

Immunogenic sequences from a Phage Tail Length Tape Measure Protein, bacteria expressing the same and their use in treating a cancer

FIELD OF THE INVENTION

5 The present invention relates to the field of probiotic adjuvantization of anticancer treatments. In particular, the present invention concerns immunogenic sequences from a prophage present in bacteria identified as efficient adjuvants of cancer treatments. The invention provides bacterial compositions expressing immunogenic sequences from this prophage and methods using sequences of this prophage, for increasing the anticancer armamentarium.

10 **BACKGROUND OF THE INVENTION**

Cancer incidence and progression results from a complex interplay between gene regulation and the environment (Hanahan and Weinberg, 2011). Many epithelial and hematopoietic neoplasias are thought to be under strong immunosurveillance, as indicated by numerous studies revealing that the density, composition, and functional state of immune cells that infiltrate tumors dictate patient prognosis, as well as therapeutic response to adjuvant or neoadjuvant chemotherapy (Ingold Heppner et al., 2016; Palucka and Coussens, 2016) and immune checkpoint blockers (Hodi et al., 2010; Ribas, 2015; Robert et al., 2015). The recognition of cancer cells by immune effectors relies on two parameters, namely, antigenicity (the presence of tumor-associated antigens that are derived from mutations yielding mutated proteins, or the ectopic expression of genes/proteins that are normally only present in embryonic development or in testis) and adjuvanticity (the presence of co-stimulatory signals that activate innate immune effectors) (Zitvogel et al., 2016). Commensal microbial communities inhabiting the intestine, as well as other places in the body, appear to play an unappreciated role in intestinal and extraintestinal carcinogenesis by providing yet to be characterized environmental signals (Zitvogel et al., 2015). Pioneering studies performed in germ-free, gnotobiotic, or antibiotic-treated rodents have revealed an unsuspected role for commensals in tumorigenesis, irrespective of the role of inflammation. In the genesis of colon cancer or hepatocarcinoma, microbes can be direct transforming agents (Abreu and Peek, 2014; Sears and Garrett, 2014), by providing a toxic metabolite, an oncogenic product or by inducing an inflammatory milieu which will culminate in genomic instability and/or DNA damage response and/or immune escape (Garrett, 2015; Gur et al., 2015; Louis et al., 2014). Commensals can also form cooperative biofilms that facilitate cross-feeding or cross-metabolism, redefining the

cancer landscape (Bongers et al., 2014; Dejea et al., 2014). Recently, the development of extraintestinal (breast and ovarian) neoplasias were linked to TLR5- mediated IL-6 or IL-17 driven systemic inflammation provoked by intestinal microbes (Rutkowski et al., 2015).

5 In contrast, other observations support a beneficial role for bacteria in combatting cancer. Prolonged antibiotic treatment with a combination of metronidazole and ciprofloxacin subsequently tripled breast cancer (BC) incidence in protooncogene HER2/neu driven-transgenic mice (Rossini et al., 2006). In humans, epidemiological studies suggest a dose-dependent association between antibiotic use and risk of BC
10 (Blaser, 2011). The beneficial role of intestinal microbiota was first shown *via* total body irradiation, promoting LPS/TLR4-dependent activation of antigen presenting cells that facilitated the efficacy of adoptive T cell transfer (Paulos et al., 2007). During platinum-based anticancer therapy and immunomodulatory regimens, bacterial-associated TLR4 agonists accounted for the ROS and TNF α -mediated antitumor effects of tumor
15 infiltrating myeloid cells (Iida et al., 2013).

Antitumor efficacy of metronomic dosing of the alkylating agent cyclophosphamide (CTX) was also showed to be compromised in germ-free or specific pathogen free animals treated with broad spectrum antibiotics (ATBs) (Daillère et al., 2016; Viaud et al., 2013). Indeed, CTX altered the