In one embodiment, analysing includes hybridizing bacterial nucleic acid in the sample to beads or to an array, e.g. a nucleic acid microarray.

The PCR-based techniques are performed with amplification primers designed to be specific for the sequences which are measured. The present invention hence also pertains to a set of primers suitable for performing the above method, i.e., a set of primers comprising primer pairs for amplifying sequences specific for each of the microorganism species to be detected (i.e., at least one more species selected amongst those recited in Tables 1 and 2 and 3).

16S rDNA sequence is provided herein for B1-B15 and this can be used to generate primers for such an analysis. In one embodiment, a plurality of the bacteria is detected. In one embodiment, the sample is analysed for nucleic acid of the bacteria using genome sequencing.

In one embodiment, the subject is a cancer patient, such as melanoma patient. The cancer patient may or may not have received anti-cancer treatment. Thus, the subject may be one that is in need of treatment with an immune checkpoint inhibitor. In one embodiment, the subject is a healthy individual, for example a healthy individual with a family history of cancer, such as melanoma.

In one embodiment, if the subject is a cancer patient and has been identified as a subject that will respond to therapy with an immune checkpoint inhibitor, then the method may include the further step of administering an immune checkpoint inhibitor to said patient.

In one embodiment, the method also comprises the prior step of obtaining the biological sample that comprises gut flora.

In one embodiment, the method also includes the initial step of identifying a subject in need of treatment with the immune checkpoint inhibitor.

In one embodiment of the methods, if the subject is identified as a responder, e.g. if one or more of the bacteria listed in Table 1 has been shown to have an increased abundance in the sample, an anti-cancer therapy comprising an immune checkpoint inhibitor is administered to the subject.

Checkpoint inhibitors are as defined herein. In one embodiment of the methods, the immune checkpoint inhibitor inhibits PD-1 activity, i.e. acts as a PD-1 antagonist. In one embodiment of the methods, the immune checkpoint inhibitor inhibits PD-L1 activity, i.e. acts as a PD-L1 antagonist. In one embodiment of the methods, the immune checkpoint inhibitor inhibits CTLA-4 activity, i.e. acts as a CTLA-4 antagonist. In one embodiment of the methods, the immune checkpoint inhibitor inhibits LAG3, TIGIT or TIM3- activity.

In one embodiment the immune checkpoint inhibitor is an anti PD-1, PD-L1 or CTLA-4 antibody. In one embodiment, the anti PD-1 antibody is selected from nivolumab, pembrolizumab, cemiplimab, avelumab, durvalumab, atezolizumab, Spartalizumab, Camrelizumab, Sintilimab, Tislelizumab, Pidilizumab or Toripalimab, Ipilimumab or Tremelimumab.

In one embodiment, the immune checkpoint inhibitor is an interfering nucleic acid molecule. In one embodiment, the interfering nucleic acid molecule is an siRNA molecule, an shRNA molecule or an antisense RNA molecule.

In one embodiment, the immune checkpoint inhibitor is a small molecule or PROteolysis TArgeting Chimera (PROTAC) or another immune checkpoint inhibitor as defined above.

In one embodiment, in a further step of the method, surgical, radiation, and/or chemotherapeutic cancer intervention is carried out or a second anti-cancer therapeutic is administered to said subject.

In another aspect, the invention relates to a method of detecting the risk that a subject will not respond to therapy with an immune checkpoint inhibitor. The method comprising determining the abundance of one or more of the bacteria listed in Table 1 in a biological sample from said subject that comprises gut intestinal flora wherein a decrease in the abundance or an abundance below a reference level of one or more of the bacteria listed in Table 1 is indicative that the subject will not respond to therapy with an immune checkpoint inhibitor. The method may also comprise comparing the abundance of one or more of the bacteria listed in Table 1 to one or more reference value. A reference value is as described above. The abundance that is determined is relative abundance. In a further step, if the subject has been identified as a subject that will not respond to therapy with an immune checkpoint inhibitor, alternative anti-cancer treatment is administered. Alternatively, in a further step, if the subject has been identified as a subject that will not respond to therapy with an immune checkpoint inhibitor, a therapeutic bacterial composition as described herein is administered together with a checkpoint inhibitor therapy, e.g. an anti PD-1 therapy.

In another aspect, the invention relates to a method of discriminating between subjects that respond to therapy with an immune checkpoint inhibitor and subjects that do not respond to therapy with an immune checkpoint inhibitor. The method comprising determining the abundance of one or more of the bacteria listed in Table 1 in a biological sample from said subject that comprises gut intestinal flora wherein a decrease in the abundance or a abundance below a reference level of one or more of the bacteria listed in Table 1 is indicative that the subject will not respond to therapy with an immune checkpoint inhibitor and an increase in the abundance of one or more of the bacteria listed in Table 1 is indicative that the subject will respond to therapy with an immune checkpoint inhibitor. The method may also comprise comparing the abundance one or more of the bacteria listed in Table 1 to one or more reference value. A reference value is as described above. The abundance that is determined is relative abundance. In a further step, if the subject has been identified as a subject that will not respond to therapy with an immune checkpoint inhibitor, alternative anti-cancer treatment is administered.

In one embodiment of the biomarker methods above, modulated abundance of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 different bacteria selected from Table 1 is indicative of a response to treatment. In another embodiment of the biomarker methods above, modulated abundance of at least 9, 10, 11, 12, 13, 14 or 15 different bacteria selected from Table 1 is indicative of a response to treatment. Thus, establishing a composite signature that includes abundance of at least 9, 10, 11, 12, 13, 14 or 15 different bacteria is a particular embodiment of the methods. It is the totality of the bacteria, i.e. the biomarker signature, that provides a particularly powerful discriminatory tool.

In one embodiment of the various methods above, the abundance of at least 9 bacterial species / a population of 9 bacterial species selected from those in Table 1 is assessed, i.e. 9 species selected from SEQ ID Nos. 1, 2, 3, 4, 5 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15. In one embodiment, the subset of 9 corresponds to a consortium as shown in Table 3, i.e. consortia 2, 4, 5, 6 or 10. In one embodiment, the 9 species comprise bacteria as defined by SEQ ID NO. 1 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. In one embodiment, the 9 species comprise bacteria as defined by SEQ ID NO. 2 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. = In one embodiment, the 9 species comprise bacteria as defined by SEQ ID NO. 3 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. In one embodiment, the 9 species comprises bacteria as defined by SEQ ID NO. 4 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. In one embodiment, the 9 species comprise bacteria as defined by SEQ ID NO. 5 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. In one embodiment, the 9 species comprise bacteria as defined by SEQ ID NO. 6 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. In one embodiment, the 9 species comprise bacteria as defined by SEQ ID NO. 7 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. In one embodiment, the 9 species comprise bacteria as defined by SEQ ID NO. 8 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. In one embodiment, the 9 species comprise bacteria as defined by SEQ ID NO. 9 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. In one embodiment, the 9 species comprise bacteria as defined by SEQ ID NO. 10 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. In one embodiment, the 9 species comprise bacteria as defined by SEQ ID NO. 11 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. In one embodiment, the 9 species comprise bacteria as defined by SEQ ID NO. 12 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. In one embodiment, the 9 species comprise bacteria as defined by SEQ ID NO. 13 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. In one embodiment, the 9 species comprise bacteria as defined by SEQ ID NO. 14 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. In one embodiment, the 9 species comprise bacteria as defined by SEQ ID NO. 15 or a sequence with at least 97%, 98%, 98.7% or 99% sequence identity thereto. In one embodiment, the 9 species do not comprise an *Alistipes* species.

In one embodiment, the biomarker methods above may comprise a further step of determining another biomarker that is predictive of tumor response with an immune checkpoint inhibitor, for example a PD-1, PD-L1 or CTLA-4 antagonist. For example, the biomarker may be a Programmed Death Ligand 1 (PD-L1) or Programmed Death Ligand 1 (PD-L2) gene signature. Thus, the method may comprise the step of obtaining a sample from the tumor of a test subject, measuring RNA expression level in the tumor sample of one or more gene in a PD-1 and/or PD-L1 gene signature and comparing the RNA expression level to a reference level. Expression can be measured by any appropriate methods, including immunohistochemistry.

In another aspect, the invention relates to one or more of the bacteria listed in Table 1 for use as a predictive biomarker in determining the efficacy of therapeutic intervention with checkpoint inhibitor, e.g. PD-1 therapy. The term predictive biomarker as used herein is to describe a biomarker that gives information about the effect of a therapeutic intervention, i.e. responsiveness to treatment with an immune checkpoint inhibitor. Thus, the invention also relates to the use of one or more bacterium; e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 different bacteria selected from one or more of the bacterial species listed in Table 1 in identifying a patient that will respond to therapy with an immune checkpoint inhibitor.

The invention also relates to a biomarker signature, that is a consortium of one or more of the bacteria listed in Table 1, e.g. as in Table 3, which can be used to predict the efficacy of therapeutic intervention with checkpoint inhibitor therapy, e.g. with a PD-1 inhibitor or with another checkpoint inhibitor therapy.

Systems and computer readable media

Embodiments of the invention also provide for systems (and computer readable media for causing computer systems) to perform a method for determining responsiveness to treatment with an immune checkpoint inhibitor in a subject. In another aspect, a computer-implemented method is provided for indicating a likelihood that a subject responds to treatment with an immune checkpoint inhibitor. The method comprises: retrieving on a computer biomarker information for an individual, wherein the biomarker information comprises biomarker values that each correspond to the abundance of one or more bacteria selected from the group of bacteria set forth in Table 1; performing with the computer a classification of each of the biomarker values; and indicating a likelihood that the subject responds to treatment with an immune checkpoint inhibitor based upon a plurality of classifications.

In another aspect, a computer program product is provided for indicating a likelihood that a subject responds to treatment with an immune checkpoint inhibitor. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises biomarker values that each correspond to the abundance of one or more bacteria selected from the group of bacteria set forth in Table 1; and code that executes a classification method that indicates a likelihood that the individual responds to treatment with an immune checkpoint inhibitor as a function of the biomarker values.

In one embodiment the reference data stored in the storage device to be read by the comparison module is compared, e.g., relative abundance of a particular bacterium in a reference sample as described herein. The "comparison module" can use a variety of available software programs and formats for the comparison operative to compare bacteria abundance information data determined in

the determination system to reference samples and/or stored reference data, e.g. a predetermined threshold value. In one embodiment, the comparison module is configured to use pattern recognition techniques to compare information from one or more entries to one or more reference data patterns. The comparison module may be configured using existing commercially-available or freely- available software for comparing patterns and may be optimized for particular data comparisons that are conducted. The comparison module provides computer readable information related to the response-associated bacteria.

The comparison module provides a computer readable comparison result that can be processed in computer readable form by predefined criteria, or criteria defined by a user, to provide a content based in part on the comparison result that may be stored and output.

The methods described herein therefore provide for systems (and computer readable media for causing computer systems) to perform methods for determining responsiveness to treatment with an immune checkpoint inhibitor in a subject.

FMT

Implantation or administration of human microbiota into the bowel of a sick patient is called Faecal Microbiota Transplantation (FMT), also commonly known as faecal bacteriotherapy. FMT is believed to repopulate the gut with a diverse array of microbes that bring missing beneficial functions or microbiota to the resident gut bacteria, displace harmful microbiota or control key pathogens by creating an unfavourable ecological environment.

In another aspect, the invention relates to a method for screening/ identifying a faecal donor comprising assessing a faecal sample of a subject for the presence of one or more bacteria associated with response to cancer; e.g. response to cancer when a patient is treated with an immune checkpoint inhibitor and identifying the faecal donor based on the presence and/or abundance of one or more bacteria.

For example, in such a method, the one or more bacteria selected from Table 1 and the faecal donor is identified based on the presence and/or abundance of one or more bacteria selected from Table 1.

In another aspect, the invention relates to a method for screening/ identifying a faecal donor comprising assessing a faecal sample of a subject for the presence of one or more bacteria selected from Table 1 and identifying the faecal donor based on the presence and/or abundance of one or more bacteria selected from Table 1. The method may also comprise obtaining a faecal sample from a donor. Assessing a faecal sample of a subject for the presence of one or more bacteria can be done by methods known in the art, e.g. sequence analysis of bacterial genomes, e.g. using a shotgun

sequencing approach. For example, one or more of the bacteria is present above a predetermined threshold, the donor is selected as a donor for bacteriotherapy purposes. The predetermined threshold may be based on the average abundance of the one or more bacteria in faecal samples obtained from a donor population. A higher than average abundance indicates that the faeces are suitable for FMT therapy.

The invention also relates to a use of one or more bacteria selected from Table 1 in a method for identifying a donor for FMT therapy.

The invention relates to a method for treating a faecal transplant prior to administration to a subject comprising supplementing the faecal transplant with one or more bacterial isolates selected from Table 1 or with a faecal sample obtained from a donor by the method described above.

According to another aspect of the present invention, an individual in need of a treatment with an immune checkpoint inhibitor therapy is treated by FMT, using faecal microbiota from healthy individual(s) that has been shown to be abundant in one or more of the species in table 1, and/or faecal microbiota from one or several individual(s) treated with an immune checkpoint inhibitor therapy and who proved to respond to this therapy, and/or faecal microbiota from one or several individual(s) exhibiting a gut microbiota profile that identifies him/her/them as likely to respond to the envisioned treatment or from a responding patient.

In the aspects above, the FMT therapy is for the treatment of a disease as mentioned herein, e.g. a cancer such as melanoma.

Composition and methods for increasing abundance of bacteria in a host

In another aspect of the invention, a subject's microbiome may be altered to increase the abundance of bacteria listed in Table 1 or a subset thereof. Glycan metabolism has been shown to influence the human gut microbiota and prebiotics can enrich bacterial taxa that promote anti-tumor immunity (Koropatkin et al, Nature Reviews Microbiology volume 10, pages 323–335 (2012); Li et al, Cell Report, Volume 30, ISSUE 6, P1753-1766.e6, February 11, 2020). Thus, there is provided a method for increasing the abundance of bacteria listed in table 1 in a subject or a subset thereof by administration of a composition comprising oligosaccharides, such as glycans. Compositions comprising oligosaccharides, such as glycans for use in such a method are also envisaged.

Kits

In a further aspect, the invention relates to a kit. The kit includes a composition described herein and optionally an anti-cancer treatment that includes an immune checkpoint inhibitor as described herein.

In an example, the kit can include materials to ship the collected material without harming the samples (e.g., packaged in lyophilized form, or packaged in an aqueous medium etc.). The kit may include the processed material or treatment in a sterile container, such as a nasogastric (NG) tube, a vial (e.g., for use with a retention enema), a gastro-resistant capsule (e.g., acid-bio resistant to reach the intestinal tract, having a sterile outside), etc. The kit may also comprise instructions for use.

In an alternative aspect, the kit comprises

- a sealable container configured to receive a biological sample, such as a faecal sample;
- polynucleotide primers for amplifying a 16S rDNA polynucleotide sequence from at least one gut associated bacterium to form an amplified 16S rDNA polynucleotide sequence, wherein the amplified 16S rDNA sequence has at least 97%, 98%, 98.7% or 99% homology to a polynucleotide sequence selected from SEQ ID NOs 1 to SEQ ID NO 15, e.g. SEQ ID NOs 16 to 29;
- a detecting reagent to detect the amplified 16S rDNA sequence; and
- instructions for use.

The invention also relates to as kit comprising a composition comprising oligosaccharides, such as glycans for use in a method for increasing the abundance of bacteria listed in table 1 in a subject or a subset thereof by administration of the composition.

The invention also relates to the use of a composition of the invention, i.e. comprising or consisting of one or more a bacterial isolate as shown in Table 1 with reference to a SEQ ID NO. shown therein, in increasing efficacy of an anti-cancer treatment with an immune checkpoint inhibitor. The invention also relates to the use of a composition of the invention, i.e. comprising or consisting of one or more a bacterial isolate as shown in Table 1 with reference to a SEQ ID NO. as shown in the Table, in enhancing immune checkpoint blockade. The invention also relates to a method for increasing efficacy of an anti-cancer treatment with an immune checkpoint inhibitor comprising administering a composition of the invention, i.e. comprising or consisting of one or more bacterial isolate as shown in Table 1 with reference to a SEQ ID NO., to a subject. The invention also relates to a method for enhancing immune checkpoint blockade comprising administration of a composition of the invention, i.e. comprising or consisting of one or more bacterial isolate as shown in Table 1 with reference to a SEQ ID NO. as shown in the Table to a subject.

The invention also relates to the use of a composition of the invention in providing an immunostimulatory effect.

The invention also relates to a method for determining if a cancer patient needs a bacterial composition of the invention, i.e. comprising or consisting of one or more a bacterial isolate as shown in Table 1 with reference to a SEQ ID NO. as shown therein, before administration of an immune

checkpoint inhibitor comprising assessing, in a faeces sample from said patient, the presence or absence or one or more bacterial isolates selected from the species in Table 1.

Aspects

The invention is further described in the following aspects.

1. A composition comprising isolated bacteria selected from at least two species wherein the bacteria from the first species comprise a 16S rDNA sequence having at least 98.7% sequence identity with a nucleic acid sequence according to SEQ ID NO: 1, and the bacteria from the second species comprise a 16S rDNA sequence having at least 98.7% sequence identity with a nucleic acid sequence according to SEQ ID NO: 2.
2. The composition according to aspect 1, further comprising isolated bacteria from at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13 different species wherein the bacteria comprise a 16S rDNA sequence selected from a sequence having at least 98.7% sequence identity with a nucleic acid sequence according to SEQ ID NO: 3 to 15.
3. The composition according to aspect 1 further comprising isolated bacteria from at least 4 different species wherein the bacteria comprise a 16S rDNA sequence selected from a sequence having at least 98.7% sequence identity with a nucleic acid sequence according to SEQ ID NO: 3 to 15.
4. The composition according to aspect 1, further comprising isolated bacteria from at least 7 different species wherein the bacteria comprise a 16S rDNA sequence selected from a sequence having at least 98.7% sequence identity with a nucleic acid sequence according to SEQ ID NO: 3 to 15.
5. The composition according to aspect 1, further comprising a bacterial isolate comprising a 16S rDNA sequence selected from a sequence having at least 98.7% sequence identity with a nucleic acid sequence according to SEQ ID NO: 7.
6. The composition according to aspect 1 comprising a consortium selected from consortia 1 to 4 or 6 to 10 as shown in Table 3.
7. The composition according to any preceding aspect, wherein said composition is formulated for oral or rectal administration.
8. The composition according to aspect 7, wherein said composition is in the form of a capsule, tablet, gel or liquid.
9. The composition according to aspect 8, wherein said composition is encapsulated in an enteric coating.
10. The composition according to any preceding aspect, wherein the composition comprises live, attenuated or killed bacteria.
11. The composition according to any preceding aspect, wherein the composition comprises bacterial spores.

12. The composition according to any of aspects 1 to 11, wherein the composition does not comprise bacterial spores.
13. The composition according to any preceding aspect, wherein the composition comprises bacterial strains that originate from one or more human donor.
14. The composition according to any preceding aspect, wherein the bacteria are lyophilized.
15. The composition according to any preceding aspect, wherein the composition comprises at least about $1x10^3$ to $1x10^{13}$ CFU of bacteria.
16. The composition according to any preceding aspect, wherein administration of the composition induces an immune response in a subject and/or increases the efficacy of an anti cancer therapy that includes an immune checkpoint inhibitor.
17. A pharmaceutical composition comprising a composition of any of aspects 1 to 16 and a pharmaceutical carrier.
18. The pharmaceutical composition according to aspect 17, further comprising an effective amount of an immune checkpoint inhibitor or a vaccine.
19. The pharmaceutical composition according to aspect 18, wherein the immune checkpoint inhibitor inhibits PD-1, PDL-1, CTLA-4, LAG3 or TIM-3 activity.
20. The pharmaceutical composition according to aspect 19 wherein the immune checkpoint inhibitor is an anti PD-1, PDL-1 or CTLA-4 antibody or fragment thereof.
21. The pharmaceutical composition according to aspect 20 wherein the anti PD-1, PDL-1 or CTLA4 antibody is selected from nivolumab, pembrolizumab, cemiplimab, avelumab, durvalumab, atezolizumab, spartalizumab, camrelizumab, sintilimab, tislelizumab, pidilizumab toripalimab, Ipilimumab or Tremelimumab.
22. The pharmaceutical composition according to aspect 18, wherein the immune checkpoint inhibitor is an interfering nucleic acid molecule, a small molecule or PROteolysis TArgeting Chimera (PROTAC), alternative protein scaffold or other immune checkpoint inhibitor.
23. The pharmaceutical composition according to aspect 22, wherein the interfering nucleic acid molecule is an siRNA molecule, an shRNA molecule or an antisense RNA molecule.
24. A composition according to any of aspects 1 to 16, or a pharmaceutical composition of any of aspects 17 to 23 for use in the treatment of disease.
25. A composition according to any of aspects 1 to 16, or a pharmaceutical composition of any of aspects 17 to 23 for use in the treatment of cancer or an infectious disease or for use as a vaccine adjuvant or for increasing the efficacy of a cancer treatment.
26. A method for treating cancer or an infectious disease in a subject in need thereof, comprising administering a composition according to any of aspects 1 to 17 or a pharmaceutical composition of aspect 17, to said subject.
27. The method of aspect 26 wherein said subject is receiving, has received or will receive therapy with an immune checkpoint inhibitor, thereby treating the cancer or infectious disease.
28. A method for treating cancer in a subject in need thereof comprising administering a composition according to aspect 18 to said subject.

29. The method according to aspect 26 or 27, wherein administration of the composition enhances an immune response by the subject and/or inhibits immune evasion by the cancer and/or increases efficacy of an anti cancer treatment with an immune checkpoint inhibitor.
30. The composition for use according to aspect 25, or the method according to any of aspects 26 to 29 wherein the cancer is selected from melanoma melanoma, such as Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, nodular melanoma, subungual melanoma, Cutaneous melanoma, uveal/intraocular melanoma and superficial spreading melanoma or bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, breast cancer, brain cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, kidney cancer, sarcoma of soft tissue, cancer of the urethra, cancer of the bladder, renal cancer, lung cancer, non-small cell lung cancer, thymoma, urothelial carcinoma leukemia, prostate cancer, mesothelioma, adrenocortical carcinoma, lymphomas, such as such as Hodgkin's disease, non-Hodgkin's, gastric cancer, and multiple myelomas.
31. The composition for use according to aspect 25 or the method according to any of aspects 26 to 29, wherein the composition or pharmaceutical composition is administered by oral administration or rectal administration.
32. The composition for use according to aspect 25 or the method according to any of aspects 26 to 29, wherein said subject has received prior anti cancer therapy with an immune checkpoint inhibitor.
33. The composition for use according to aspect 25 or the method according to any of aspects 26 or 29 further comprising administering an anti cancer therapy with an immune checkpoint inhibitor.
34. The composition for use or method according to aspect 33, wherein the immune checkpoint inhibitor is administered before, after or at the same time as the bacterial formulation.
35. The composition for use or method according to aspect 33 or 34, wherein the immune checkpoint inhibitor is administered by injection.
36. The composition for use or method according to any of aspects 33 to 35, wherein the injection is an intravenous, intramuscular, intratumoural or subcutaneous injection.
37. The composition for use or method according to any of aspects 33 to 36 wherein the immune checkpoint inhibitor inhibits PD-1, PDL-1 or CTLA-4 activity.
38. The composition for use or method according to aspect 37, wherein the immune checkpoint inhibitor is an anti PD-1, PDL-1 or CTLA-4 antibody.

39. The composition for use method according to aspect 38, wherein the anti PD-1, PDL-1 or CTLA4 antibody is selected from nivolumab, pembrolizumab, cemiplimab, avelumab, durvalumab, atezolizumab, Spartalizumab, Camrelizumab, Sintilimab, Tislelizumab, Pidilizumab, Toripalimab, Ipilimumab or Tremelimumab.
40. The composition for use or method according to any of aspects 33 to 37, wherein the immune checkpoint inhibitor is an interfering nucleic acid molecule, a small molecule or PROteolysis TArgeting Chimera (PROTAC) or other immune checkpoint inhibitor.
41. The composition for use or method according to aspect 40, wherein the interfering nucleic acid molecule is an siRNA molecule, an shRNA molecule or an antisense RNA molecule or a small molecule or peptide.
42. The composition for use according to aspect 25 or 30 to 41, or the method according to any of aspects 26 to 41, further comprising surgical, radiation, and/or chemotherapeutic cancer intervention or administration of a second anti cancer therapeutic.
43. The composition for according to aspect 25 or 30 to 42 or method according to any of aspects 26 to 42, further comprising administering to the subject an antibiotic.
44. The composition for use according to aspect 25 or 30 to 43 or method according to any of aspects 26 to 43, wherein the subject is identified as at risk of developing a cancer.
45. A kit comprising a composition according to any of aspects 1 to 17, and optionally an anti cancer treatment that includes an immune checkpoint inhibitor.
46. A food product or a vaccine adjuvant comprising the composition of any of aspects 1 to 17.
47. A method for treating faecal transplant prior to administration to a subject comprising supplementing the faecal transplant with isolated bacteria selected from at least two species wherein the bacteria from the first species comprise a 16S rDNA sequence having at least 98.7% sequence identity with a nucleic acid sequence according to SEQ ID NO: 1, and the bacteria from the second species comprise a 16S rDNA sequence having at least 98.7% sequence identity with a nucleic acid sequence according to SEQ ID NO: 2.
48. A use of a composition of any of aspects 1 to 17 or a pharmaceutical composition of aspect 18, in increasing efficacy of an anti cancer treatment with an immune checkpoint inhibitor.
49. A use of a composition of any of aspects 1 to 17 or a pharmaceutical composition of aspect 18, in enhancing immune checkpoint blockade.
50. A method for enhancing immune checkpoint blockade comprising administering a composition of any of aspects 1 to 17 or a pharmaceutical composition of aspect 18.
51. A composition comprising a bacterium selected from one or more bacteria selected from Table 1.
52. A method for treating or preventing cancer comprising modulating the level of one or more bacteria selected from those of Table 1 in a subject.

The invention is also further described in the following additional aspects.

1. A method for identifying a subject that will respond to therapy with an immune checkpoint inhibitor comprising determining the abundance of bacteria from at least 9 different species in a biological sample from said subject that comprises gut flora wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15 wherein said abundance is indicative of a response of a subject to therapy with an immune checkpoint inhibitor.
2. The method for identifying a subject that will respond to therapy with an immune checkpoint inhibitor according to aspect 1, the method comprising:

   a) determining the abundance of the bacteria in a biological sample obtained from the subject and

   b) comparing the abundance to a reference level from cancer patients that do not respond to therapy with an immune checkpoint inhibitor or cancer patients that respond to therapy with an immune checkpoint inhibitor;

   wherein if the reference level is from patients that do not respond to therapy with an immune checkpoint inhibitor, then an increase in the abundance of each of the bacteria compared to the reference level, is indicative that the subject will respond to therapy with an immune checkpoint inhibitor or

   wherein if the reference level is from patients that do respond to therapy with an immune checkpoint inhibitor, then the same or substantially the same or an increase in abundance of each of the bacteria, is indicative that the subject will respond to therapy with an immune checkpoint inhibitor.
3. The method for identifying a subject that will respond to therapy with an immune checkpoint inhibitor according to aspect 1, the method comprising:

   a) determining the abundance of the bacteria in a biological sample obtained from the subject;

   b) comparing the abundance to a reference level from cancer patients and

   c) applying random forest analysis.
4. The method according to a preceding aspect, wherein the bacterial species comprise a 16S rDNA sequence selected from SEQ ID NO: 1 or 2 or a 16S rDNA sequence having at least 98.7% sequence identity thereto.
5. The method according to a preceding aspect, wherein the method comprises determining the abundance of bacteria from 10, 11, 12, 13, 14 or 15 species wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15.
6. The method according to a preceding aspect wherein said subject is a cancer patient.
7. The method according to aspect 6, wherein the cancer is selected from melanoma, bone cancer, pancreatic cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach

cancer, testicular cancer, breast cancer, brain cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, kidney cancer, sarcoma of soft tissue, cancer of the urethra, cancer of the bladder, renal cancer, lung cancer, non-small cell lung cancer, thymoma, urothelial carcinoma leukemia, prostate cancer, mesothelioma, adrenocortical carcinoma, lymphomas, such as such as Hodgkin's disease, non-Hodgkin's, gastric cancer, and multiple myelomas.

8. The method according to aspect 7, wherein the melanoma is selected from Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, nodular melanoma, subungual melanoma, Cutaneous melanoma, uveal/intraocular melanoma and superficial spreading melanoma.
9. The method according to a preceding aspect, further comprising the step of identifying a subject in need of treatment with the immune checkpoint inhibitor.
10. The method according to a preceding aspect, further comprising administering an immune checkpoint inhibitor to said subject.
11. The method according to a preceding aspect wherein the immune checkpoint inhibitor inhibits PD-1, PD-L1 or CTLA-4 activity.
12. The method according to aspect 11, wherein the immune checkpoint inhibitor is an anti PD-1, PDL-1 or CTLA-4 antibody.
13. The method according to aspect 12, wherein the anti PD-1, PDL-1 or CTLA-4 antibody is selected from nivolumab, pembrolizumab, cemiplimab, avelumab, durvalumab, atezolizumab, Spartalizumab, Camrelizumab, Sintilimab, Tislelizumab, Pidilizumab, Toripalimab, Ipilimumab or Tremelimumab.
14. The method according to any of aspects 1 to 11, wherein the immune checkpoint inhibitor is an interfering nucleic acid molecule, a small molecule or a PROteolysis TArgeting Chimera (PROTAC) or other immune checkpoint inhibitor.
15. The method according to aspect 14, wherein the interfering nucleic acid molecule is an siRNA molecule, an shRNA molecule or an antisense RNA molecule or a small molecule or peptide.
16. The method according to a preceding aspect, wherein the abundance is the abundance of the bacteria in the sample as a proportion of the total microbiota in the sample.
17. The method according to a preceding aspect, further comprising the step of obtaining a biological sample that comprises gut flora from said subject.
18. The method according to a preceding aspect, wherein the sample is a faecal sample.
19. Use of bacteria selected from at least 9 different bacterial species wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15 in identifying a patient that will respond to therapy with an immune checkpoint inhibitor.

20. A kit comprising

    a sealable container configured to receive a biological sample;

    polynucleotide primers for amplifying a 16S rDNA polynucleotide sequence from at least 9 different bacterial species wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15;

    a detecting reagent to detect the amplified 16S rDNA sequence; and

    instructions for use.

21. A method for identifying a faecal donor comprising assessing a faecal sample of a subject for the presence of bacteria from at least 9 different bacterial species wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15 and identifying the faecal donor based on the presence and/or abundance of the bacteria.

22. The use of bacteria from at least 9 different bacterial species wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15 in a method for identifying a donor for FMT therapy.

23. A method for determining if a cancer patient needs a bacterial compensation before administration of an immune checkpoint inhibitor comprising assessing, in a faeces sample from said patient, the presence or absence of bacteria from at least 9 different bacterial species wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15.

24. A method for predicting a response to an immune checkpoint inhibitor therapy in a subject having cancer comprising determining the abundance of bacteria from at least 9 different species wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15 in a biological sample from said subject that comprises gut flora wherein said abundance is indicative of a response or non-response of a subject to therapy with an immune checkpoint inhibitor.

25. A method for predicting a response to an immune checkpoint inhibitor therapy in a subject having cancer according to aspect 24, the method comprising:

    a) determining the abundance of the bacteria a biological sample obtained from the subject;

    b) comparing the abundance to a reference level from patients that do not respond to immune checkpoint inhibitor therapy; or patients that respond to immune checkpoint inhibitor therapy;

    wherein if the reference level is from patients that do not respond to immune checkpoint inhibitor therapy, then an increase in the abundance of each of the bacteria compared to the reference level, is indicative that the subject will respond to therapy with an immune checkpoint inhibitor or

wherein if the reference level is from patients that do respond to immune checkpoint inhibitor therapy, then the same or substantially the same or an increase in abundance of each of the bacteria, is indicative that the subject will respond to therapy with an immune checkpoint inhibitor.

26. The method according to aspect 24, the method comprising:
    a) determining the abundance of the bacteria a biological sample obtained from the subject and;
    b) comparing the abundance to a reference level from cancer patients or healthy subjects and
    c) applying random forest analysis.
27. The method according to any of aspects 24 to 26, the method comprising the step of predicting a response.
28. The method according to any of aspects 24 to 27, where if the subject is predicted to be a non-responder, an anti cancer therapy is administered which is not an immune checkpoint inhibitor.
29. The method according to any of aspects 24 to 28, where if the subject is predicted to be a non-responder, a composition comprising isolated bacteria from one or more species is administered wherein the bacteria comprise a sequence selected from SEQ ID 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15.
30. A method according to aspect 29, where if the subject is predicted to be a non-responder, a composition comprising isolated bacteria selected from at least two species is administered wherein the bacteria from the first species comprise a 16S rDNA sequence having at least 98.7% sequence identity with a nucleic acid sequence according to SEQ ID NO: 1, and the bacteria from the second species comprise a 16S rDNA sequence having at least 98.7% sequence identity with a nucleic acid sequence according to SEQ ID NO: 2.
31. A method according to aspect 29 or 31, wherein the composition further comprises isolated bacteria from at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13 different species wherein the bacteria comprise a 16S rDNA sequence selected from a sequence having at least 98.7% sequence identity with a nucleic acid sequence according to SEQ ID NO: 3 to 15.
32. The method according to any of aspects 24 to 27, where if the subject is predicted to be a responder, an immune checkpoint inhibitor therapy is administered.

Further aspects and embodiments of the invention will be apparent to those skilled in the art given the present disclosure including the following experimental exemplification.

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. While the foregoing disclosure provides a general description of the subject matter encompassed

within the scope of the present invention, including methods, as well as the best mode thereof, of making and using this invention, the following examples are provided to further enable those skilled in the art to practice this invention and to provide a complete written description thereof. However, those skilled in the art will appreciate that the specifics of these examples should not be read as limiting on the invention, the scope of which should be apprehended from the claims and equivalents thereof appended to this disclosure. Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

All documents mentioned in this specification are incorporated herein by reference in their entirety, including any references to gene accession numbers and references to patent publications.

"and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example, "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein. Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

The invention is further described in the non-limiting examples.

Example 1 Identification of gut bacteria and isolates driving response to immunotherapy

The inventors have analysed how the microbiome of melanoma patients impacts response to immune checkpoint inhibitor therapy in the MELRESIST study. This study was conducted at Cambridge University Hospitals and was performed with the best standards in sample collection and processing. The study involved 69 patients many of which had longitudinal faecal sampling. The inventors analysed the relative abundance of gut bacteria in the baseline MELRESIST faecal samples by performing shotgun metagenomic sequencing. The metagenomic sequencing was analysed using a comprehensive and highly curated reference genome database primarily built on reference-quality genomes from cultured isolates. This reference-based metagenomic analysis gives highly sensitive and accurate identification of bacteria (Forster et al Nat Biotechnol. 2019; 37: 186). To support this analysis, the inventors re-analysed three additional shotgun metagenomic datasets from melanoma patients about to receive immune checkpoint inhibitor therapy using the same analysis platform.

The microbiome was examined by machine learning approaches to select the specific bacterial species most predictive of response to immune checkpoint inhibitor therapy. For the first time in the field, a consistent microbiome signature associated with and highly predictive of response across multiple studies was identified. The size and quality of the MELRESIST dataset, the comprehensive and accurate identification of bacteria by reference-based metagenomic analysis and machine

learning analysis all contributed to the discovery of this cross-study microbiome signature. The signature also further validates the central importance of the gut microbiome as a primary driver of immune checkpoint inhibitor response. This provides the basis for both a predictive biomarker and Live Bacterial Therapeutic co-therapy to increase the proportion of patients responding to checkpoint inhibitors. Using feature reduction steps, this microbiome signature was reduced to small consortia of bacteria comprising species more abundant in patients that response to immunotherapy. These smaller consortia are still predictive of response across studies, so can act as a biomarker. In addition, these consortia can form a live bacterial therapeutic for the co-administration with immune checkpoint inhibitors in the treatment of cancer.

This analysis enabled the identification of strains isolates representing thirteen species in the consortia. Dendritic cells stimulated with these strains, individually or as consortia of up to nine, potently activated Cytotoxic T Lymphocytes. Two consortia of nine were also tested in a syngeneic mouse model of cancer and both consortia demonstrated tumour growth inhibition. These results validate the bacteria as drivers of anti-tumour response.

1.1 Discovery based on MELRESIST clinical study

MELRESIST is a study performed at Cambridge University Hospitals in which 69 advanced melanoma patients gave a faecal sample prior to and/or following treatment with anti-PD1 based immunotherapy. Complete clinical metadata, including response to therapy, antibiotic use and toxicities, was also recorded. A rigorous sample collection protocol was used to ensure the highest possible standards. The DNA was extracted in a single batch at Microbiotica, and shotgun metagenomics performed. Shotgun metagnomics sequencing is well known in the art and for example described in Quince, C. et al, Shotgun metagenomics, from sampling to analysis. Nat Biotechnol 35, 833–844 (2017).

Reference-based metagenomics was used to analysis the sequences of the baseline stool samples to give more sensitive and specific identification of bacteria. The accuracy is further improved by a bioinformatic tool to mask mobile elements thereby reducing spurious signals caused by horizontal gene transfer. Suitable methods are also described in WO2020065347 incorporated by reference. Additional classification filtering removes mis-assigned reads caused by contamination and gene duplication. The platform can accurately classify over 95% of the metagenomic reads leading to a precise mapping of the abundance of almost every bacterium in the sample.
To support and validate the analysis, three additional datasets from melanoma patients about to undergo immune checkpoint inhibitor therapy were reanalysed using the Microbiotica high-precision platform. These were:

- Frankel *Neoplasia* (2017) 19:848, Advanced melanoma, 39 patients

- Gopalakrishnan et al and Wargo *Science* (2018) 359:97, Metastatic melanoma, 25 patients (referenced as Wargo in figures)
- Matson et al and Gajewski *Science* (2018) 359:104, Metastatic melanoma, 39 patients (referenced as Gajewski in figures)

1.2 Bioinformatic analysis to derive microbiome-signatures predictive of response to immunotherapy

The baseline samples from MELRESIST were used to define a signature of response by linking the relative abundance of each bacteria in a sample to the clinical outcome data. In the primary analysis stable disease, partial response and complete response at 6 months were all determined to be a response and progressive disease was considered non-response. Machine learning approaches, including Random Forest models, were used to select species providing the most power as part of a signature to predict response.

The random forest classifier is an algorithm based on the results of many decision trees. In a single decision tree, features are selected iteratively that best separate samples into responder and non-responder categories, until all features are utilized. In the case of prevalence data, these features could be presence or absence of a given species, where presence of a single species might be preferentially associated with responder samples, or vice versa. Alternatively, relative abundance of a given species might be predictive of response, in which it could be either more or less abundant in responder samples. Since a single decision tree typically overfits data and does not produce robust results, random forests are often used instead. A random forest classifier is based on many different decision trees, where each tree only uses a subset of the available data, for example randomly leaving out 20% of the observed species for each tree. In some cases, a subset of the samples is used for training the random forest. The random forest classifier thus learns which signals are strongest across all possible features and samples. For all random forest models, out-of-bag error was used to prevent overoptimistic performance and improve generalizability.

The inventors expanded the analysis by including the additional melanoma datasets to identify the bacteria linked to response across multiple studies. First, the data from the different studies was standardised, for example the response criteria was changed to be consistent with the MELRESIST study where necessary. A signature was then generated using the machine learning process on the combined dataset of all four melanoma datasets. The ability of this signature to function as a biomarker was then tested on the combined dataset, and it predicted whether a patient would respond to therapy with an accuracy of 91% (Figure 1A). The Receiver Operating Characteristic (ROC) curve of this analysis gave an area under the curve (AUC) of 0.98 (Figure 1C) thereby confirming how highly predictive this signature is. Importantly, the signature was 83-100% accurate when tested against the studies individually (Figure 1B), and the ROC curves gave AUCs from 0.96 to 1 (Figure 1D). This is

the first demonstration of a microbiome based predictive biomarker that accurately predicts response across studies.

To progress the signature as a biomarker and select bacteria for inclusion in a Live Bacterial Therapeutic, the inventors identified the bacteria most robustly associated with response. The species that were consistently increased in abundance in responding patients from three or all four studies were selected to be advanced. Subsequently, a filtering step was applied to choose the bacteria with the cleanest signal by excluding species where the metagenomic reads did not broadly and evenly cover the genome.

The entire analysis was repeated from the start but excluding patients with stable disease, where possible, to focus on bacteria linked to a better clinical response. This reanalysis overlapped considerably with the first thereby validating it and was used to refine the final list of species. These analyses produced a list of 15 bacterial species, consortium 1, all increased in abundance in melanoma patients that subsequently responded to immune checkpoint inhibitor therapy across multiple studies (see Table 1 and 3). The robustness of this reduced signature was demonstrated by repeating the test as a biomarker in the combined dataset, and it predicted whether a patient would respond to therapy with an accuracy of 77% (Figure 2A). The Receiver Operating Characteristic (ROC) curve of this analysis gave an area under the curve (AUC) of 0.8 (Figure 2C) thereby confirming how highly predictive this signature is. Importantly, the signature was 67-84% accurate when tested against the studies individually (Figure 2B), and the ROC gave AUCs from 0.73 to 0.88 (Figure 2D). Six additional consortia 2, 3, 4, 5, 6 and 10 composed of 9 or 12 species (Table 3) from the 15 were also tested as biomarkers, and had good predictivity of response both in the combined dataset and the individual studies (Figures 3-7 and 17).

Thus, the results show that the bacteria identified can be used as predictive biomarker for response to anti-PD1 therapy in melanoma patients and also as a bacterial co-therapy to increase the proportion of melanoma patients responding to checkpoint inhibitors.

To understand if the bacteria could have utility in other cancer indications where checkpoint inhibitors are used, the inventors analysed the predictive value of the full signature in a Non-Small Cell Lung Cancer (NSCLC) patient cohort (Routy et al 2018 Science 359:91-97). The study sampled patients stool prior to anti-PD1 based therapy, and subjected it to shotgun metagenomic sequencing. This was reanalysed using the Microbiotica high-precision platform. The fifteen species in consortium 1 were predictive of whether NSCLC patients would respond to anti-PD1 therapy (ROC AUC= 0.722; Figure 8). Therefore, the bacteria described herein and discovered in melanoma patients are also linked to response in NSCLC. This shows that the bacteria described herein can be used as predictive biomarkers in another cancer indication. Moreover, this also suggests that the bacteria described herein can be used as a bacterial co-therapy in other cancer indications.

1.3 Selection of Bacterial Isolates

The reference-based metagenomic analysis using genomes from cultured isolates enables the identified bacteria to be linked back to isolates of the specific strains and/or closely related strains in the associated culture collection. All available strains representing the species in table 1 underwent in silico characterisation to select for strains with a desirable developability and safety profile. The primary selection criteria consisted of anti-microbial resistance, bacteriophage production, and sporulation. Strains with a good profile were selected for further testing. These were expanded, cell banks generated, and growth characterised to enable testing in in vitro assays and in vivo models. In addition, each strain has undergone full developability and safety testing by laboratory testing and in silico analysis. For each genome assembly, 16S rDNA regions were identified in two ways. Firstly, using barrnap (https://github.com/tseemann/barrnap), and secondly by extracting, *in silico*, sequences of the desired length (between 1200 and 1800 bp) by searching for DNA matches to the 7F (5'-AGAGTTTGATYMTGGCTCAG-3') (SEQ ID NO. 30) 1510R (5'-ACGGYTACCTTGTTACGACTT-3') (SEQ ID NO. 31) universal 16S primers. Where multiple overlapping 16S sequences were extracted from an assembly, the longest was retained.

1.4 Host interaction

The lead bacteria have been selected based on a strong association with clinical response across multiple studies, and, therefore, are considered suitable candidates for inclusion in a Live Bacterial Therapeutic. To understand the mechanism of action, the bacteria have been profiled individually, as a complete consortium and as sub-consortia in several in vitro assays with human cells. Cytotoxic T Lymphocytes (CTLs) are a significant effector cell in anti-tumour immune responses by directly lysing tumour cells via granzyme B and perforin release and production of cytokines such as IFNγ. CTLs can express co-stimulatory and co-inhibitory receptors. Immune checkpoint inhibitor therapies block the suppression of CTL activity by blocking the interaction between co-inhibitory receptor (eg PD-1) and their ligands (eg PD-L1). The later can be expressed by tumour cells as a mechanism to escape immune-mediated depletion, which is reversed by checkpoint inhibitor therapy. CTLs are activated and educated by dendritic cells, which are a sentinel innate immune cells that have many receptors to sense and respond to bacteria. Therefore, the bacteria identified (individually or as consortia) were tested for an ability to stimulate dendritic cells (DCs), and then if these DCs activate CTLs.

Bacterial strains representing thirteen of the fifteen species were identified and grown in bacterial media. These were washed and added in co-cultured with human monocyte-derived DCs in anaerobic conditions. Antibiotics were then added and the DCs cultured in an aerobic environment. The activation of DCs was measured by upregulation of the maturation markers CD86 (a co-stimulatory ligand) and CD83. Eleven of the thirteen species robustly induced expression of both markers with

Gordonibacter urolithinfaciens and *Alistipes indictincus* being poor activators of DC maturation (Figure 9A and 9B). Indeed, many induced a similar level of CD86 and CD83 express as the positive controls, lipopolysaccharide (LPS), Poly I:C and *Salmonella typhimurium*, all of which are known to be very potent activators of DCs. Two consortia of nine species (Consortia 5 and 6), as well as sub-consortia of consortium 6 containing six, three and two species all also triggered DC maturation as measured by CD86 and CD83 upregulation (Figure 9B-9E). The bacteria also triggered cytokine release from the DCs. IL-12 is a very important cytokine for the priming of a CTL response, and IL-10 is associated with suppression of T cell response. Therefore, the ratio of IL-12 to IL-10 was used to measure whether the DCs could be a strong inducer of a positive CTL response. The nine of the ten species tested and Consortia 5 and 6 triggered higher levels of IL-12 than IL-10 even when compared to strong inflammatory stimuli like LPS and Poly I:C (Figure 9G). The data from *Gordonibacter urolithinfaciens* is not shown because the levels of cytokines released was too low to make a ratio meaningful. These data indicate that the bacteria identified as being associated with response to immune checkpoint inhibitor therapy are by enlarge potent activators of DC maturation, and release cytokines that could direct an enhanced T cell response.

To understand how effective these DCs were at stimulating CTLs, the mature DCs were co-cultured with allogenic CD8 expressing T cells (CTLs) for 6 days. CTLs activation was quantified by upregulation of Granzyme B, perforin and IFNγ. Thirteen of the bacterial species were tested, and all were shown to induce DCs that can potentially activate CTLs (Figure 10). The level of activation was comparable to or better than the strong inflammatory stimuli LPS, Poly I:C and *Salmonella typhimurium*. Interesting, even *Gordonibacter urolithinfaciens* and *Alistipes indictincus* were shown to lead to robust CTLs activation despite being poor stimulators of CD86 and CD83 expression by DCs. The consortia tested (5, 6,7, 8 and 9) also all induced strong CTL activation (Figure 11 and 12). These data demonstrate that the bacteria identified as being associated with response to immune checkpoint inhibitor therapy are potent activators of a CTL response via stimulation of DCs. This is true of the individual species and of consortia of two to nine species. This induction of CTL activation could be a key mechanism by which the raised abundance of these bacteria leads to enhanced anti-tumour immunity in the presence of anti-PD1. It could also indicate that a therapeutic composition comprising these bacteria could enhance a vaccine response and/or anti-viral immunity.

The bacteria lead to potent CTL activation, so their ability to kill tumour cells was tested next. In this assay, the CTLs activated by bacteria-stimulated DCs were co-cultured with the tumour cell line SKOV-3 cells. All ten of the species tested led to potent cytolysis of the tumour cells by CTLs as measured by a decrease in electric impedance. The level of tumour cell killing compared favourably to the other known strong innate stimuli. The consortia tested (5, 6, 7, 8 and 9) also led to high levels of tumour cell killing (Figures 13 and 14). These data demonstrate that the bacteria identified as being associated with response to immune checkpoint inhibitor therapy are potent activators of an immune-

mediated tumour cell killing. This is true of the individual species tested and of consortia of two to nine species.

In total, the above data show that the bacterial species identified as being associated anti-PD-1 response are able to stimulate DCs to trigger CTLs activation and tumour cell killing. This mechanism is likely to explain, at least in part, why these bacteria are associated with response to anti-PD-1 based therapy in melanoma. This mechanism is associated with immune checkpoint inhibitor efficacy in multiple tumours indicating that the bacteria described herein can be used as a bacterial co-therapy in other cancer indications. Indeed, the bacteria described herein are likely to be an effective co-therapy with any immunotherapy that enhances CTL response for example adoptive T cell transfer therapy and CAR-T cell therapy. Interestingly, two of the strains tested (*Gordonibacter urolithinfaciens* and *Alistipes indictincus*) did not induce classical markers of DC activation (CD86 and CD83), but the DCs still induced CTL activation.

The type 1 interferons (IFNs), IFNα and IFNβ, are potent inducers of CTL immunity and can have direct anti-tumour effects. Plasmacytoid dendritic cells are capable of producing very high levels of IFNα and IFNβ. To test if the isolated bacteria associated with anti-PD-1 response induced IFNα release, plasmacytoid dendritic cells were stimulated with strains representing nine of the species. Plasmacytoid dendritic cells did not tolerate anaerobic conditions, so heat-killed bacteria were used in an aerobic environment. Seven of the nine strain induced IFNα release from plasmacytoid DCs (Figure 15). This could be another potential mechanism by which these bacteria enhance anti-tumour immune responses. Interesting a tonic type 1 interferon signal from the microbiome has been implicated in enhancing anti-viral responses in the lung (Bradley et al 2019 Cell Reports 28:245-256). Therefore, the species identified may also potential drive an anti-viral response and/or anti-viral vaccine efficacy.

In addition, to the above mechanistic assays two selected consortia were tested for efficacy in a syngeneic model of cancer. SPF mice were treated with antibiotics before engrafting the microbiome of a melanoma patient one day prior to implanting MCA205 tumour cells. Dosing consortium 5 or 6 by oral gavage induce tumour growth inhibition (Figure 16) although not to the same degree as the positive control (anti-PD1). This shows the anti-tumour potential of these consortia, and validates the selection of these species by association with improved clinical outcome. MCA-205 is a fibrosarcoma cell lines, which further demonstrates that the bacteria described herein have potential in cancer indications beyond melanoma. Together the data presented here shows we have identified bacterial species predictive of response to checkpoint inhibitor therapy in multiple melanoma studies and in NSCLC. These species are able to stimulate DCs leading to the activation of CTLs and tumour cell killing. Two consortia of these species are further validated in an in vivo cancer model.

Table 2 Sequences

SEQ ID	16S rDNA Sequence
SEQ ID No. 1	AGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAAGT CGAACGGAGTTATGCAGAGGAAGTTTTCGGATGGAATCGGCGTAACTTAGTGGCGGA CGGGTGAGTAACGCGTGGGAAACCTGCCCTGTACCGGGGGATAACACTTAGAAATAG GTGCTAATACCGCATAAGCGCACAGCTTCACATGAGGCAGTGTGAAAAACTCCGGTG GTACAGGATGGTCCCGCGTCTGATTAGCCAGTTGGCAGGGTAACGGCCTACCAAAGC GACGATCAGTAGCCGGCCTGAGAGGGTGAACGGCCACATTGGGACTGAGACACGGC CCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGAT GCAGCGACGCCGCGTGAGTGAAGAAGTATTTCGGTATGTAAAGCTCTATCAGCAGGG AAGAAAATGACGGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCG GTAATACGTAGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGTAGAC GGCATGACAAGCCAGATGTGAAAACCCAGGGCTCAACCCTGGGACTGCATTTGGAAC TGCCAGGCTGGAGTGCAGGAGAGGTAAGCGGAATTCCTAGTGTAGCGGTGAAATGCG TAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTACTGGACTGTAACTGACGTT GAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCGGTA AACGATGATTGCTAGGTGTAGGTGGGTATGGACCCATCGGTGCCGCAGCTAACGCAA TAAGCAATCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGG GACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC CAAGTCTTGACATCCCAATGACGTGTCCGTAACGGGGCATTCTCTTCGGAGCATTGGA GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG CAACGAGCGCAACCCTTATCCTTAGTAGCCAGCAGGTAGAGCTGGGCACTCTAGGGA GACTGCCGGGGATAACCCGGAGGAAGGCGGGGATGACGTCAAATCATCATGCCCCTT ATGATTTGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCGAGACAGTGAT GTTGAGCAAATCCCAGAAATAACGTCTCAGTTCGGATTGTAGTCTGCAACTCGACTAC ATGAAGCTGGAATCGCTAGTAATCGCGAATCAGCATGTCGCGGTGAATACGTTCCCGG GTCTTGTACACACCGCCCGTCACACCATGGGAGTTGGAAATGCCCGAAGCCTGTGAC CTAACCGCAAGGGAGGAGCAGTCGAAGGCAGGTCTAATAACTGGGGTGAAGTCGTAA CAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID No. 2	TTTAGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAA GTCGAACGGAGTTATTTTGGAAATCTCTTCGGGGATGGAATTCATAACTTAGTGGCGG ACGGGTGAGTAACGCGTGAGCAATCTGCCCTTAGGTGGGGGATAACAGCCGGAAACG GCTGCTAATACCGCATAACACATTGAAGCCGCATGGTTTTGATGTCAAAGATTTATTGC CTTTGGATGAGCTCGCGTCTGATTAGCTGGTTGGCGGGGTAACGGCCCACCAAGGCG ACGATCAGTAGCCGGACTGAGAGGTTGAACGGCCACATTGGGACTGAGACACGGCCC AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCGCAATGGGGGAAACCCTGACG CAGCAACGCCGCGTGATTGAAGAAGGCCTTCGGGTTGTAAAGATCTTTAATTGGGGAC GAATTTTGACGGTACCCAAAGAATAAGCTCCGGCTAACTACGTGCCAGCAGCCGCGG TAATACGTAGGGAGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGCGAGTAGGCG GGCTGGCAAGTTGGGAGTGAAATCCCGGGGCTTAACCCCGGAACTGCTTTCAAAACT GCTGGTCTTGAGTGATGGAGAGGCAGGCGGAATTCCGTGTGTAGCGGTGAAATGCGT AGATATACGGAGGAACACCAGTGGCGAAGGCGGCCTGCTGGACATTAACTGACGCTG AGGAGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA ACGATGGATACTAGGTGTGGGAGGTATTGACCCCTTCCGTGCCGGAGTTAACACAATA AGTATCCCACCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG CCCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAG GTCTTGACATCCCTCTGACCGCCCTAGAGATAGGGTTTCCCTTCGGGGCAGAGGTGA CAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA ACGAGCGCAACCCTTACGGTTAGTTGATACGAAAGATCACTCTAGCCGGACTGCCGTT

	GACAAAACGGAGGAAGGTGGGGACGACGTCAAATCATCATGCCCCTTATGACCTGGG CTACACACGTACTACAATGGCAGTCATACAGAGGGAAGCAAAACAGTGATGTGGAGCA AATCCCTAAAAGCTGTCCCAGTTCAGATTGCAGGCTGCAACTCGCCTGCATGAAGTCG GAATTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTA CACACCGCCCGTCACACCATGAGAGCCGGTAATACCCGAAGTCCGTAGCCTAACCGC AAGGAGGGCGCGGCCGAAGGTAGGACTGGTAATTAGGGTGAAGTCGTAACAAGGTAG CCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID No. 3	TTTAAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAACACATGCAA GTCGAACGAAGCTTGATTTCTGATTTTTTCGGAATGACGAATGATATGACTGAGTGGC GGACGGGTGAGTAACGCGTGAGCAACCTGCCCTTCGGAACGGGATAGTGTCTGGAAA CGGACAGTAATACCGTATAATATATATTGATCGCATGGTTGATATATCAAAACTGAGGT GCCGAAGGATGGGCTCGCGTCTGATTAGATAGTTGGTGGGGTAACGGCCTACCAAGT CGACGATCAGTAGCCGGACTGAGAGGTTGAACGGCCACATTGGGACTGAGACACGGC CCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGAT GCAGCAACGCCGCGTGAAGGAAGACGGTTTTCGGATTGTAAACTTCTGTTCTTAGTGA AGAATAATGACGGTAGCTAAGGAGCAAGCCACGGCTAACTACGTGCCAGCAGCCGCG GTAATACGTAGGTGGCAAGCGTTGTCCGGAATTACTGGGTGTAAAGGGAGCGTAGGC GGGATGCCAAGTCAGCTGTGAAAACTATGGGCTTAACTTGTAGACTGCAGTTGAAACT GGTATTCTTGAGTGAAGTAGAGGTTGGCGGAATTCCGAGTGTAGCGGTGAAATGCGTA GATATTCGGAGGAACACCGGTGGCGAAGGCGGCCAACTGGGCTTTAACTGACGCTGA GGCTCGAAAGTGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACACTGTAAA CGATGATAACTAGGTGTGGGGGGTCTGACCCCTTCCGTGCCGCAGCTAACGCAATAA GTTATCCACCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGAC CCGCACAAGCAGTGGATTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGC ACTTGACATCCGACTAACGAAGTAGAGATACATTAGGTGCCCTTCGGGGAAAGTCGAG ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC AACGAGCGCAACCCCTGCCATTAGTTGCTACGCAAGAGCACTCTAATGGGACCGCTA CCGACAAGGTGGAGGAAGGTGGGGACGACGTCAAATCATCATGCCCCTTATGTGCTG GGCTACACACGTAATACAATGGCCATCAACAAAGAGAAGCAATACCGCGAGGTGGAG CAAAACTATAAAAATGGTCTCAGTTCGGACTGCAGGCTGCAACCCGCCTGCACGAAGT TGGAATTGCTAGTAATCGTGGATCAGCATGCCACGGTGAATACGTTCCCGGGTCTTGT ACACACCGCCCGTCACACCATGGGAGTTGGTAACACCCGAAGTCAGTAGTCTAACCG CAAGGAGGACGCTGCCGAAGGTGGGATTGACGACTGGGGTGAAGTCGTAACAAGGTA GCCGTATCAGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID No. 4	ATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTAGCGGCAGGCTTAACACATGCAA GTCGAGGGGCAGCATAATGGTAGCAATACTATTGATGGCGACCGGCGGACGGGTGC GTAACGCGTATGCAACCTACCCTTTACAGGGGGATAACACTGAGAAATCGGTACTAAT ACCCCATAATATTCTGGGAGGCATCTTTCGGAGTTGAAAGCTTTGGTGGTAAAGGATG GGCATGCGTTGTATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGACGATACATA GGGGGACTGAGAGGTTAACCCCCCACATTGGTACTGAGACACGGACCAAACTCCTAC GGGAGGCAGCAGTGAGGAATATTGGTCAATGGACGGAAGTCTGAACCAGCCATGCCG CGTGCAGGAAGACGGCTCTATGAGTTGTAAACTGCTTTTGTACGAGGGTAAACGCAGA TACGTGTATCTGCCTGAAAGTATCGTACGAATAAGGATCGGCTAACTCCGTGCCAGCA GCCGCGGTAATACGGAGGATCCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGTGC GTAGGCGGTTTAGTAAGTCAGCGGTGAAATTTTGGTGCTTAACACCAAACGTGCCGTT GATACTGCTGGGCTAGAGAGTAGTTGCGGTAGGCGGAATGTATGGTGTAGCGGTGAA ATGCTTAGAGATCATACAGAACACCGATTGCGAAGGCAGCTTACCAAACTATATCTGA CGTTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC

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SEQ ID No. 5	ATGGAGAGTTTGATCCTGGCTCAGGATAAACGCTAGCGGCAGGCCTAACACATGCAA GTCGAGGGGCAGCGGGTGGAGTATTTCGGTACTCCTGCCGGCGACCGGCGCACGGG TGCGTAACGCGTATGCAACCTACCTTTAACAGGGGGATAATCCGAAGAAATTTGGTCT AATACCCCATAATATCATTTAAGGCATCTTAGATGGTTGAAAATTCCGATGGTTAGAGA TGGGCATGCGTTGTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCTACGATACA TAGGGGGACTGAGAGGTTTTCCCCCCACACTGGTACTGAGACACGGACCAGACTCCT ACGGGAGGCAGCAGTGAGGAATATTGGTCAATGGACGCAAGTCTGAACCAGCCATGC CGCGTGCAGGATGAAGGTGCTATGCATTGTAAACTGCTTTTGTACGAGGGTAAATGCA GGTACGTGTACCTGTTTGAAAGTATCGTACGAATAAGGGTCGGCTAACTCCGTGCCAG CAGCCGCGGTAATACGGAGGACCCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGT GCGTAGGCGGATTAGTAAGTTAGAGGTGAAAGCTCGATGCTCAACATCGAAATTGCCT CTGATACTGTTAGTCTAGAGTATAGTTGCGGAAGGCGGAATGTGTGGTGTAGCGGTGA AATGCTTAGATATCACACAGAACACCGATTGCGAAGGCAGCTTTCCAAGCTATTACTGA CGCTGATGCACGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGC CGTAAACGATGATAACTCGTTGCAGGCGATACACAGTCTGTGACTTAGCGAAAGCGTT AAGTTATCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGG GCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACC CGGGCTTGAAAGTTAGCGACGGATCCTGAAAGGGGTCTTCTCTTCGGAGCGCGAAAC TAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTTAAGTCCCATA ACGAGCGCAACCCCTACTGTTAGTTACCAGCACGTCAAGGTGGGCACTCTAGCAGGA CTGCCGGTGTAAGCCGAGAGGAAGGTGGGGATGACGTCAAATCAGCACGGCCCTTAC GTCCGGGGCGACACACGTGTTACAATGGTCGGTACAGAGGGTCGCTACCCCGTGAG GGGATGCCAATCTCGAAAGCCGATCTCAGTTCGGATTGGAGGCTGAAACTCGCCTCC ATGAAGTTGGATTCGCTAGTAATCGCGCATCAGCCATGGCGCGGTGAATACGTTCCCG GGCCTTGTACACACCGCCCGTCAAGCCATGGGAGTTGGGGGTGCCTGAAGTACGTGA CCGCAAGGAGCGTCCTAGGGCAAAACCGATGACTGGGGCTAAGTCGTAACAAGGTAG CCGTACCGGAAGGTGCGGCTGGAACACCTCCTT
SEQ ID No. 6	ACGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAA GTCGAACGGTTAAGGCGCCTTCGGGCGCGAATAGAGTGGCGAACGGGTGAGTAACA CGTGACCAACCTGCCCCCCTCCCCGGGATAACGCGAGGAAACCCGCGCTAATACCGG ATACTCCGCCCCTCCCGCATGGGAGGGGCGGGAAAGCCCCGACGGAGGGGGATGG GGTCGCGGCCCATTAGGTAGACGGCGGGGCAACGGCCCACCGTGCCTGCGATGGGT AGCCGGGTTGAGAGACCGACCGGCCACATTGGGACTGAGATACGGCCCAGACTCCTA CGGGAGGCAGCAGTGGGGAATTTTGCGCAATGGGGGGAACCCTGACGCAGCAACGC CGCGTGCGGGACGAAGGCCTTCGGGTTGTAAACCGCTTTCAGCAGGGAAGAAGTTGA

	CGGTACCTGCAGAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA GGGGGCGAGCGTTATCCGGATTCATTGGGCGTAAAGCGCGCGTAGGCGGCCCGTCA AGCGGAACCTCTAACCCGAGGGCTCAACCCCCGGCCGGGTTCCGAACTGGCAGGCT CGAGTTTGGTAGAGGAAGATGGAATTCCCGGTGTAGCGGTGGAATGCGCAGATATCG GGAAGAACACCGATGGCGAAGGCAGTCTTCTGGGCCATCAACTGACGCTGAGGCGC GAAAGCTGGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCCAGCCGTAAACGATG GGTGCTAGGTGTGGGGGGATCATCCCTCCGTGCCGCAGCCAACGCATTAAGCACCCC GCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCAC AAGCAGCGGAGCATGTGGCTTAATTCGAAGCAACGCGAAGAACCTTACCAGGGCTTG ACATGCTGGTGAAGCCGGGGAAACCCGGTGGCCGAGAGGAGCCAGCGCAGGTGGTG CATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA ACCCCTGCCATATGTTGCCAGCATTCAGTTGGGGACTCATATGGGACTGCCGGCGTC AAGCCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCTTTATGCCCTGGGCTG CACACGTGCTACAATGGCCGGTACAACGGGCCGCGACCTGGCGACAGGAAGCGAAT CCCTCAAAGCCGGCCCCAGTTCGGATCGGAGGCTGCAACCCGCCTCCGTGAAGTCG GAGTTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTA CACACCGCCCGTCACACCACCCGAGTCGTCTGCACCCGAAGCCGCCGGCCGAACCC GCAAGGGGCGGAGGCGTCGAAGGTGTGGAGGGTAAGGGGGGTGAAGTCGTAACAAG GTAGCCGTACCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID No. 7	ATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTAATACATGCAA GTCGAACGAAGTCTTTAGGAAGCTTGCTTCCAAAGAGACTTAGTGGCGAACGGGTGA GTAACACGTAGGTAACCTGCCCATGTGCCCGGGATAACTGCTGGAAACGGTAGCTAA AACCGGATAGGTATGAGGGAGGCATCTTCCTCATATTAAAGCACCTTCGGGTGTGAAC ATGGATGGACCTGCGGCGCATTAGCTGGTTGGTGAGGTAACGGCCCACCAAGGCGAT GATGCGTAGCCGACCTGAGAGGGTGAACGGCCACATTGGGACTGAGACACGGCCCA AACTCCTACGGGAGGCAGCAGTAGGGAATTTTCGTCAATGGGGGGAACCCTGAACGA GCAATGCCGCGTGTGTGAAGAAGGTCTTCGGATCGTAAAGCACTGTTGTAAGTGAAGA ATGCCATATAGAGGAAATGCTATGTGGGTGACGGTAGCTTACCAGAAAGCCACGGCTA ACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATCATTG GGCGTAAAGGGTGCGTAGGTGGCACGATAAGTCTGAAGTAAAAGGCAACAGCTCAAC TGTTGTATGCTTTGGAAACTGTCGAGCTAGAGTGCAGAAGAGGGCGATGGAATTCCAT GTGTAGCGGTAAAATGCGTAGATATATGGAGGAACACCAGTGGCGAAGGCGGTCGCC TGGTCTGTAACTGACACTGATGCACGAAAGCGTGGGGAGCAAATAGGATTAGATACCC TAGTAGTCCACGCCGTAAACGATGAGAACTAAGTGTTGGAGAGATTCAGTGCTGCAGT TAACGCAATAAGTTCTCCGCCTGGGGAGTATGCACGCAAGTGTGAAACTCAAAGGAAT TGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGA ACCTTACCAGGCCTTGACATGGATATAAATGTTCTAGAGATAGAAAGATAGCTATATAT CACACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCC CGCAACGAGCGCAACCCTTGTCTTCTGTTACCAGCATTAAGTTGGGGACTCAGGAGAG ACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTA TGGCCTGGGCTACACACGTACTACAATGGCGCCTACAAAGAGCAGCGACACCGCGAG GTGGAGCGAATCTCATAAAGGGCGTCTCAGTTCGGATTGAAGTCTGCAACTCGACTTC ATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATGCTGCGGTGAATACGTTCTCGG GCCTTGTACACACCGCCCGTCAAACCATGGGAGTTGGTAATACCCGAAGCCGGTGGC ATAACCGCAAGGAGTGAGCCGTCGAAGGTAGGACCGATGACTGGGGTTAAGTCGTAA CAAGGTATCCCTACGGGAACGTGGGGATGGATCACCTCCTTT
SEQ ID No. 8	TCAGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAA GTCGAGCGAAGCACTTAAGTGGATCTCTTCGGATTGAAACTTATTTGACTGAGCGGCG

	GACGGGTGAGTAACGCGTGGGTAACCTGCCTCATACAGGGGGATAACAGTTAGAAAT GGCTGCTAATACCGCATAAGCGCACAGGACCGCATGGTCTGGTGTGAAAAACTCCGG TGGTATGAGATGGACCCGCGTCTGATTAGCTAGTTGGAGGGGTAACGGCCCACCAAG GCGACGATCAGTAGCCGGCCTGAGAGGGTGAACGGCCACATTGGGACTGAGACACG GCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTG ATGCAGCGACGCCGCGTGAAGGAAGAAGTATCTCGGTATGTAAACTTCTATCAGCAGG GAAGAAAATGACGGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGC GGTAATACGTAGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGTAGA CGGAAGAGCAAGTCTGATGTGAAAGGCTGGGGCTTAACCCCAGGACTGCATTGGAAA CTGTTTTTCTAGAGTGCCGGAGAGGTAAGCGGAATTCCTAGTGTAGCGGTGAAATGCG TAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTACTGGACGGTAACTGACGTT GAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA AACGATGAATACTAGGTGTCGGGTGGCAAAGCCATTCGGTGCCGCAGCAAACGCAAT AAGTATTCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGA CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCA AGTCTTGACATCCCTCTGACCGGCCCGTAACGGGGCCTTCCCTTCGGGGCAGAGGAG ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC AACGAGCGCAACCCCTATCCTTAGTAGCCAGCAGGTAGAGCTGGGCACTCTAGGGAG ACTGCCGGGGATAACCCGGAGGAAGGCGGGGACGACGTCAAATCATCATGCCCCTTA TGATTTGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCGAGACAGCGAT GTTGAGCAAATCCCAAAAATAACGTCCCAGTTCGGACTGCAGTCTGCAACTCGACTGC ACGAAGCTGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCG GGTCTTGTACACACCGCCCGTCACACCATGGGAGTCAGTAACGCCCGAAGTCAGTGA CCCAACCTTACAGGAGGGAGCTGCCGAAGGCGGGACCGATAACTGGGGTGAAGTCG TAACAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID No. 9	CGAAGAGTTTGATCCTGGCTCAGGATGAACGCTAGCGACAGGCCTAACACATGCAAG TCGAGGGGCAGCGGAGAGGTAGCAATACCTTTGCCGGCGACCGGCGCACGGGTGAG TAACACGTATGCAATCCACCTGTAACAGGGGGATAACCCGGAGAAATCCGGACTAATA CCCCATAATATGGGCGCTCCGCATGGAGAGTCCATTAAAGAGAGCAATTTTGGTTACA GACGAGCATGCGCTCCATTAGCCAGTTGGCGGGGTAACGGCCCACCAAAGCGACGAT GGATAGGGGTTCTGAGAGGAAGGTCCCCCACATTGGAACTGAGACACGGTCCAAACT CCTACGGGAGGCAGCAGTGAGGAATATTGGTCAATGGTCGGCAGACTGAACCAGCCA AGTCGCGTGAGGGAAGACGGCCCTACGGGTTGTAAACCTCTTTTGTCGGAGAGTAAA GTACGCTACGTGTAGTGTATTGCAAGTATCCGAAGAAAAAGCATCGGCTAACTCCGTG CCAGCAGCCGCGGTAATACGGAGGATGCGAGCGTTATCCGGATTTATTGGGTTTAAA GGGTGCGTAGGCGGCACGCCAAGTCAGCGGTGAAATTTCCGGGCTCAACCCGGACT GTGCCGTTGAAACTGGCGAGCTAGAGTGCACAAGAGGCAGGCGGAATGCGTGGTGTA GCGGTGAAATGCATAGATATCACGCAGAACCCCGATTGCGAAGGCAGCCTGCTAGGG TGCGACAGACGCTGAGGCACGAAAGCGTGGGTATCGAACAGGATTAGATACCCTGGT AGTCCACGCAGTAAACGATGAATACTAACTGTTTGCGATACAATGTAAGCGGTACAGC GAAAGCGTTAAGTATTCCACCTGGGGAGTACGCCGGCAACGGTGAAACTCAAAGGAA TTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGG AACCTTACCCGGGCTCAAACGCAGGGGGAATGCCGGTGAAAGTCGGCAGCTAGCAAT AGTCACCTGCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGCTT AAGTGCCATAACGAGCGCAACCCCTATGGACAGTTACTAACGGGTGAAGCCGAGGAC TCTGTCTAGACTGCCGGCGCAAGCCGCGAGGAAGGTGGGGATGACGTCAAATCAGCA CGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCAGGTACAGAAGGCAGCCA GTCAGCAATGACGCGCGAATCCCGAAAACCTGTCTCAGTTCGGATTGGAGTCTGCAAC

	CCGACTCCATGAAGCTGGATTCGCTAGTAATCGCGCATCAGCCATGGCGCGGTGAAT ACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCCGGGAGTACCTGA AGCATGCAACCGCAAGGAGCGTACGAAGGTAATACCGGTAACTGGGGCTAAGTCGTA ACAAGGTAGCCGTACCGGAAGGTGCGGCTGGAACACCTCCTTT
SEQ ID No. 10	ATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTAGCGGCAGGCCTAACACATGCAA GTCGAGGGGCAGCGGGATTGAAGCTTGCTTCAATCGCCGGCGACCGGCGCACGGGT GCGTAACGCGTATGCAACCTACCCAGAACAGGGGGATAACACTGAGAAATTGGTACTA ATATCCCATAACATCATAAGGGGCATCCCTTTTGGTTGAAAACTCCGGTGGTTCTGGAT GGGCATGCGTTGTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATACAT AGGGGGACTGAGAGGTTAACCCCCCACATTGGTACTGAGACACGGACCAAACTCCTA CGGGAGGCAGCAGTGAGGAATATTGGTCAATGGACGCAAGTCTGAACCAGCCATGCC GCGTGCAGGAAGACGGCTCTATGAGTTGTAAACTGCTTTTGTACTAGGGTAAACTCAG ATACGTGTATCTGACTGAAAGTATAGTACGAATAAGGATCGGCTAACTCCGTGCCAGC AGCCGCGGTAATACGGAGGATTCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGTGC GTAGGCGGTTTGATAAGTTAGAGGTGAAATACCGGTGCTTAACACCGGAACTGCCTCT AATACTGTTGAGCTAGAGAGTAGTTGCGGTAGGCGGAATGTATGGTGTAGCGGTGAAA TGCTTAGAGATCATACAGAACACCGATTGCGAAGGCAGCTTACCAAACTATATCTGAC GTTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCA GTAAACGATGATAACTCGCTGTCGGCGATACACAGTCGGTGGCTAAGCGAAAGCGAT AAGTTATCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGG GCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACC CGGGCTTGAAAGTTAGTGACGGATCTGGAAACAGGTCTTCCCTTCGGGGCGCGAAAC TAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTTAAGTCCCATA ACGAGCGCAACCCCTACCGTTAGTTGCCATCAGGTCAAGCTGGGCACTCTGACGGGA CTGCCGGTGTAAGCCGAGAGGAAGGTGGGGATGACGTCAAATCAGCACGGCCCTTAC GTCCGGGGCCACACACGTGTTACAATGGTAGGTACAGAGGGCAGCTACCCAGCGATG GGATGCGAATCTCGAAAGCCTATCTCAGTTCGGATCGGAGGCTGAAACCCGCCTCCG TGAAGTTGGATTCGCTAGTAATCGCGCATCAGCCATGGCGCGGTGAATACGTTCCCG GGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCTGGGGGTGCCTGAAGTTCGTGA CCGCAAGGAGCGACCTAGGGCAAAACCGGTGACTGGGGCTAAGTCGTAACAAGGTAG CCGTACCGGAAGGTGCGGCTGGAACACCTCCTTT
SEQ ID No. 11	TCAGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAA GTCGAGCGAAGCACTTGCCATTGACTCTTCGGAAGATTTGGCATTTGACTGAGCGGCG GACGGGTGAGTAACGCGTGGGTAACCTGCCTCATACAGGGGAATAACAGTTAGAAAT GGCTGCTAATGCCGCATAAGCGCACAGGACCGCATGGTCTGGTGTGAAAAACTGAGG TGGTATGAGATGGGCCCGCGTCTGATTAGGTAGTTGGCGGGGTAACGGCCCACCAAG CCGACGATCAGTAGCCGACCTGAGAGGGTGACCGGCCACATTGGGACTGAGACACG GCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGAGGAAACTCTG ATGCAGCGACGCCGCGTGAAGGAAGAAGTATCTCGGTATGTAAACTTCTATCAGCAGG GAAGAAAATGACGGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGC GGTAATACGTAGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGTAGA CGGACGGGCAAGTCTGATGTGAAAGCCCGGGGCTTAACCCCGGGACTGCATTGGAAA CTGTCCATCTTGAGTGCCGGAGAGGTAAGCGGAATTCCTAGTGTAGCGGTGAAATGC GTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTACTGGACGGTAACTGACGT TGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT AAACGATGAATACTAGGTGTCGGGTTGCAAAGCAATCCGGTGCCGCAGCAAACGCAG TAAGTATTCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGG ACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC

	AAGTCTTGACATCTGCCTGACCGTTCCTTAACCGGAACTTTCCTTCGGGACAGGCAAG ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC AACGAGCGCAACCCCTGTCCTTAGTAGCCAGCAGTCCGGCTGGGCACTCTAGGGAGA CTGCCGGGGATAACCCGGAGGAAGGCGGGGACGACGTCAAATCATCATGCCCCTTAT GATTTGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCGGAGTGGTGACA CTGAGCAAATCTCAAAAATAACGTCCCAGTTCGGACTGCAGTCTGCAACTCGACTGCA CGAAGCTGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGG GTCTTGTACACACCGCCCGTCACACCATGGGAGTCAGTAACGCCCGAAGTCAGTGAC CTAACCGCAAGGGAGGAGCTGCCGAAGGCGGGACCGATAACTGGGGTGAAGTCGTA ACAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID No. 12	TTATGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAA GTCGAACGAAGCATTTAAGACGGATTCTTTCGGGATGAAGACTTTTATGACTGAGTGG CGGACGGGTGAGTAACGCGTGGGTAACCTGCCTCACACAGGGGGATAGCAGTTGGA AACGGCTGATAATACCGCATAAGCGCACAGTACCGCATGGTACAGTGTGAAAAACTCC GGTGGTGTGAGATGGACCCGCGTCTGATTAGCTTGTTGGCAGGGTAACGGCCTACCA AGGCAACGATCAGTAGCCGACCTGAGAGGGTGACCGGCCACATTGGGACTGAGACAC GGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGAGGAAACTCT GATGCAGCGACGCCGCGTGAGTGAAGAAGTAATTCGTTATGTAAAGCTCTATCAGCAG GGAAGATAGTGACGGTACCTGACTAAGAAGCTCCGGCTAAATACGTGCCAGCAGCCG CGGTAATACGTATGGAGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGTGTAGG TGGCATCACAAGTCAGAAGTGAAAGCCCGGGGCTCAACCCCGGGACTGCTTTTGAAA CTGTGGAGCTGGAGTGCAGGAGAGGCAAGTGGAATTCCTAGTGTAGCGGTGAAATGC GTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTGCTGGACTGTAACTGACAC TGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT AAACGATGAATACTAGGTGTCGGGGCTCATAAGAGCTTCGGTGCCGCAGCAAACGCA ATAAGTATTCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGG GACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC CAAGTCTTGACATCCTCTTGACCGGTCAGTAATGTGACCTTTTCTTCGGAACAAGAGTG ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC AACGAGCGCAACCCCTATTCTTAGTAGCCAGCATTTAAGGTGGGCACTCTAGGAAGAC TGCCAGGGATAACCTGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATG ACTTGGGCTACACACGTGCTACAATGGCGTAAACAAAGTGAAGCGAGAGTGTGAGCTT AAGCAAATCACAAAAATAACGTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGA AGCTGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGTCT TGTACACACCGCCCGTCACACCATGGGAGTCGGAAATGCCCGAAGTCGGTGACCTAA CGAAAGAAGGAGCCGCCGAAGGCAGGTCTGATAACTGGGGTGAAGTCGTAACAAGGT AGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID No. 13	TAAAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCACGCTTAACACATGCAAGT CGAACGGAGAATATCGAAGCTTGCTTTGATATTCTTAGTGGCGGACGGGTGAGTAACA CGTGAGTAACCTGCCTCTGAGAGTGGGATAGCTTCTGGAAACGGATGGTAATACCGC ATGAAATCATAGTATCGCATGGTACAATGATCAAAGATTTATCGCTCAGAGATGGACTC GCGTCTGATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGACGATCAGTAGCCG GACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGA GGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCGATGCCGCGT GGAGGAAGAAGGTTTTCGGATTGTAAACTCCTGTTGAAGAGGACGATAATGACGGTAC TCTTTTAGAAAGCTCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGAGC GAGCGTTGTCCGGAATTACTGGGTGTAAAGGGAGCGTAGGCGGGACGGCAAGTCAG ATGTGAAAACTATGGGCTCAACCCATAGACTGCATTTGAAACTGTTGTTCTTGAGTGAG

	GTAGAGGTAAGCGGAATTCCTGGTGTAGCGGTGAAATGCGTAGAGATCAGGAGGAAC ATCGGTGGCGAAGGCGGCTTACTGGGCCTTTACTGACGCTGAGGCTCGAAAGCGTGG GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATTACTAGGTG TGGGGGGACTGACCCCTTCCGTGCCGCAGTTAACACAATAAGTAATCCACCTGGGGA GTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCAGTGGA GTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCGAGTGA CGTACCTAGAGATAGGTATTTTCTTCGGAACACAAAGACAGGTGGTGCATGGTTGTCG TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTACCATT AGTTGCTACGCAAGAGCACTCTAATGGGACTGCCGTTGACAAAACGGAGGAAGGTGG GGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTACTACAATGGC AATATAACAGAGGGAAGCAATACAGCGATGTGGAGCAAATCCCCAAAAATTGTCCCAG TTCAGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTGCTAGTAATCGCAGAT CAGCATGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATG GGAGTCGGTAACACCCAAAGCCGGTCGTCTAACCTTCGGGAGGACGCCGTCTAAGGT GGGATTGATGACTGGGGTGAAGTCGTAACAAGGTAGCCGTATCGGAAGGTGCGGCTG GATCACCTCCTTT
SEQ ID No. 14	ATGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGT CGAACGAAGCACTCTATTTGATTTTCTTCGGAAATGAAGATTTTGTGACTGAGTGGCGG ACGGGTGAGTAACGCGTGGGTAACCTGCCTCATACAGGGGGATAACAGTTGGAAACG ACTGCTAATACCGCATAAGCGCACAGGATCGCATGGTCCGGTGTGAAAAACTCCGGT GGTATGAGATGGACCCGCGTCTGATTAGCCAGTTGGCAGGGTAACGGCCTACCAAAG CGACGATCAGTAGCCGACCTGAGAGGGTGACCGGCCACATTGGGACTGAGACACGG CCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGA TGCAGCGACGCCGCGTGAGCGAAGAAGTATTTCGGTATGTAAAGCTCTATCAGCAGG GAAGAAGAATGACGGTACCTGACTAAGAAGCACCGGCTAAATACGTGCCAGCAGCCG CGGTAATACGTATGGTGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGCAG GCGGTGCGGCAAGTCTGATGTGAAAGCCCGGGGCTCAACCCCGGTACTGCATTGGAA ACTGTCGTACTAGAGTGTCGGAGGGGTAAGTGGAATTCCTAGTGTAGCGGTGAAATG CGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTACTGGACGATAACTGACG CTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG TAAACGATGAATACTAGGTGTCGGGGAGCATTGCTCTTCGGTGCCGCAGCAAACGCA ATAAGTATTCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGG GACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC CAAGTCTTGACATCCCGATGACAGAGTATGTAATGTACTTTCTCTTCGGAGCATCGGT GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG CAACGAGCGCAACCCCTGTTCTTAGTAGCCAGCGGTTCGGCCGGGCACTCTAGGGAG ACTGCCAGGGATAACCTGGAGGAAGGCGGGGATGACGTCAAATCATCATGCCCCTTA TGACTTGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCGGAGCCGTGAG GCCGAGCAAATCTCAAAAATAACGTCTCAGTTCGGACTGTAGTCTGCAACCCGACTAC ACGAAGCTGGAATCGCTAGTAATCGCAGATCAGAATGCTGCGGTGAATACGTTCCCG GGTCTTGTACACACCGCCCGTCACACCATGGGAGTTGGAAATGCCCGAAGTCAGTGA CCCAACCGCAAGGAGGGAGCTGCCGAAGGCAGGTTCGATAACTGGGGTGAAGTCGT AACAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID No. 15	AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGCGCCTAACACATGCAAGTCG AACGAGAGAGAGGGAGCTTGCTTCCTTGATCGAGTGGCGAACGGGTGAGTAACGCGT GAGGAACCTGCCTCAAAGAGGGGGACAACAGTTGGAAACGACTGCTAATACCGCATA AGCCCACGACCCGGCATCGGGAAGAGGGAAAAGGAGCAATCCGCTTTGAGATGGCCT CGCGTCCGATTAGCTAGTTGGTGAGGTAACGGCCCACCAAGCGACGATCGGTAGCCG

	GACTGAGAGGTTGAACGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGA GGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCGACGCCGCGT GGAGGAAGAAGGTCTTCGGATTGTAAACTCCTGTTGTTGAGGAAGATAATGACGGTAC TCAACAAGGAAGTGACGGCTAACTACGTGCCAGCAGCCGCGGTAAAACGTAGGTCAC AAGCGTTGTCCGGAATTACTGGGTGTAAAGGGAGCGCAGGCGGGCGATCAAGTTGGA AGTGAAATCCATGGGCTCAACCCATGAACTGCTTTCAAAACTGGTCGTCTTGAGTAGT GCAGAGGTAGGCGGAATTCCCGGTGTAGCGGTGGAATGCGTAGATATCGGGAGGAA CACCAGTGGCGAAGGCGGCCTACTGGGCACCAACTGACGCTGAGGCTCGAAAGTGT GGGTAGCAAACAGGATTAGATACCCTGGTAGTCCACACCGTAAACGATGATTACTAGG TGTTGGGAGATTGACCCTCTCAGTGCCGCAGTTAACACAATAAGTAATCCACCTGGGG AGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCAGTGG AGTATGTGGTTTAATTCGACGCAACGCGAAGAACCTTACCAAGTCTTGACATCCCTTGA CGATGCTGGAAACAGTATTTCTCTTCGGAGCAAGGAGACAGGTGGTGCATGGTTGTC GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATGG TCAGTTACTACGCAAGAGGACTCTGGCCAGACTGCCGTTGACAAAACGGAGGAAGGT GGGGATGACGTCAAATCATCATGCCCTTTATGACTTGGGCTACACACGTACTACAATG GCGTTAAACAAAGAGAAGCAAGACCGCGAGGTGGAGCAAAACTCAGAAACAACGTCC CAGTTCGGACTGCAGGCTGCAACTCGCCTGCACGAAGTCGGAATTGCTAGTAATCGT GGATCAGCATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACAC CATGAGAGCCGGGGGGGACCCGAAGTCGGTAGTCTAACCGCAAGGAGGACGCCGCCG AAGGTAAAACTGGTGATTGGGGTGAAGTCGTAACAAGGTAGCCGT
SEQ ID No. 16	ATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTAGCGGCAGGCTTAACACATGCAA GTCGAGGGGCAGCATAATGGTAGTAATACTATTGATGGCGACCGGCGGACGGGTGCG TAACGCGTATGCAACCTACCCTTTACAGGGGGATAACACTGAGAAATCGGTACTAATA CCCCATAATATTCTGGGAGGCATCTTTCGGAGTTGAAAGCTTTGGTGGTAAAGGATGG GCATGCGTTGTATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGACGATACATAG GGGGACTGAGAGGTTAACCCCCCACATTGGTACTGAGACACGGACCAAACTCCTACG GGAGGCAGCAGTGAGGAATATTGGTCAATGGACGGAAGTCTGAACCAGCCATGCCGC GTGCAGGAAGACGGCTCTATGAGTTGTAAACTGCTTTTGTACGAGGGTAAACGCAGAT ACGTGTATCTGCCTGAAAGTATCGTACGAATAAGGATCGGCTAACTCCGTGCCAGCAG CCGCGGTAATACGGAGGATCCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGTGCGT AGGCGGTTTAGTAAGTCAGCGGTGAAATTTTGGTGCTTAACACCAAACGTGCCGTTGA TACTGCTGGGCTAGAGAGTAGTTGCGGTAGGCGGAATGTATGGTGTAGCGGTGAAAT GCTTAGAGATCATACAGAACACCGATTGCGAAGGCAGCTTACCAAACTATATCTGACG TTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCAG TAAACGATGATAGCTCGTTGTCGGCGATACACAGTCGGTGACTAAGAGAAATCGATAA GCTATCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGGC CCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCG GGCTTGAAAGTTACTGACGATTCTGGAAACAGGATTTCCCTTCGGGGCAGGAAACTAG GTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTTAAGTCCCATAACG AGCGCAACCCCTACTGATAGTTGCCATCAGAGCGTTTGAGCGATCAAACAAGCTGGG CACTCTATCGGGACTGCCGGTGTAAGCCGAGAGGAAGGTGGGGATGACGTCAAATCA GCACGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGTAGGTACAGAGGGCAG CCACCCAGTGATGGGGAGCGAATCTCGAAAGCCTATCTCAGTTCGGATTGGAGGCTG AAACTCGCCTCCATGAAGTTGGATTCGCTAGTAATCGCGCATCAGCCATGGCGCGGT GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGGAGTTGGGGGTGC CTGAAGTTCGTGACCGAAAGGAGCGACCTAGGGCAAAACCGATGACTGGGGCTAAGT CGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGAACACCTCCTTT

SEQ ID No. 17	TTTAGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAA GTCGAACGGAGTTATTTTGGAAATCTCTTCGGAGATGGAATTCATAACTTAGTGGCGG ACGGGTGAGTAACGCGTGAGCAATCTGCCCTTAGGTGGGGGATAACAGCCGGAAACG GCTGCTAATACCGCATAACACATTGAAGCCGCATGGTTTTGATGTCAAAGATTTATTGC CTTTGGATGAGCTCGCGTCTGATTAGCTGGTTGGCGGGGTAACGGCCCACCAAGGCG ACGATCAGTAGCCGGACTGAGAGGTTGAACGGCCACATTGGGACTGAGACACGGCCC AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCGCAATGGGGGAAACCCTGACG CAGCAACGCCGCGTGATTGAAGAAGGCCTTCGGGTTGTAAAGATCTTTAATTGGGGAC GAAAAATGACGGTACCCAAAGAATAAGCTCCGGCTAACTACGTGCCAGCAGCCGCGG TAATACGTAGGGAGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGCGAGTAGGCG GGCTGGCAAGTTGGGAGTGAAATCCCGGGGCTTAACCCCGGAACTGCTTTCAAAACT GCTGGTCTTGAGTGATGGAGAGGCAGGCGGAATTCCGTGTGTAGCGGTGAAATGCGT AGATATACGGAGGAACACCAGTGGCGAAGGCGGCCTGCTGGACATTAACTGACGCTG AGGAGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA ACGATGGATACTAGGTGTGGGAGGTATTGACCCCTTCCGTGCCGGAGTTAACACAATA AGTATCCCACCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG CCCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAG GTCTTGACATCCCTCTGACCGCCCTAGAGATAGGGTTTCCCTTCGGGGCAGAGGTGA CAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA ACGAGCGCAACCCTTACGGTTAGTTGATACGCAAGATCACTCTAGCCGGACTGCCGTT GACAAAACGGAGGAAGGTGGGGACGACGTCAAATCATCATGCCCCTTATGACCTGGG CTACACACGTACTACAATGGCAGTCATACAGAGGGAAGCAAAACAGTGATGTGGAGCA AATCCCTAAAAGCTGTCCCAGTTCAGATTGCAGGCTGCAACTCGCCTGCATGAAGTCG GAATTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTA CACACCGCCCGTCACACCATGAGAGCCGGTAATACCCGAAGTCCGTAGCCTAACCGC AAGGAGGGCGCGGCCGAAGGTAGGACTGGTAATTAGGGTGAAGTCGTAACAAGGTAG CCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID No. 18	ACGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAA GTCGAACGGTTAAGGCGCCTTCGGGCGCGAATAGAGTGGCGAACGGGTGAGTAACA CGTGACCAACCTGCCCCCCTCCCCGGGATAACGCGAGGAAACCCGCGCTAATACCGG ATACTCCGCCCCTCCCGCATGGGAGGGGCGGGAAAGCCCCGACGGAGGGGGATGG GGTCGCGGCCCATTAGGTAGACGGCGAGGCAACGGCCCACCGTGCCTGCGATGGGT AGCCGGGTTGAGAGACCGACCGGCCACATTGGGACTGAGATACGGCCCAGACTCCTA CGGGAGGCAGCAGTGGGGAATTTTGCGCAATGGGGGGAACCCTGACGCAGCAACGC CGCGTGCGGGACGAAGGCCTTCGGGTTGTAAACCGCTTTCAGCAGGGAAGAAGTTGA CGGTACCTGCAGAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA GGGGGCGAGCGTTATCCGGATTCATTGGGCGTAAAGCGCGCGTAGGCGGCCCGTCA AGCGGAACCTCTAACCCGAGGGCTCAACCCCCGGCCGGGTTCCGAACTGGCAGGCT CGAGTTTGGTAGAGGAAGATGGAATTCCCGGTGTAGCGGTGGAATGCGCAGATATCG GGAAGAACACCGATGGCGAAGGCAGTCTTCTGGGCCATCAACTGACGCTGAGGCGC GAAAGCTGGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCCAGCCGTAAACGATG GGTGCTAGGTGTGGGGGGATCATCCCTCCGTGCCGCAGCCAACGCATTAAGCACCCC GCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCAC AAGCAGCGGAGCATGTGGCTTAATTCGAAGCAACGCGAAGAACCTTACCAGGGCTTG ACATGCTGGTGAAGCCGGGGAAACCCGGTGGCCGAGAGGAGCCAGCGCAGGTGGTG CATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA ACCCCTGCCATATGTTGCCAGCATTCAGTTGGGGACTCATATGGGACTGCCGGCGTC AAGCCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCTTTATGCCCTGGGCTG CACACGTGCTACAATGGCCGGTACAACGGGCCGCGACCTGGCGACAGGAAGCGAAT CCCTCAAAGCCGGCCCCAGTTCGGATCGGAGGCTGCAACCCGCCTCCGTGAAGTCG GAGTTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTA CACACCGCCCGTCACACCACCCGAGTCGTCTGCACCCGAAGCCGCCGGCCGAACCC GCAAGGGGCGGAGGCGTCGAAGGTGTGGAGGGTAAGGGGGGTGAAGTCGTAACAAG GTAGCCGTACCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID No. 19	TCAGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAA GTCGAGCGAAGCACTTGCCATTGACTCTTCGGAAGATTTGGCATTTGACTGAGCGGCG GACGGGTGAGTAACGCGTGGGTAACCTGCCTCATACAGGGGAATAACAGTTAGAAAT GGCTGCTAATGCCGCATAAGCGCACAGGACCGCATGGTCTGGTGTGAAAAACTGAGG

	TGGTATGAGATGGGCCCGCGTCTGATTAGGTAGTTGGCGGGGTAACGGCCCACCAAG CCGACGATCAGTAGCCGACCTGAGAGGGTGACCGGCCACATTGGGACTGAGACACG GCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGAGGAAACTCTG ATGCAGCGACGCCGCGTGAAGGAAGAAGTATCTCGGTATGTAAACTTCTATCAGCAGG GAAGAAAATGACGGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGC GGTAATACGTAGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGTAGA CGGACGGGCAAGTCTGATGTGAAAGCCCGGGGCTTAACCCCGGGACTGCATTGGAAA CTGTCCATCTTGAGTGCCGGAGAGGTAAGCGGAATTCCTAGTGTAGCGGTGAAATGC GTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTACTGGACGGTAACTGACGT TGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT AAACGATGAATACTAGGTGTCGGGTTGCAAAGCAATCCGGTGCCGCAGCAAACGCAG TAAGTATTCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGG ACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC AAGTCTTGACATCTGCCTGACCGTTCCTTAACCGGAACTTTCCTTCGGGACAGGCAAG ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC AACGAGCGCAACCCCTGTCCTTAGTAGCCAGCAGTCCGGCTGGGCACTCTAGGGAGA CTGCCGGGGATAACCCGGAGGAAGGCGGGGACGACGTCAAATCATCATGCCCCTTAT GATTTGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCGGAGTGGTGACA CTGAGCAAATCTCAAAAATAACGTCCCAGTTCGGACTGCAGTCTGCAACTCGACTGCA CGAAGCTGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGG GTCTTGTACACACCGCCCGTCACACCATGGGAGTCAGTAACGCCCGAAGTCAGTGAC CTAACCGCAAGGGAGGAGCTGCCGAAGGCGGGACCGATAACTGGGGTGAAGTCGTA ACAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID No. 20	TTATGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAA GTCGAACGAAGCATTTAAGACGGATTCTTTCGGGATGAAGACTTTTATGACTGAGTGG CGGACGGGTGAGTAACGCGTGGGTAACCTGCCTCACACAGGGGGATAGCAGTTGGA AACGGCTGATAATACCGCATAAGCGCACAGTACCGCATGGTACAGTGTGAAAAACTCC GGTGGTGTGAGATGGACCCGCGTCTGATTAGCTTGTTGGCAGGGTAACGGCCTACCA AGGCAACGATCAGTAGCCGACCTGAGAGGGTGACCGGCCACATTGGGACTGAGACAC GGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGAGGAAACTCT GATGCAGCGACGCCGCGTGAGTGAAGAAGTAATTCGTTATGTAAAGCTCTATCAGCAG GGAAGATAGTGACGGTACCTGACTAAGAAGCTCCGGCTAAATACGTGCCAGCAGCCG CGGTAATACGTATGGAGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGTGTAGG TGGCATCACAAGTCAGAAGTGAAAGCCCGGGGCTCAACCCCGGGACTGCTTTTGAAA CTGTGGAGCTGGAGTGCAGGAGAGGCAAGTGGAATTCCTAGTGTAGCGGTGAAATGC GTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTGCTGGACTGTAACTGACAC TGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT AAACGATGAATACTAGGTGTCGGGGCTCATAAGAGCTTCGGTGCCGCAGCAAACGCA ATAAGTATTCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGG GACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC CAAGTCTTGACATCCTCTTGCCCGGTCAGTAATGTGACCTTTTCTTCGGAACAAGAGT GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG CAACGAGCGCAACCCCTATTCTTAGTAGCCAGCATATAAGGTGGGCACTCTAGGAAGA CTGCCAGGGATAACCTGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTAT GACTTGGGCTACACACGTGCTACAATGGCGTAAACAAAGTGAAGCGAGAGTGTGAGC TTAAGCAAATCACAAAAATAACGTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACAT GAAGCTGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGG TCTTGTACACACCGCCCGTCACACCATGGGAGTCGGAAATGCCCGAAGTCGGTGACC TAACGAAAGAAGGAGCCGCCGAAGGCAGGTCTGATAACTGGGGTGAAGTCGTAACAA GGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT

SEQ ID NO. 21	AGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAAGT CGAACGGAGTTATGCAGAGGAAGTTTTCGGATGGAATCGGCGTAACTTAGTGGCGGA CGGGTGAGTAACGCGTGGGAAACCTGCCCTGTACCGGGGGATAACACTTAGAAATAG GTGCTAATACCGCATAAGCGCACAGCTTCACATGARGCAGTGTGAAAAACTCCGGTGG TACAGGATGGTCCCGCGTCTGATTAGCCAGTTGGCAGGGTAAYGGCCTACCAAAGCG ACGATCAGTAGCCGGCCTGAGAGGGTGAACGGCCACATTGGGACTGAGACACGGCC CAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGATG

	CAGCGACGCCGCGTGAGTGAAGAAGTATTTCGGTATGTAAAGCTCTATCAGCAGGGA AGAAAATGACGGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGG TAATACGTAGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGTAGACG GCATGACAAGCCAGATGTGAAAACCCAGGGCTCAACCCTGGGACTGCATTTGGAACT GCCAGGCTGGAGTGCAGGAGAGGTAAGCGGAATTCCTAGTGTAGCGGTGAAATGCGT AGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTACTGGACTGTAACTGACGTTG AGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCGGTAA ACGATGATTGCTAGGTGTAGGTGGGTATGGACCCATCGGTGCCGCAGCTAACGCAAT AAGCAATCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGG ACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC AAGTCTTGACATCCCAATGACGTGTCCGTAACGGGGCATTCTCTTCGGAGCATTGGAG ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC AACGAGCGCAACCCTTATCCTTAGTAGCCAGCAGGTARAGCTGGGCACTCTAGGGAG ACTGCCGGGGATAACCCGGAGGAAGGCGGGGAYGACGTCAAATCATCATGCCCCTTA TGATTTGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCGAGACAGTGAT GTTGAGCAAATCCCAGAAATAACGTCTCAGTTCGGATTGTAGTCTGCAACTCGACTAC ATGAAGCTGGAATCGCTAGTAATCGCGAATCAGCATGTCGCGGTGAATACGTTCCCGG GTCTTGTACACACCGCCCGTCACACCATGGGAGTTGGAAATGCCCGAAGCCTGTGAC CTAACCGCAAGGGAGGAGCAGTCGAAGGCAGGTCTAATAACTGGGGTGAAGTCGTAA CAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID NO. 22	TTTAGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAA GTCGAACGGAGTTATTTTGGAAATCTCTTCGGGGATGGAATTCATAACTTAGTGGCGG ACGGGTGAGTAACGCGTGAGCAATCTGCCCTTAGGTGGGGGATAACAGCCGGAAACG GCTGCTAATACCGCATAACACATTGAAGCCGCATGGTTTTGATGTCAAAGATTTATTGC CTTTGGATGAGCTCGCGTCTGATTAGCTGGTTGGCGGGGTAACGGCCCACCAAGGCG ACGATCAGTAGCCGGACTGAGAGGTTGAACGGCCACATTGGGACTGAGACACGGCCC AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCGCAATGGGGGAAACCCTGACG CAGCAACGCCGCGTGATTGAAGAAGGCCTTCGGGTTGTAAAGATCTTTAATTGGGGAC GAAWWWTGACGGTACCCAAAGAATAAGCTCCGGCTAACTACGTGCCAGCAGCCGCG GTAATACGTAGGGAGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGCGAGTAGGC GGGCTGGCAAGTTGGGAGTGAAATCCCGGGGCTTAACCCCGGAACTGCTTTCAAAAC TGCTGGTCTTGAGTGATGGAGAGGCAGGCGGAATTCCGTGTGTAGCGGTGAAATGCG TAGATATACGGAGGAACACCAGTGGCGAAGGCGGCCTGCTGGACATTAACTGACGCT GAGGAGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA AACGATGGATACTAGGTGTGGGAGGTATTGACCCCTTCCGTGCCGGAGTTAACACAAT AAGTATCCCACCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGG GCCCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCA GGTCTTGACATCCCTCTGACCGCCCTAGAGATAGGGTTTCCCTTCGGGGCAGAGGTG ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC AACGAGCGCAACCCTTACGGTTAGTTGATACGAAAGATCACTCTAGCCGGACTGCCGT TGACAAAACGGAGGAAGGTGGGGACGACGTCAAATCATCATGCCCCTTATGACCTGG GCTACACACGTACTACAATGGCAGTCATACAGAGGGAAGCAAAACAGTGATGTGGAG CAAATCCCTAAAAGCTGTCCCAGTTCAGATTGCAGGCTGCAACTCGCCTGCATGAAGT CGGAATTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTG TACACACCGCCCGTCACACCATGAGAGCCGGTAATACCCGAAGTCCGTAGCCTAACC GCAAGGAGGGCGCGGCCGAAGGTAGGACTGGTAATTAGGGTGAAGTCGTAACAAGG TAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID NO. 23	ACGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAA GTCGAACGGTTAAGGCGCCTTCGGGCGCGAATAGAGTGGCGAACGGGTGAGTAACA CGTGACCAACCTGCCCCCCTCCCCGGGATAACGCGAGGAAACCCGCGCTAATACCGG ATACTCCGCCCCTCCCGCATGGGAGGGGCGGGAAAGCCCCGACGGAGGGGGATGG GGTCGCGGCCCATTAGGTAGACGGCGGGGCAACGGCCCACCGTGCCTGCGATGGGT AGCCGGGTTGAGAGACCGACCGGCCACATTGGGACTGAGATACGGCCCAGACTCCTA CGGGAGGCAGCAGTGGGGAATTTTGCGCAATGGGGGGAACCCTGACGCAGCAACGC CGCGTGCGGGACGAAGGCCTTCGGGTTGTAAACCGCTTTCAGCAGGGAAGAAGTTGA CGGTACCTGCAGAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA GGGGGCGAGCGTTATCCGGATTCATTGGGCGTAAAGCGCGCGTAGGCGGCCCGTCA AGCGGAACCTCTAACCCGAGGGCTCAACCCCCGGCCGGGTTCCGAACTGGCAGGCT

	CGAGTTTGGTAGAGGAAGATGGAATTCCCGGTGTAGCGGTGGAATGCGCAGATATCG GGAAGAACACCGATGGCGAAGGCAGTCTTCTGGGCCATCAACTGACGCTGAGGCGC GAAAGCTGGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCCAGCCGTAAACGATG GGYGCTAGGTGTGGGGGGATCATCCCTCCGTGCCGCAGCCAACGCATTAAGCRCCC CGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCA CAAGCAGCGGAGCATGTGGCTTAATTCGAAGCAACGCGAAGAACCTTACCAGGGCTT GACATGCTGGTGAAGCCGGGGAAACCCGGTGGCCGAGAGGAGCCAGCGCAGGTGGT GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC AACCCCTGCCATATGTTGCCAGCATTCAGTTGGGGACTCATATGGGACTGCCGGCGT CAAGCCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCTTTATGCCCTGGGCT GCACACGTGCTACAATGGCCGGTACAACGGGCCGCGACCTGGCGACAGGAAGCGAA TCCCTCAAAGCCGGCCCCAGTTCGGATCGGAGGCTGCAACCCGCCTCCGTGAAGTCG GAGTTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTA CACACCGCCCGTCACACCACCCGAGTCGTCTGCACCCGAAGCCGCCGGCCGAACCC GCAAGGGGCGGAGGCGTCGAAGGTGTGGAGGGTAAGGGGGGTGAAGTCGTAACAAG GTAGCCGTACCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID NO. 24	ATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTAATACATGCAA GTCGAACGAAGTCTTTAGGAAGCTTGCTTCCAAAGAGACTTAGTGGCGAACGGGTGA GTAACACGTAGGTAACCTGCCCATGTGCCCGGGATAACTGCTGGAAACGGTAGCTAA AACCGGATAGGTATGAGGGAGGCATCTTCCTCATATTAAAGCACCTTCGGGTGTGAAC ATGGATGGACCTGCGGCGCATTAGCTGGTTGGTGAGGTAACGGCCCACCAAGGCGAT GATGCGTAGCCGACCTGAGAGGGTGAACGGCCACATTGGGACTGAGACACGGCCCA AACTCCTACGGGAGGCAGCAGTAGGGAATTTTCGTCAATGGGGGGAACCCTGAACGA GCAATGCCGCGTGTGTGAAGAAGGTCTTCGGATCGTAAAGCACTGTTGTAAGTGAAGA ATGCCATATAGAGGAAATGCTATGTGGGTGACGGTAGCTTACCAGAAAGCCACGGCTA ACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATCATTG GGCGTAAAGGGTGCGTAGGTGGCACGATAAGTCTGAAGTAAAAGGCAACAGCTCAAC TGTTGTATGCTTTGGAAACTGTCGAGCTAGAGTGCAGAAGAGGGCGATGGAATTCCAT GTGTAGCGGTAAAATGCGTAGATATATGGAGGAACACCAGTGGCGAAGGCGGTCGCC TGGTCTGTAACTGACACTGATGCACGAAAGCGTGGGGAGCAAATAGGATTAGATACCC TAGTAGTCCACGCCGTAAACGATGAGAACTAAGTGTTGGAGAGATTCAGTGCTGCAGT TAACGCAATAAGTTCTCCGCCTGGGGAGTATGCACGCAAGTGTGAAACTCAAAGGAAT TGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGA ACCTTACCAGGCCTTGACATGGATATAAATGTTCTAGAGATAGAAAGATAGCTATATAT CACACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCC CGCAACGAGCGCAACCCTTGTCTTCTGTTACCAGCATTAAGTTGGGGACTCAGGAGAG ACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTA TGGCCTGGGCTACACACGTACTACAATGGCGCCTACAAAGAGCAGCGACACCGCGAG GTGGAGCGAATCTCATAAAGGGCGTCTCAGTTCGGATTGAAGTCTGCAACTCGACTTC ATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATGCTGCGGTGAATACGTTCTCGG GCCTTGTACACACCGCCCGTCAAACCATGGGAGTTGGTAATACCCGAAGCCGGTGGC ATAACCGCAAGGAGTGAGCCGTCGAAGGTAGGACCGATGACTGGGGTTAAGTCGTAA CAAGGTATCCCTACGGGAACGTGGGGATGGATCACCTCCTTT
SEQ ID NO. 25	TCAGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAA GTCGAGCGAAGCRCTTRARYGGATCTCTTCGGATTGAARYTTWTKTGACTGAGCGGC GGACGGGTGAGTAACGCGTGGGTAACCTGCCTCATACAGGGGGATAACAGTTAGAAA TGGCTGCTAATACCGCATAAGCGCACAGGACCGCATGGTCTGGTGTGAAAAACTCCG GTGGTATGAGATGGACCCGCGTCTGATTAGCTAGTTGGAGGGGTAACGGCCCACCAA GGCGACGATCAGTAGCCGGCCTGAGAGGGTGAACGGCCACATTGGGACTGAGACAC GGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCT GATGCAGCGACGCCGCGTGAAGGAAGAAGTATCTCGGTATGTAAACTTCTATCAGCAG GGAAGAAAATGACGGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCG CGGTAATACGTAGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGTAG ACGGAAGAGCAAGTCTGATGTGAAAGGCTGGGGCTTAACCCCAGGACTGCATTGGAA ACTGTTTTTCTAGAGTGCCGGAGAGGTAAGCGGAATTCCTAGTGTAGCGGTGAAATGC GTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTACTGGACGGTAACTGACGT TGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT AAACGATGAATACTAGGTGTCGGGTGGCAAAGCCATTCGGTGCCGCAGCAAACGCAA

	TAAGTATTCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGG ACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC AAGTCTTGACATCCCTCTGACCGGCCCGTAACGGGGCCTTCCCTTCGGGGCAGAGGA GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG CAACGAGCGCAACCCCTATCCTTAGTAGCCAGCAGGTRRAGCTGGGCACTCTAGGGA GACTGCCGGGGATAACCCGGAGGAAGGCGGGGACGACGTCAAATCATCATGCCCCTT ATGATTTGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCGAGACAGCGA TGTTGAGCAAATCCCAAAAATAACGTCCCAGTTCGGACTGCAGTCTGCAACTCGACTG CACGAAGCTGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCG GGTCTTGTACACACCGCCCGTCACACCATGGGAGTCAGTAACGCCCGAAGTCAGTGA CCCAACCTTAYAGGAGGGAGCTGCCGAAGGCGGGACCGATAACTGGGGTGAAGTCG TAACAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID NO. 26	CGAAGAGTTTGATCCTGGCTCAGGATGAACGCTAGCGACAGGCCTAACACATGCAAG TCGAGGGGCAGCGGRGAGGYAGCAATACCTTTGCCGGCGACCGGCGCACGGGTGAG TAACACGTATGCAATCCACCTGTAACAGGGGGATAACCCGGAGAAATCCGGACTAATA CCCCATAATATGGGCGCTCCGCATGGAGRGTCCATTAAAGAGAGCAATTTTGGTTACA GACGAGCATGCGCTCCATTAGCCAGTTGGCGGGGTAACGGCCCACCAAAGCGACGAT GGATAGGGGTTCTGAGAGGAAGGTCCCCCACATTGGAACTGAGACACGGTCCAAACT CCTACGGGAGGCAGCAGTGAGGAATATTGGTCAATGGTCGGCAGACTGAACCAGCCA AGTCGCGTGAGGGAAGACGGCCCTACGGGTTGTAAACCTCTTTTGTCGGAGAGTAAA GTRCGCTACGTGTAGYGTATTGCAAGTATCCGAAGAAAAAGCATCGGCTAACTCCGTG CCAGCAGCCGCGGTAATACGGAGGATGCGAGCGTTATCCGGATTTATTGGGTTTAAA GGGTGCGTAGGCGGCACGCCAAGTCAGCGGTGAAATTTCCGGGCTCAACCCGGACT GTGCCGTTGAAACTGGCGAGCTAGAGTGCACAAGAGGCAGGCGGAATGCGTGGTGTA GCGGTGAAATGCATAGATATCACGCAGAACCCCGATTGCGAAGGCAGCCTGCTAGGG TGCGACAGACGCTGAGGCACGAAAGCGTGGGTATCGAACAGGATTAGATACCCTGGT AGTCCACGCAGTAAACGATGAATACTAACTGTTTGCGATACAATGTAAGCGGTACAGC GAAAGCGTTAAGTATTCCACCTGGGGAGTACGCCGGCAACGGTGAAACTCAAAGGAA TTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGG AACCTTACCCGGGCTCAAACGCAGGGGGAATGCCGGTGAAAGTCGGCAGCTAGCAAT AGTCACCTGCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGCTT AAGTGCCATAACGAGCGCAACCCCTATGGACAGTTACTAACGGGTGAAGCCGAGGAC TCTGTCTAGACTGCCGGCGCAAGCCGCGAGGAAGGTGGGGATGACGTCAAATCAGCA CGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCAGGTACAGAAGGCAGCCA GTCAGCAATGACGCGCGAATCCCGAAAACCTGTCTCAGTTCGGATTGGAGTCTGCAAC CCGACTCCATGAAGCTGGATTCGCTAGTAATCGCGCATCAGCCATGGCGCGGTGAAT ACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCCGGGAGTACCTGA AGCATGCAACCGCAAGGAGCGTACGAAGGTAATACCGGTAACTGGGGCTAAGTCGTA ACAAGGTAGCCGTACCGGAAGGTGCGGCTGGAACACCTCCTTT
SEQ ID NO. 27	TCAGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAA GTCGAGCGAAGCACTTRYYATTGAMTCTTCGGARGATTTRGCATKTGACTGAGCGGCG GACGGGTGAGTAACGCGTGGGTAACCTGCCTCATACAGGGGAATAACAGTTAGAAAT GGCTGCTAATGCCGCATAAGCGCACAGGRCCGCATGGTCYGGTGTGAAAAACTSMGG TGGTATGAGATGGRCCCGCGTCTGATTAGGTAGTTGGCGGGGTAACGGCCCACCAAG CCGACGATCAGTAGCCGACCTGAGAGGGTGACCGGCCACATTGGGACTGAGACACG GCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGAGGAAACTCTG ATGCAGCGACGCCGCGTGAAGGAAGAAGTATCTCGGTATGTAAACTTCTATCAGCAGG GAAGAAAATGACGGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGC GGTAATACGTAGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGTAGA CGGACGGGCAAGTCTGATGTGAAAGCCCGGGGCTTAACCCCGGGACTGCATTGGAAA CTGTCCATCTTGAGTGCCGGAGAGGTAAGCGGAATTCCTAGTGTAGCGGTGAAATGC GTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTACTGGACGGTAACTGACGT TGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT AAACGATGAATACTAGGTGTCGGGTTGCAAAGCAATCCGGTGCCGCAGCAAACGCAG TAAGTATTCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGG ACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC AAGTCTTGACATCTGCCTGACCGTTCCTTAACCGGAACTTTCCTTCGGGACAGGCAAG ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC

	AACGAGCGCAACCCCTGTCCTTAGTAGCCAGCAGTCCGGCTGGGCACTCTAGGGAGA CTGCCGGGGATAACCCGGAGGAAGGCGGGGACGACGTCAAATCATCATGCCCCTTAT GATTTGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCGGAGTGGTGACA CTGAGCAAATCTCAAAAATAACGTCCCAGTTCGGACTGCAGTCTGCAACTCGACTGCA CGAAGCTGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGG GTCTTGTACACACCGCCCGTCACACCATGGGAGTCAGTAACGCCCGAAGTCAGTGAC CTAACCGCAAGGGAGGAGCTGCCGAAGGCGGGACCGATAACTGGGGTGAAGTCGTA ACAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT
SEQ ID NO. 28	TTATGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAA GTCGAACGAAGCATTTGMGACRGATTYYTTCGGRWTGAAGACTTTTATGACTGAGTGG CGGACGGGTGAGTAACGCGTGGGTAACCTGCCTCACACAGGGGGATAGCAGTTGGA AACGGCTGATAATACCGCATAAGCGCACAGTACCGCATGGTACAGTGTGAAAAACTCC GGTGGTGTGAGATGGACCCGCGTCTGATTAGCTTGTTGGCRGGGTAACGGCCYACCA AGGCAACGATCAGTAGCCGACCTGAGAGGGTGACCGGCCACATTGGGACTGAGACAC GGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGAGGAAACTCT GATGCAGCGACGCCGCGTGAGTGAAGAAGTAATTCGTTATGTAAAGCTCTATCAGCAG GGAAGATAGTGACGGTACCTGACTAAGAAGCTCCGGCTAAATACGTGCCAGCAGCCG CGGTAATACGTATGGAGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGTGTAGG TGGCATCACAAGTCAGAAGTGAAAGCCCGGGGCTCAACCCCGGGACTGCTTTTGAAA CTGTGGAGCTGGAGTGCAGGAGAGGCAAGTGGAATTCCTAGTGTAGCGGTGAAATGC GTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTGCTGGACTGTAACTGACAC TGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT AAACGATGAATACTAGGTGTCGGGGCTCATAAGAGCTTCGGTGCCGCAGCAAACGCA ATAAGTATTCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGG GACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC CAAGTCTTGACATCCTCTTGRCCGGTCAGTAATGTGRYCTTTTCTTCGGAACAAGAGT GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG CAACGAGCGCAACCCCTATTCTTAGTAGCCAGCATTTAAGRTGGGCACTCTAGGAAGA CTGCCAGGGATAACCTGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTAT GACTTGGGCTACACACGTGCTACAATGGCGTAAACAAAGTGAAGCGAGAGTGTGAGC TTAAGCAAATCACAAAAATAACGTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACAT GAAGCTGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGG TCTTGTACACACCGCCCGTCACACCATGGGAGTCGGAAATGCCCGAAGTCGGTGACC TAACGAAAGAAGGAGCCGCCGAAGGCAGGTCTGATAACTGGGGTGAAGTCGTAACAA GGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT
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Table 3

No	Taxonomy	16S rDNA sequence identifier	Consortium									
			1	2	3	4	5	6	7	8	9	10
B1	Eisenbergiella sp.	SEQ ID No. 1	X	X	X	X	X	X	X	X	X	X
B2	Butyricicoccus sp.	SEQ ID No. 2	X	X	X	X		X	X	X	X	X
B3	Clostridiales sp.	SEQ ID No. 3	X	X	X	X						X
B4	Alistipes obesi	SEQ ID No. 4	X	X								
B5	Alistipes indistinctus	SEQ ID No. 5	X	X								X
B6	Gordonibacter urolithinfaciens	SEQ ID No. 6	X	X	X	X						
B7	Faecalitalea sp.	SEQ ID No. 7	X	X	X	X	X	X	X	X		X
B8	Blautia sp.	SEQ ID No. 8	X	X	X	X	X					
B9	Barnesiella intestinihominis	SEQ ID No. 9	X	X	X	X	X	X	X			
B10	Alistipes timonensis	SEQ ID No. 10	X									X
B11	Blautia sp.	SEQ ID No. 11	X		X	X						X
B12	Lachnospira sp.	SEQ ID No. 12	X		X							
B13	Ruminococcus callidus	SEQ ID No. 13	X		X		X	X				X
B14	Roseburia faecis	SEQ ID No. 14	X		X	X						X
B15	Faecalibacterium prausnitzii	SEQ ID No. 15	X		X							
B4	Alistipes obesi	SEQ ID No. 16					X	X				
B2	Butyricicoccus sp.	SEQ ID No. 17					X					
B6	Gordonibacter urolithinfaciens	SEQ ID No. 18					X	X	X			
B11	Blautia sp.	SEQ ID No. 19						X	X			
B12	Lachnospira sp.	SEQ ID No. 20					X	X				
Number of bacteria			15	9	12	9	9	9	6	3	2	9

Claims

1. A method for identifying a subject that will respond to therapy with an immune checkpoint inhibitor comprising determining the abundance of bacteria from at least 9 different species in a biological sample from said subject that comprises gut flora wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15 wherein said abundance is indicative of a response of a subject to therapy with an immune checkpoint inhibitor.

2. The method for identifying a subject that will respond to therapy with an immune checkpoint inhibitor according to claim 1, the method comprising:

a) determining the abundance of the bacteria in a biological sample obtained from the subject and

b) comparing the abundance to a reference level from cancer patients that do not respond to therapy with an immune checkpoint inhibitor or cancer patients that respond to therapy with an immune checkpoint inhibitor;

wherein if the reference level is from patients that do not respond to therapy with an immune checkpoint inhibitor, then an increase in the abundance of each of the bacteria compared to the reference level, is indicative that the subject will respond to therapy with an immune checkpoint inhibitor or

wherein if the reference level is from patients that do respond to therapy with an immune checkpoint inhibitor, then the same or substantially the same or an increase in abundance of each of the bacteria, is indicative that the subject will respond to therapy with an immune checkpoint inhibitor.

3. The method for identifying a subject that will respond to therapy with an immune checkpoint inhibitor according to claim 1, the method comprising:

a) determining the abundance of the bacteria in a biological sample obtained from the subject;

b) comparing the abundance to a reference level from cancer patients and

c) applying random forest analysis.

4. The method according to a preceding claim, wherein the bacterial species comprise a 16S rDNA sequence selected from SEQ ID NO: 1 or 2 or a 16S rDNA sequence having at least 98.7% sequence identity thereto.

5. The method according to a preceding claim, wherein the method comprises determining the abundance of bacteria from 10, 11, 12, 13, 14 or 15 species wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15.

6. The method according to a preceding claim wherein said subject is a cancer patient.

7. The method according to claim 6, wherein the cancer is selected from melanoma, bone cancer, pancreatic cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, breast cancer, brain cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium,

carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, kidney cancer, sarcoma of soft tissue, cancer of the urethra, cancer of the bladder, renal cancer, lung cancer, non-small cell lung cancer, thymoma, urothelial carcinoma leukemia, prostate cancer, mesothelioma, adrenocortical carcinoma, lymphomas, such as such as Hodgkin's disease, non-Hodgkin's, gastric cancer, and multiple myelomas.

8. The method according to claim 7, wherein the melanoma is selected from Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, nodular melanoma, subungual melanoma, Cutaneous melanoma, uveal/intraocular melanoma and superficial spreading melanoma.

9. The method according to a preceding claim, further comprising the step of identifying a subject in need of treatment with the immune checkpoint inhibitor.

10. The method according to a preceding claim, further comprising administering an immune checkpoint inhibitor to said subject.

11. The method according to a preceding claim wherein the immune checkpoint inhibitor inhibits PD-1, PD-L1 or CTLA-4 activity.

12. The method according to claim 11, wherein the immune checkpoint inhibitor is an anti PD-1, PDL-1 or CTLA-4 antibody.

13. The method according to claim 12, wherein the anti PD-1, PDL-1 or CTLA-4 antibody is selected from nivolumab, pembrolizumab, cemiplimab, avelumab, durvalumab, atezolizumab, Spartalizumab, Camrelizumab, Sintilimab, Tislelizumab, Pidilizumab, Toripalimab, Ipilimumab or Tremelimumab.

14. The method according to any of claims 1 to 11, wherein the immune checkpoint inhibitor is an interfering nucleic acid molecule, a small molecule or a PROteolysis TArgeting Chimera (PROTAC) or other immune checkpoint inhibitor.

15. The method according to claim 14, wherein the interfering nucleic acid molecule is an siRNA molecule, an shRNA molecule or an antisense RNA molecule or a small molecule or peptide.

16. The method according to a preceding claim, wherein the abundance is the abundance of the bacteria in the sample as a proportion of the total microbiota in the sample.

17. The method according to a preceding claim, further comprising the step of obtaining a biological sample that comprises gut flora from said subject.

18. The method according to a preceding claim, wherein the sample is a faecal sample.

19. Use of bacteria selected from at least 9 different bacterial species wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15 in identifying a patient that will respond to therapy with an immune checkpoint inhibitor.

20. A kit comprising

a sealable container configured to receive a biological sample;

polynucleotide primers for amplifying a 16S rDNA polynucleotide sequence from at least 9 different bacterial species wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15;

a detecting reagent to detect the amplified 16S rDNA sequence; and

instructions for use.

21. A method for identifying a faecal donor comprising assessing a faecal sample of a subject for the presence of bacteria from at least 9 different bacterial species wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15 and identifying the faecal donor based on the presence and/or abundance of the bacteria.

22. The use of bacteria from at least 9 different bacterial species wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15 in a method for identifying a donor for FMT therapy.

23. A method for determining if a cancer patient needs a bacterial compensation before administration of an immune checkpoint inhibitor comprising assessing, in a faeces sample from said patient, the presence or absence of bacteria from at least 9 different bacterial species wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15.

24. A method for predicting a response to an immune checkpoint inhibitor therapy in a subject having cancer comprising determining the abundance of bacteria from at least 9 different species wherein said bacteria comprise a 16S rDNA sequence selected from SEQ ID NOs: 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15 in a biological sample from said subject that comprises gut flora wherein said abundance is indicative of a response or non-response of a subject to therapy with an immune checkpoint inhibitor.

25. A method for predicting a response to an immune checkpoint inhibitor therapy in a subject having cancer according to claim 24, the method comprising:

a) determining the abundance of the bacteria a biological sample obtained from the subject;

b) comparing the abundance to a reference level from patients that do not respond to immune checkpoint inhibitor therapy; or patients that respond to immune checkpoint inhibitor therapy;

wherein if the reference level is from patients that do not respond to immune checkpoint inhibitor therapy, then an increase in the abundance of each of the bacteria compared to the reference level, is indicative that the subject will respond to therapy with an immune checkpoint inhibitor or

wherein if the reference level is from patients that do respond to immune checkpoint inhibitor therapy, then the same or substantially the same or an increase in abundance of each of the bacteria, is indicative that the subject will respond to therapy with an immune checkpoint inhibitor.

26. The method according to claim 24, the method comprising:

a) determining the abundance of the bacteria a biological sample obtained from the subject and;
b) comparing the abundance to a reference level from cancer patients or healthy subjects and
c) applying random forest analysis.

27. The method according to any of claims 24 to 26, the method comprising the step of predicting a response.

28. The method according to any of claims 24 to 27, where if the subject is predicted to be a non-responder, an anti cancer therapy is administered which is not an immune checkpoint inhibitor.

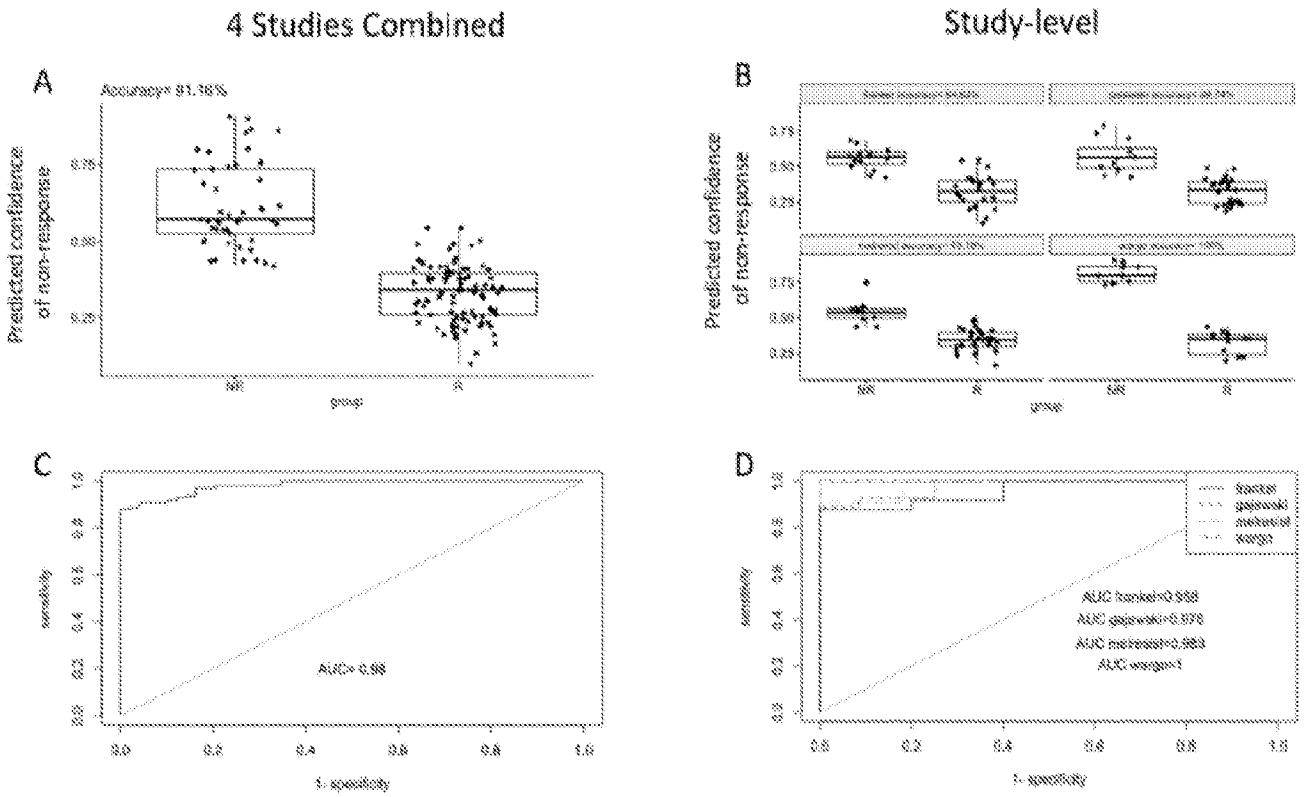
29. The method according to any of claims 24 to 28, where if the subject is predicted to be a non-responder, a composition comprising isolated bacteria from one or more species is administered wherein the bacteria comprise a sequence selected from SEQ ID 1 to 15 or a sequence having at least 98.7% sequence identity with a nucleic acid sequence selected from SEQ ID NOs: 1 to 15.

30. A method according to claim 29, where if the subject is predicted to be a non-responder, a composition comprising isolated bacteria selected from at least two species is administered wherein the bacteria from the first species comprise a 16S rDNA sequence having at least 98.7% sequence identity with a nucleic acid sequence according to SEQ ID NO: 1, and the bacteria from the second species comprise a 16S rDNA sequence having at least 98.7% sequence identity with a nucleic acid sequence according to SEQ ID NO: 2.

31. A method according to claim 29 or 31, wherein the composition further comprises isolated bacteria from at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13 different species wherein the bacteria comprise a 16S rDNA sequence selected from a sequence having at least 98.7% sequence identity with a nucleic acid sequence according to SEQ ID NO: 3 to 15.

32. The method according to any of claims 24 to 27, where if the subject is predicted to be a responder, an immune checkpoint inhibitor therapy is administered.

Figure 1


4 Studies Combined
Study-level
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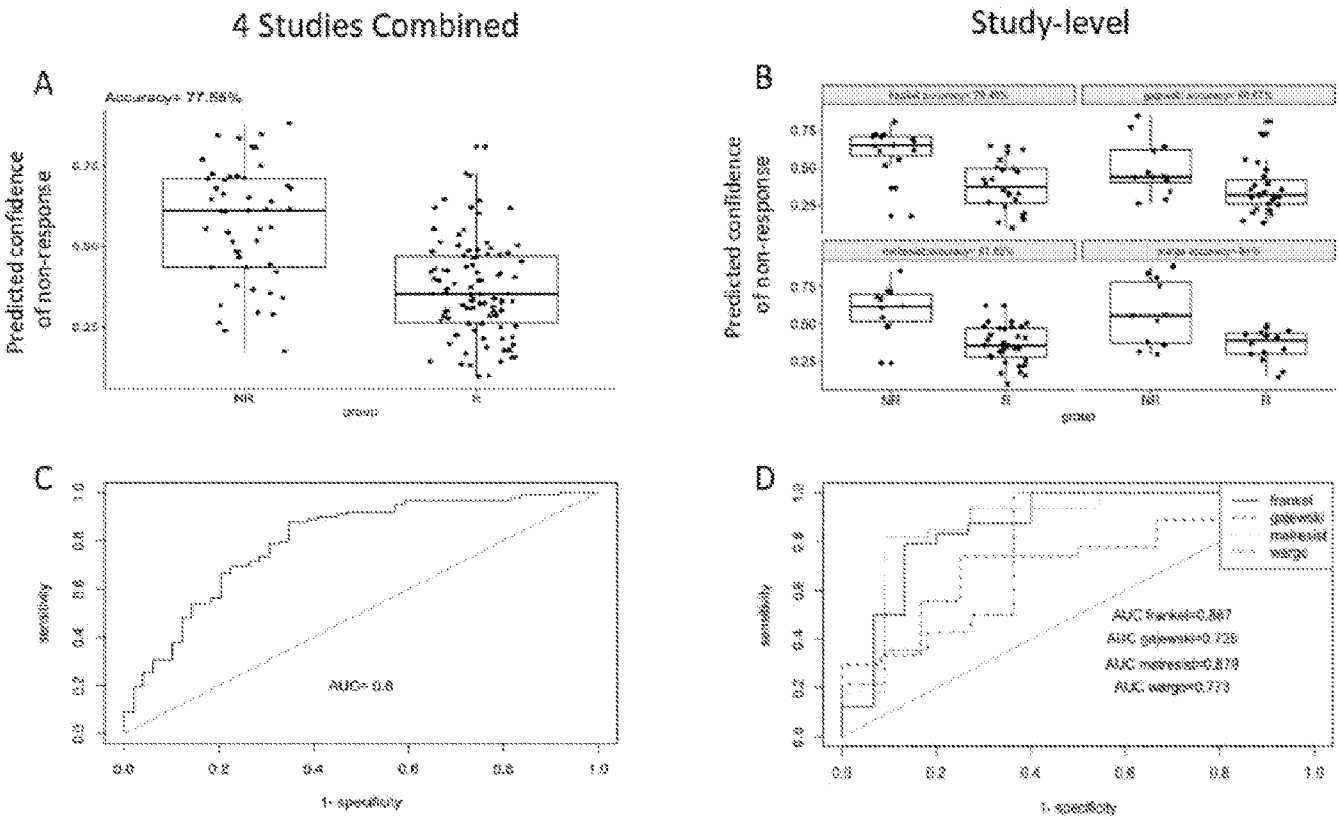
B
C
D
Predicted confidence of non-response
group
sensitivity
1- specificity
AUC= 0.98

Figure 2


4 Studies Combined
Study-level
A
B
C
D
Predicted confidence of non-response
group
sensitivity
1- specificity
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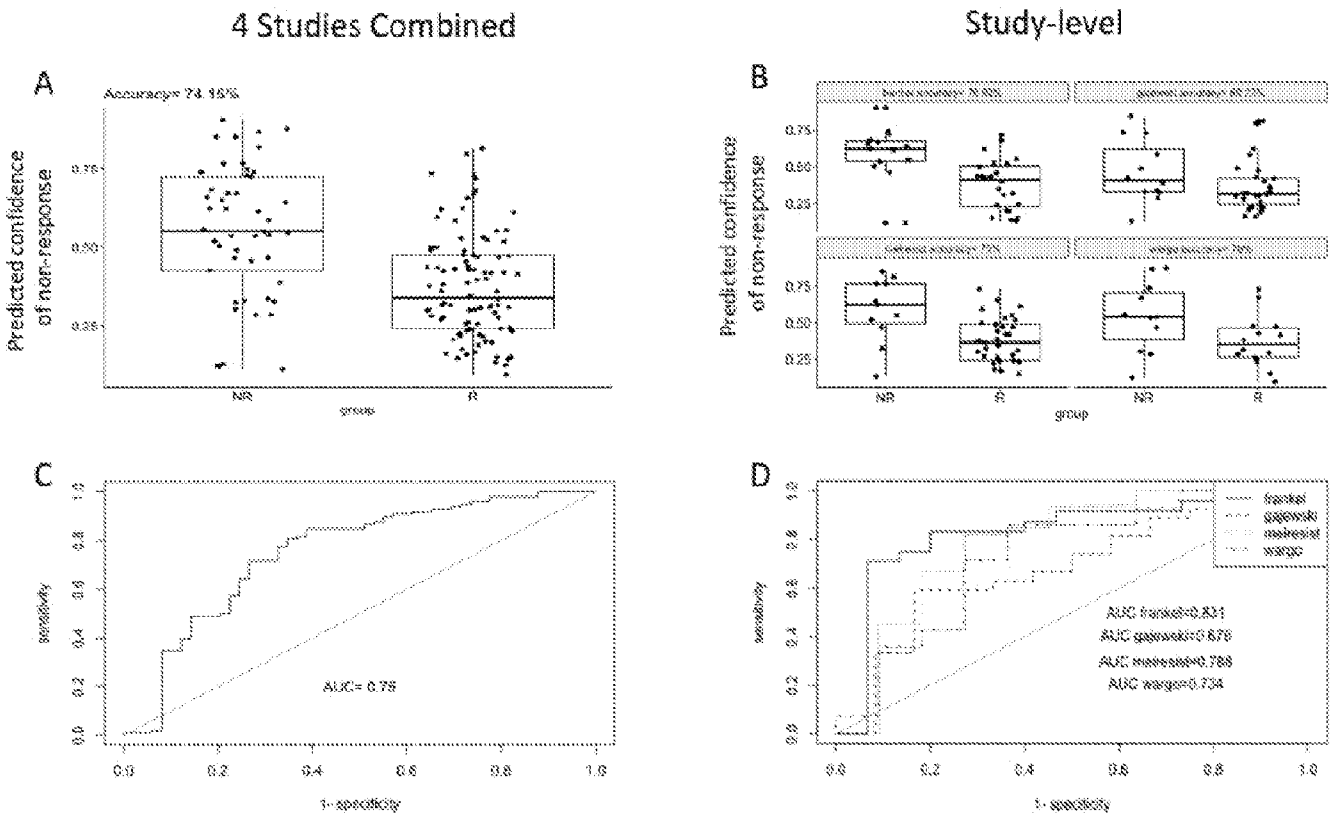
AUC gajewski=0.728
AUC matresist=0.879
AUC wargo=0.773

Figure 3


4 Studies Combined
Study-level
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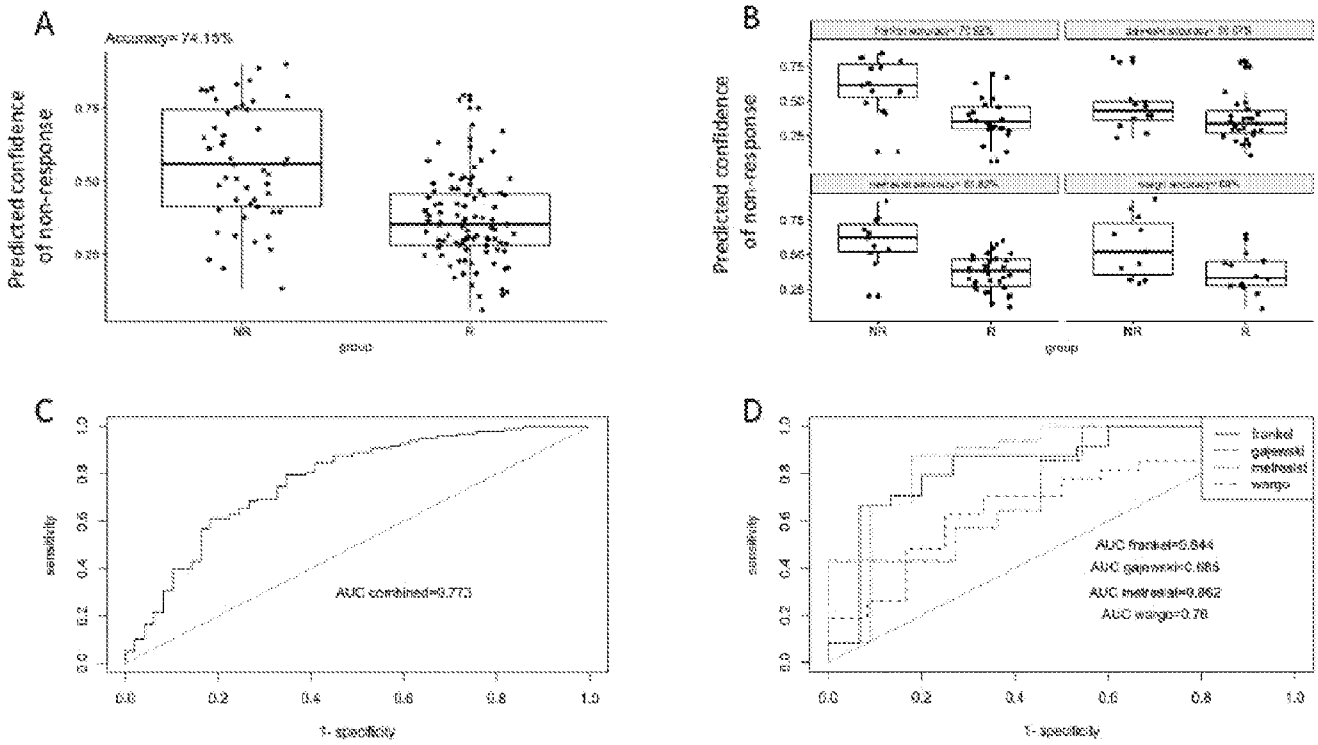
B
C
D
Predicted confidence of non-response
group
NR
R
sensitivity
1- specificity
AUC= 0.75

Figure 4


A
Accuracy= 74.15%
Predicted confidence of non-response
NR
R
group
B
Predicted confidence of non-response
NR
R
NR
R
group
C
sensitivity
AUC combined=0.773
1- specificity
D
frankel
gajewski
metresist
wargo
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AUC metresist=0.862
AUC wargo=0.78
sensitivity
1- specificity

Figure 5

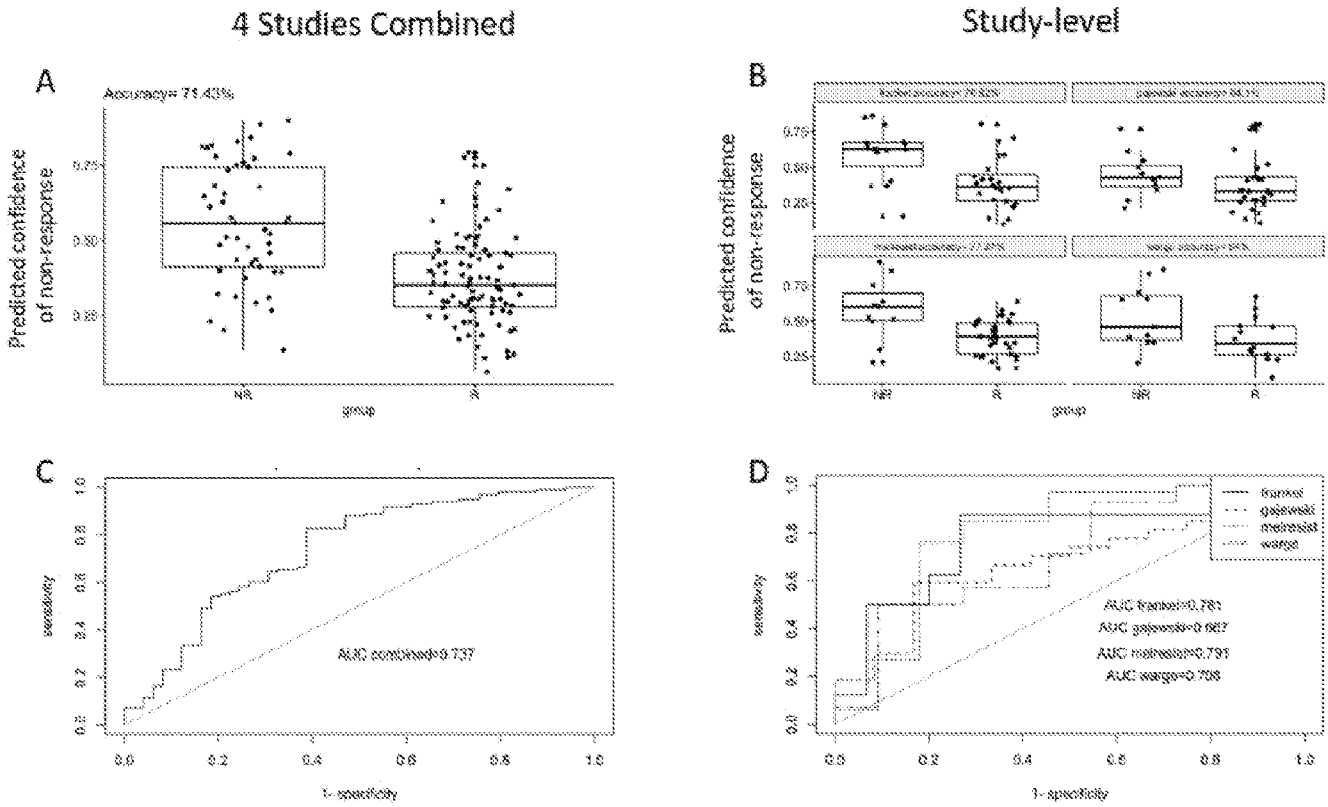

4 Studies Combined
Study-level
A
Accuracy= 71.43%
Predicted confidence of non-response
NR
R
group
B
Predicted confidence of non-response
NR
R
group
C
sensitivity
AUC combined=0.737
1- specificity
D
sensitivity
AUC frankel=0.781
AUC gajewski=0.667
AUC matrosist=0.791
AUC warga=0.708
1- specificity

Figure 6

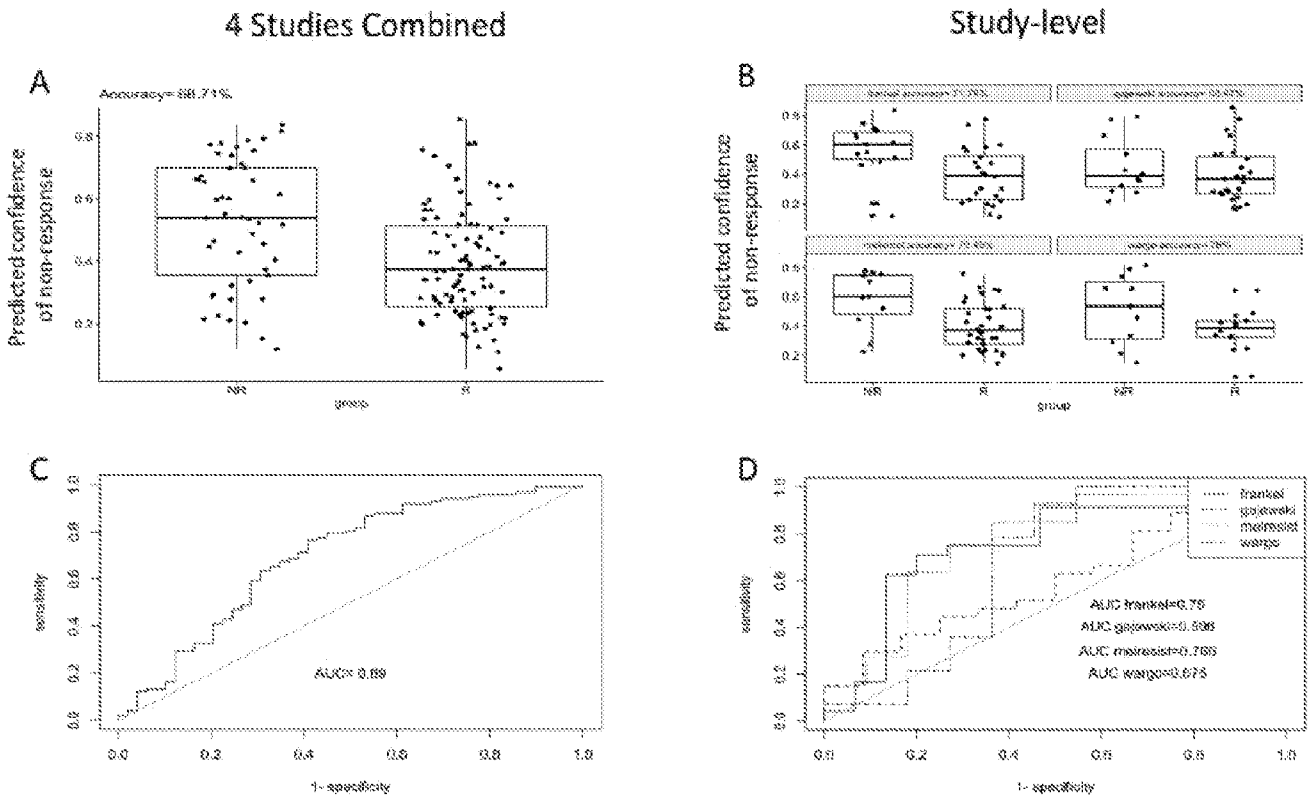

4 Studies Combined
Study-level
A
B
C
D
Predicted confidence of non-response
group
sensitivity
1- specificity
AUC= 0.89
frankel
gajewski
meiresist
wargo
AUC frankel=0.75
AUC gajewski=0.596
AUC meiresist=0.766
AUC wargo=0.675

Figure 7

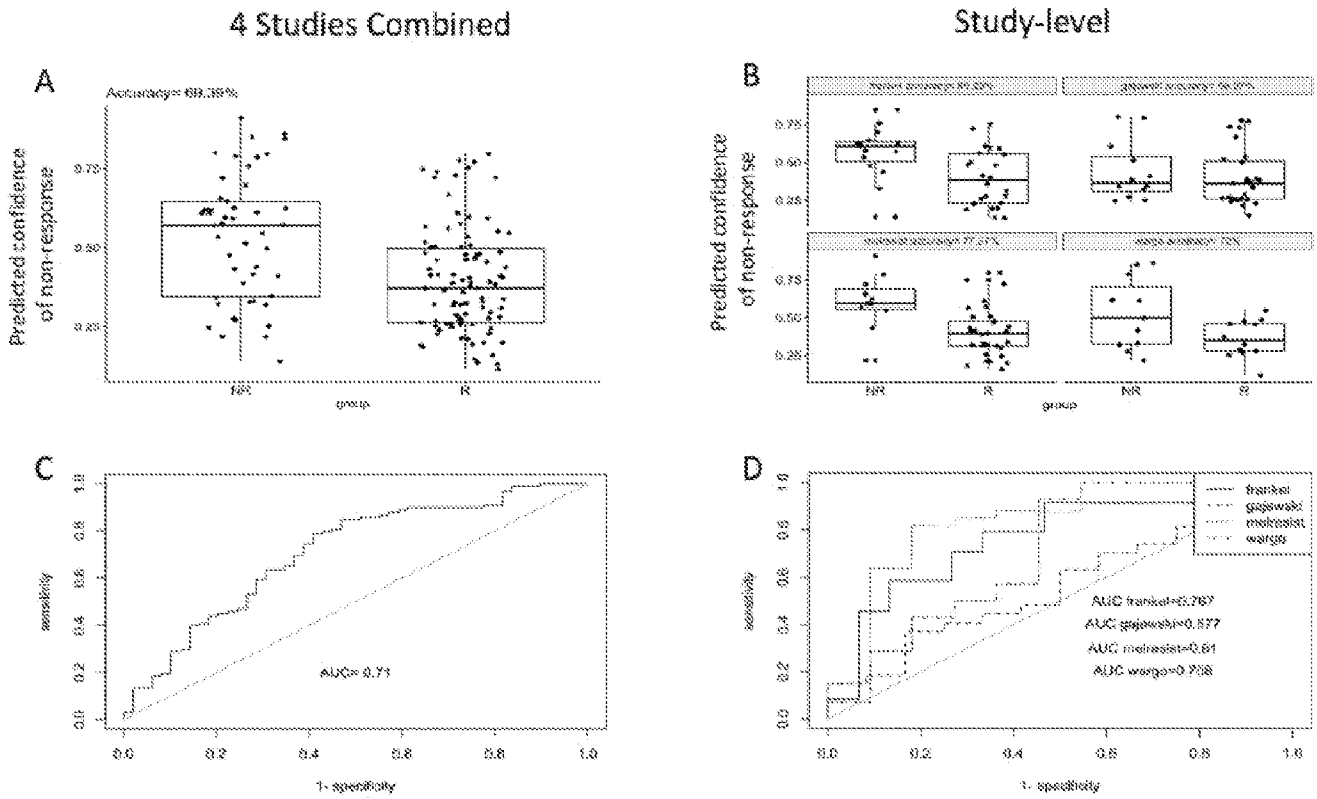

4 Studies Combined
Study-level
A
B
C
D
Predicted confidence of non-response
group
NR
R
sensitivity
1- specificity
AUC= 0.71
frankel
gajewski
melressist
wargo

Figure 8

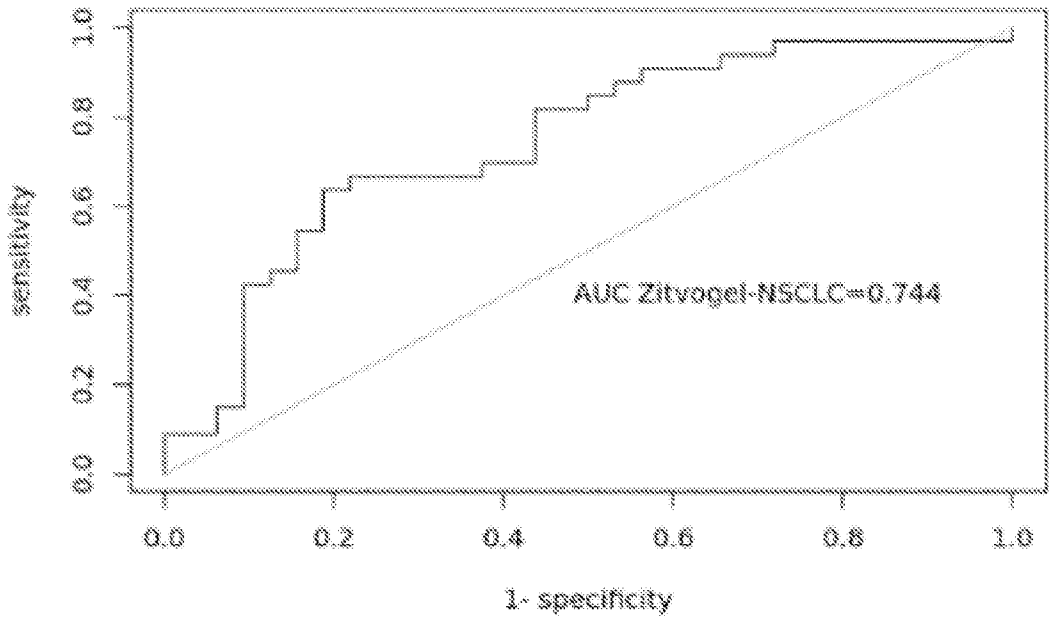

sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
AUC Zitvogel-NSCLC=0.744
1- specificity

Figure 9

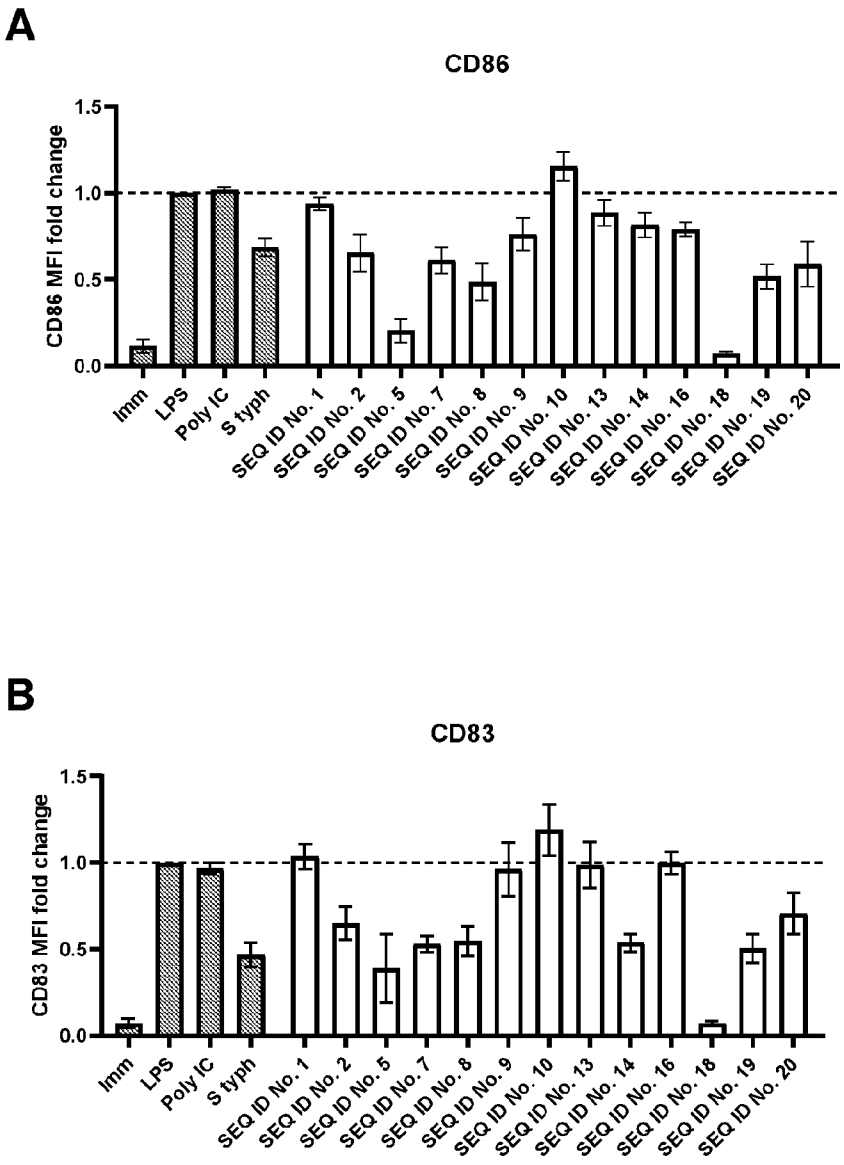
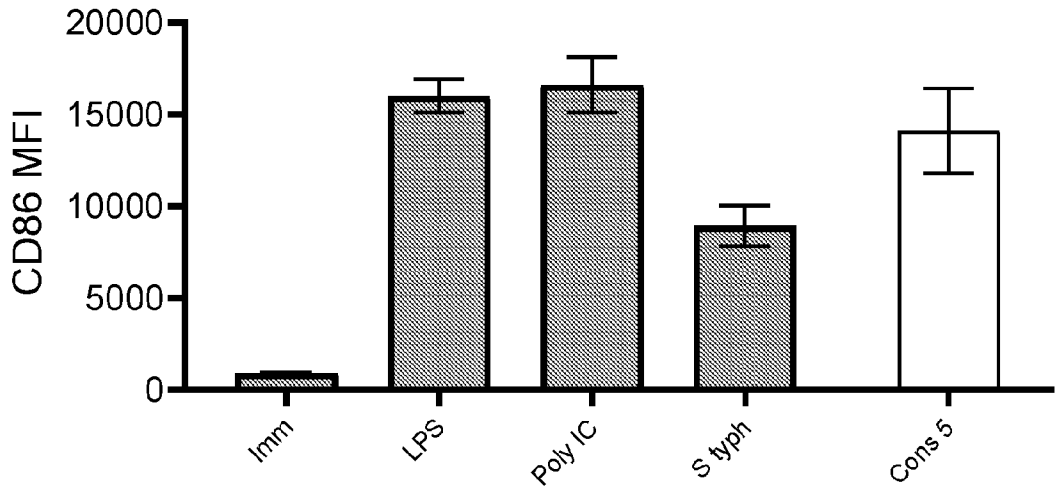

A
CD86
CD86 MFI fold change
1.5
1.0
0.5
0.0
Imm
LPS
Poly IC
S typh
SEQ ID No. 1
SEQ ID No. 2
SEQ ID No. 5
SEQ ID No. 7
SEQ ID No. 8
SEQ ID No. 9
SEQ ID No. 10
SEQ ID No. 13
SEQ ID No. 14
SEQ ID No. 16
SEQ ID No. 18
SEQ ID No. 19
SEQ ID No. 20
B
CD83
CD83 MFI fold change
1.5
1.0
0.5
0.0
Imm
LPS
Poly IC
S typh
SEQ ID No. 1
SEQ ID No. 2
SEQ ID No. 5
SEQ ID No. 7
SEQ ID No. 8
SEQ ID No. 9
SEQ ID No. 10
SEQ ID No. 13
SEQ ID No. 14
SEQ ID No. 16
SEQ ID No. 18
SEQ ID No. 19
SEQ ID No. 20

Figure 9

C


CD86 MFI
20000
15000
10000
5000
0
Imm
LPS
Poly IC
S typh
Cons 5

D

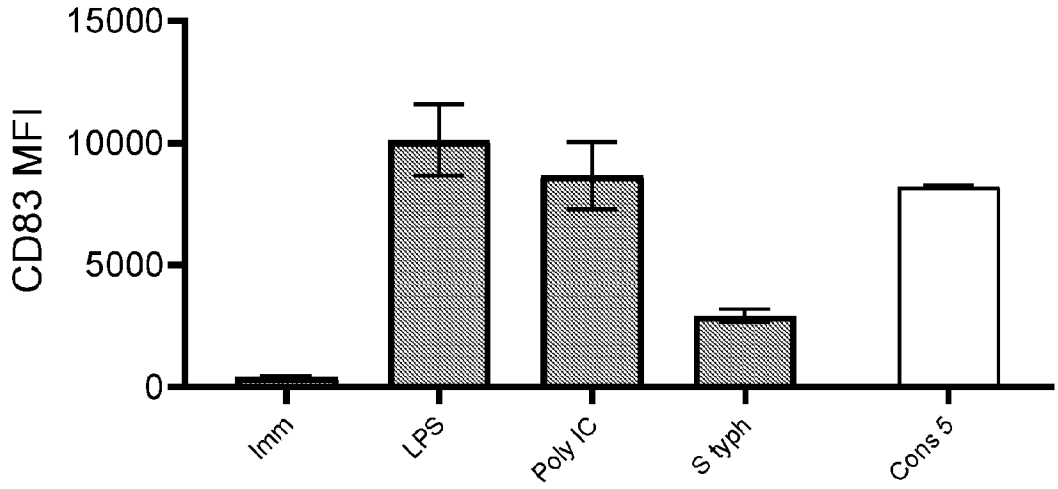
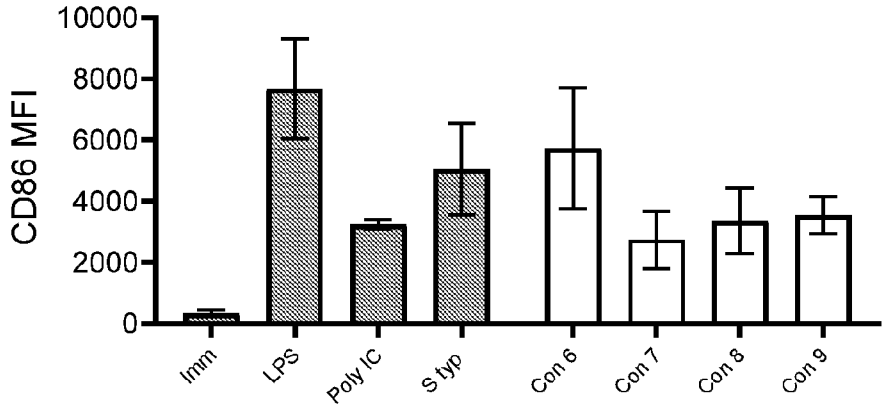
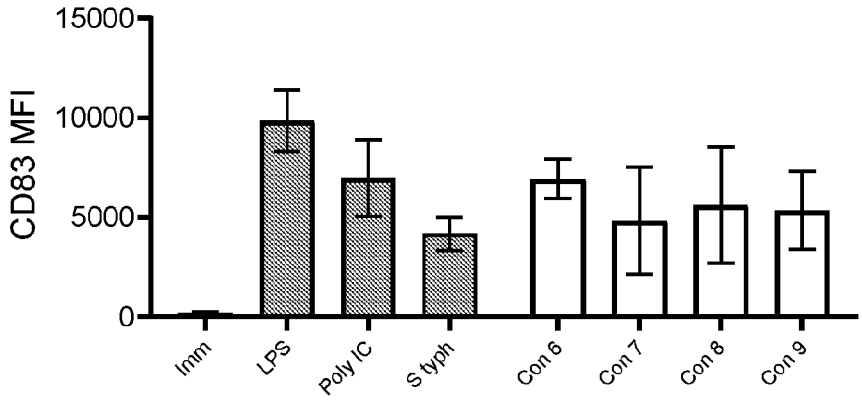

CD83 MFI
15000
10000
5000
0
Imm
LPS
Poly IC
S typh
Cons 5

Figure 9

E


CD86 MFI
10000
8000
6000
4000
2000
0
Imm
LPS
Poly IC
S typ
Con 6
Con 7
Con 8
Con 9

F


CD83 MFI
15000
10000
5000
0
Imm
LPS
Poly IC
S typh
Con 6
Con 7
Con 8
Con 9

G

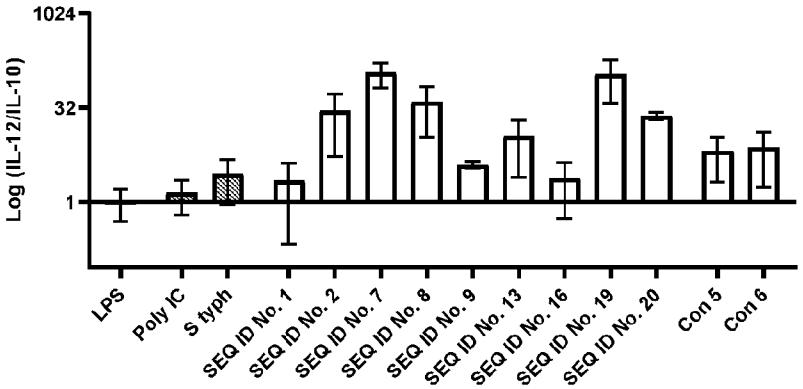
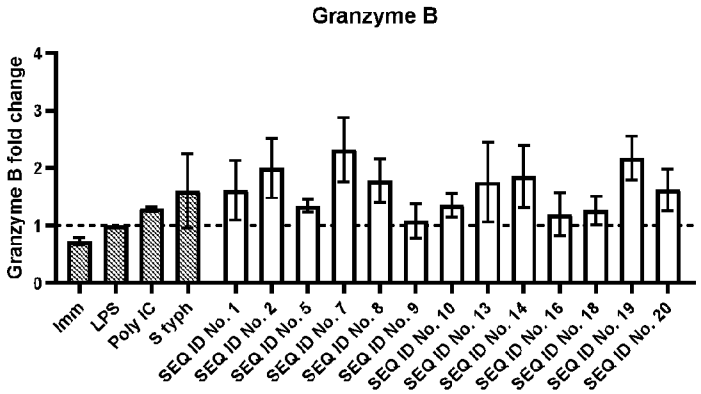
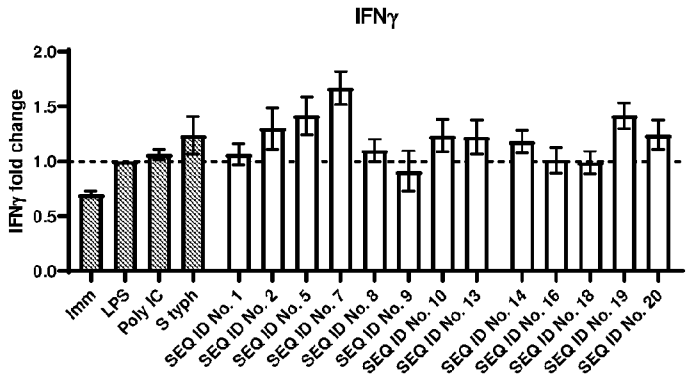

Log (IL-12/IL-10)
1024
32
1
LPS
Poly IC
S typh
SEQ ID No. 1
SEQ ID No. 2
SEQ ID No. 7
SEQ ID No. 8
SEQ ID No. 9
SEQ ID No. 13
SEQ ID No. 16
SEQ ID No. 19
SEQ ID No. 20
Con 5
Con 6

Figure 10

A



B



C

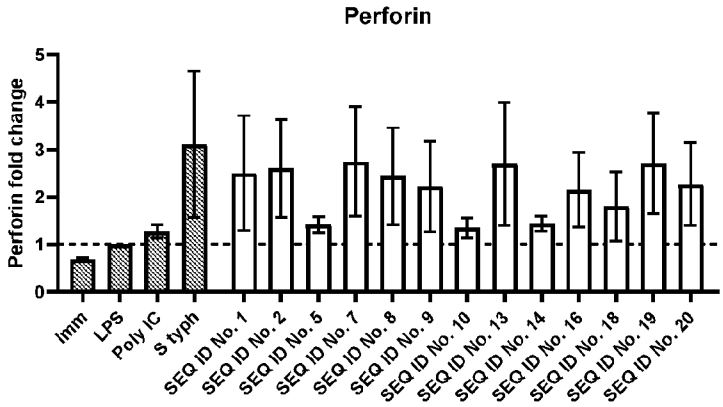


Figure 11

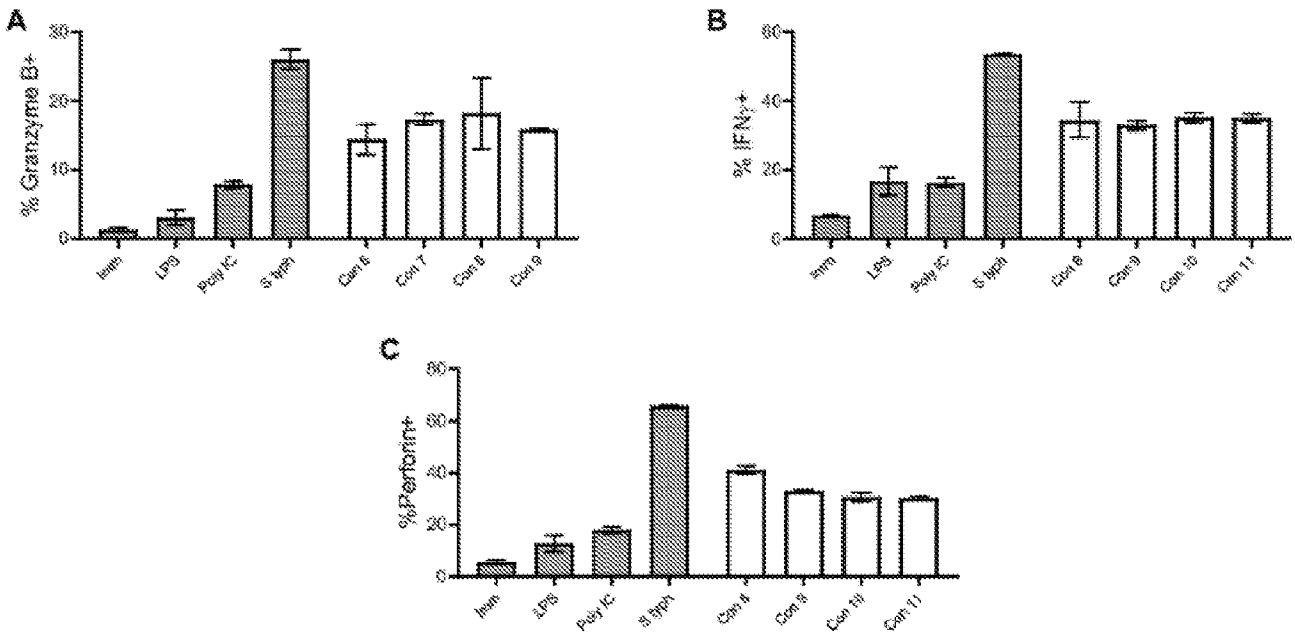

A
% Granzyme B+
Imm
LPS
Poly IC
S.typh
Con 6
Con 7
Con 8
Con 9
B
% IFNγ+
Imm
LPS
Poly IC
S.typh
Con 8
Con 9
Con 10
Con 11
C
%Perforin+
Imm
LPS
Poly IC
S.typh
Con 4
Con 5
Con 10
Con 11

Figure 12

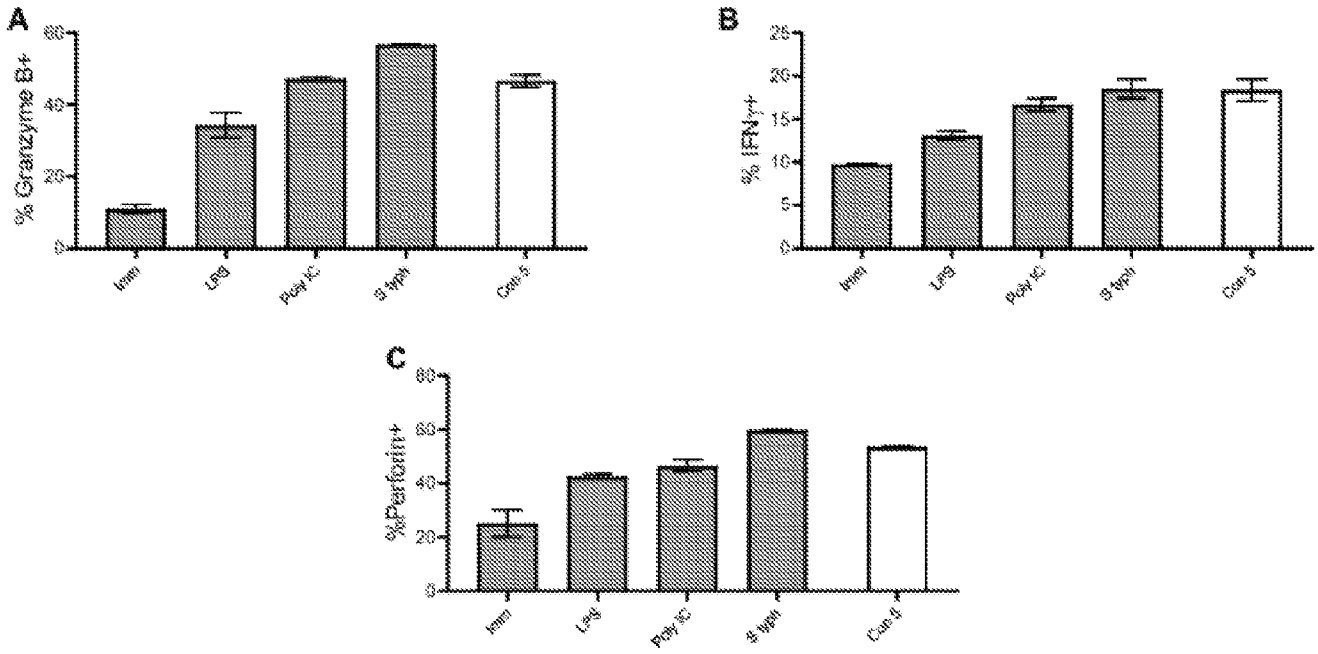

A
% Granzyme B+
0
20
40
60
Imm
LPS
Poly IC
S typh
Con-5
B
% IFNγ+
0
5
10
15
20
25
Imm
LPS
Poly IC
S typh
Con-5
C
%Perforin+
0
20
40
60
80
Imm
LPS
Poly IC
S typh
Con-5

Figure 13

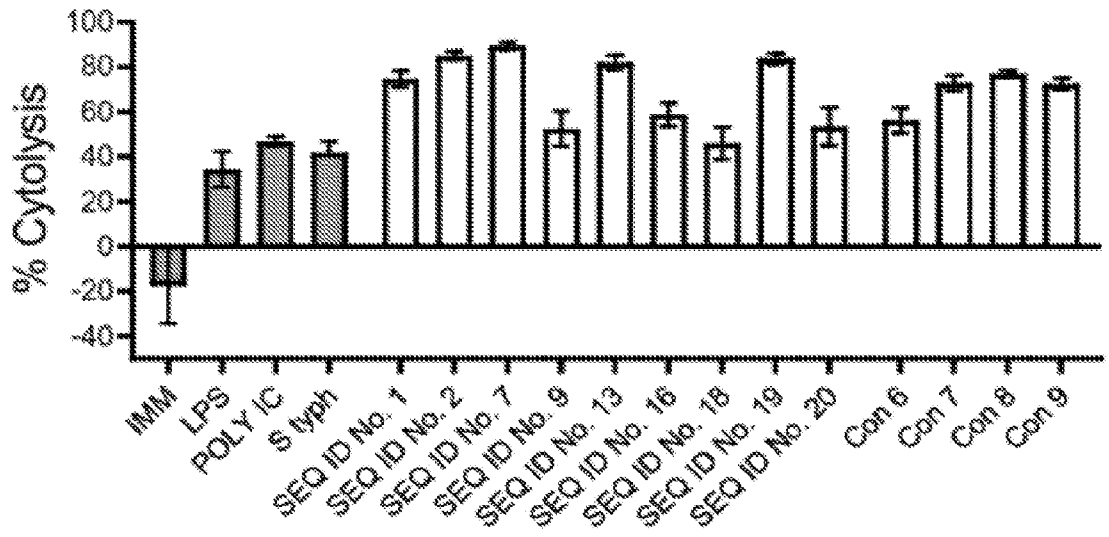

% Cytolysis
100
80
60
40
20
0
-20
-40
IMM
LPS
POLY IC
S typh
SEQ ID No. 1
SEQ ID No. 2
SEQ ID No. 7
SEQ ID No. 9
SEQ ID No. 13
SEQ ID No. 16
SEQ ID No. 18
SEQ ID No. 19
SEQ ID No. 20
Con 6
Con 7
Con 8
Con 9

Figure 14

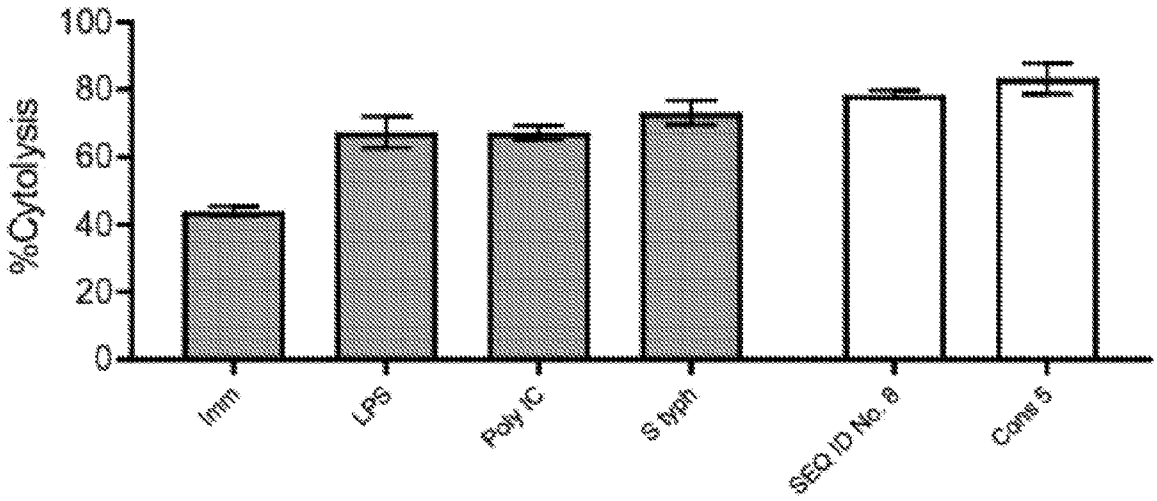

%Cytolysis
100
80
60
40
20
0
Imm
LPS
Poly IC
S typh
SEQ ID No. 8
Core 5

Figure 15

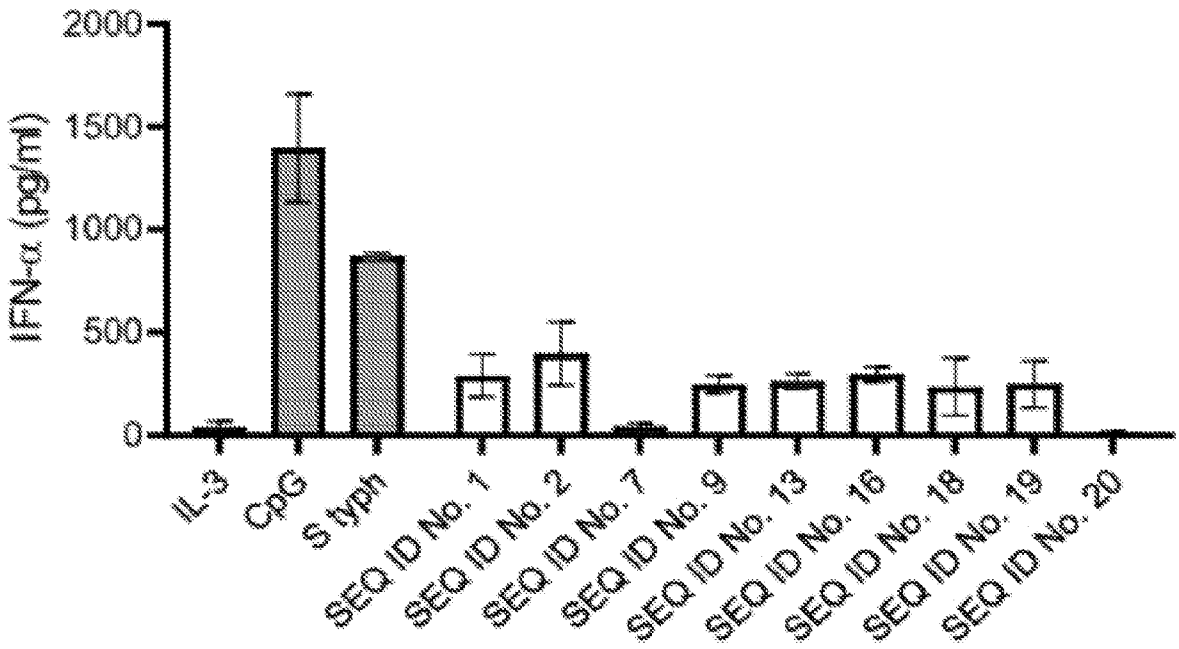
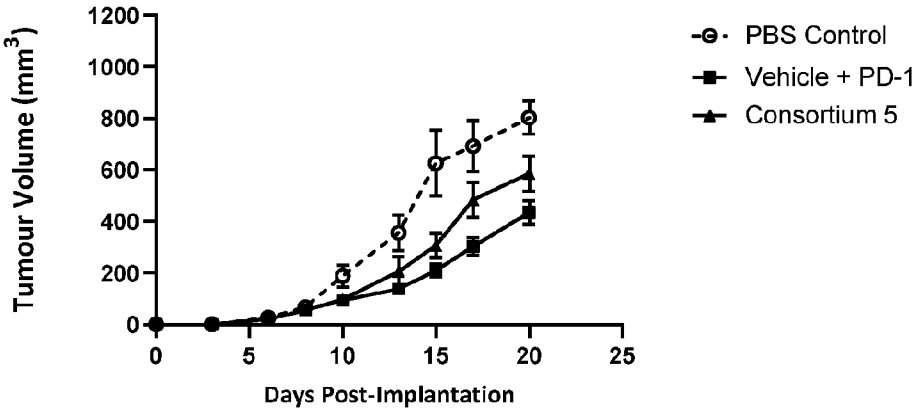

2000
1500
1000
500
0
IFN-α (pg/ml)
IL-3
CpG
S typh
SEQ ID No. 1
SEQ ID No. 2
SEQ ID No. 7
SEQ ID No. 9
SEQ ID No. 13
SEQ ID No. 16
SEQ ID No. 18
SEQ ID No. 19
SEQ ID No. 20

Figure 16

A


Tumour Volume (mm3)
1200
1000
800
600
400
200
0
0
5
10
15
20
25
Days Post-Implantation
PBS Control
Vehicle + PD-1
Consortium 5

B

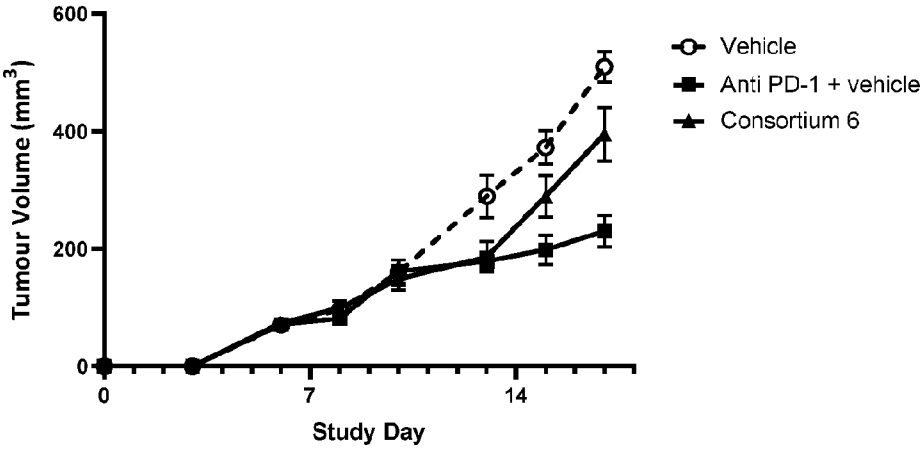
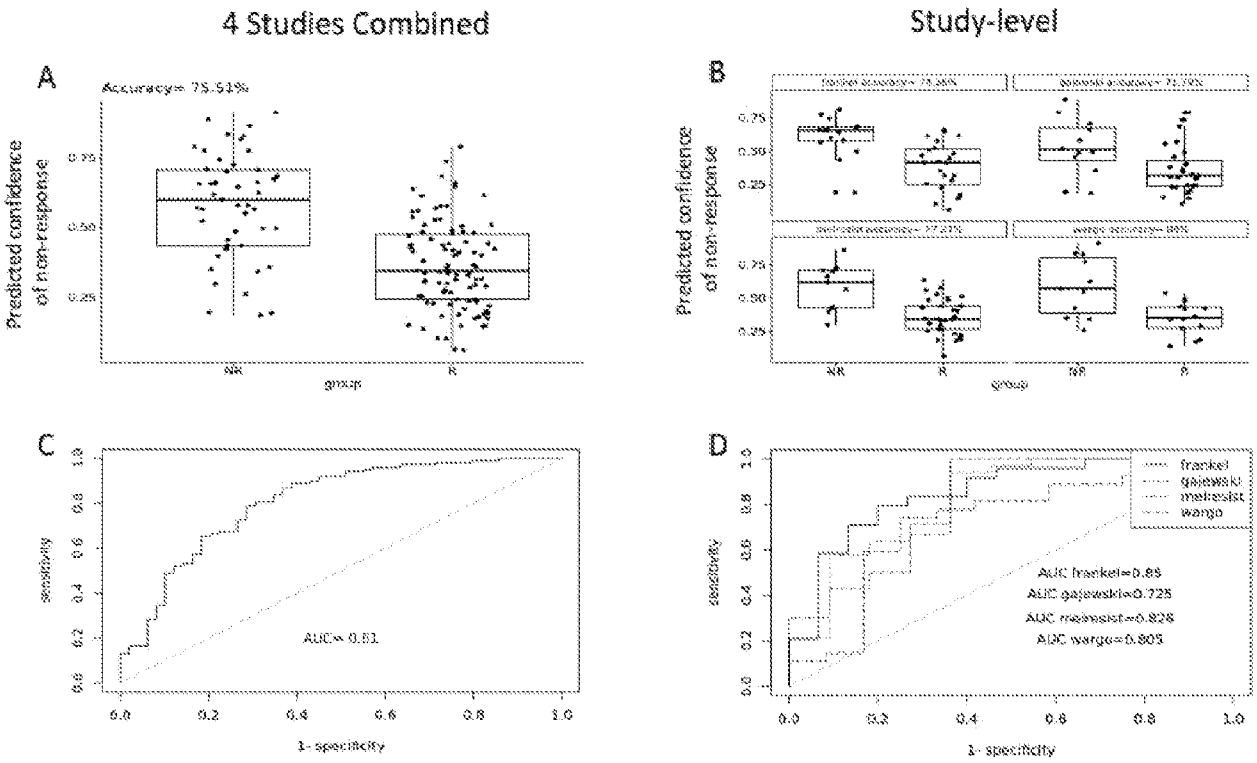

Tumour Volume (mm3)
600
400
200
0
0
7
14
Study Day
Vehicle
Anti PD-1 + vehicle
Consortium 6

Figure 17


4 Studies Combined
Study-level
A
B
C
D
Predicted confidence of non-response
group
NR
R
sensitivity
1- specificity
AUC= 0.81
frankel
gajewski
melressist
wargo
AUC frankel=0.85
AUC gajewski=0.725
AUC melressist=0.828
AUC wargo=0.805

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2021/051208

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/689
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2018/064165 A2 (UNIV TEXAS [US]) 5 April 2018 (2018-04-05) [0006],[0013] and [0014],[0008],[0028] -----	1-32
Y	WO 2018/115519 A1 (ROUSSY INST GUSTAVE [FR]; INST NAT SANTE RECH MED [FR] ET AL.) 28 June 2018 (2018-06-28) p. 6 l. 23-34,Tab. 1 p. 24-27, Tab. 2 p. 28, example 6 -----	1-20, 23-32
Y	WO 2019/191390 A2 (SERES THERAPEUTICS INC [US]; UNIV TEXAS [US]) 3 October 2019 (2019-10-03) [0005]-[0007], [0017], , tab. 1 -----	21,22
Y	EP 3 629 023 A1 (ROUSSY INST GUSTAVE [FR]) 1 April 2020 (2020-04-01) [0007], [0017] and Tab. 1 -----	11-15
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 2021	27/07/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Lapopin, Laurence

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2021/051208

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2021/051208

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JUN GONG ET AL: "The gut microbiome and response to immune checkpoint inhibitors: preclinical and clinical strategies", CLINICAL AND TRANSLATIONAL MEDICINE, vol. 8, no. 1, 18 March 2019 (2019-03-18), XP055751429, SE ISSN: 2001-1326, DOI: 10.1186/s40169-019-0225-x abstract, Tab. 2 and Fig. 2 -----	11-15

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2021/051208

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018064165 A2	05-04-2018	AU 2017335732 A1	04-04-2019
		BR 112019006041 A2	03-09-2019
		CA 3038076 A1	05-04-2018
		CN 110267651 A	20-09-2019
		EP 3518946 A2	07-08-2019
		JP 2020500151 A	09-01-2020
		KR 20190061042 A	04-06-2019
		RU 2019108925 A	29-10-2020
		US 2020129569 A1	30-04-2020
		WO 2018064165 A2	05-04-2018
WO 2018115519 A1	28-06-2018	AU 2017380257 A1	04-07-2019
		CA 3047029 A1	28-06-2018
		CN 110582291 A	17-12-2019
		EP 3559257 A1	30-10-2019
		JP 2020512294 A	23-04-2020
		KR 20190118151 A	17-10-2019
		WO 2018115519 A1	28-06-2018
WO 2019191390 A2	03-10-2019	AU 2019242875 A1	22-10-2020
		BR 112020019863 A2	23-03-2021
		CA 3095356 A1	03-10-2019
		CN 112469478 A	09-03-2021
		EP 3773915 A2	17-02-2021
		KR 20210018793 A	18-02-2021
		WO 2019191390 A2	03-10-2019
EP 3629023 A1	01-04-2020	AU 2019348525 A1	13-05-2021
		CA 3110671 A1	02-04-2020
		EP 3629023 A1	01-04-2020
		EP 3857229 A1	04-08-2021
		KR 20210068484 A	09-06-2021
		SG 11202101586Q A	29-04-2021
		WO 2020064997 A1	02-04-2020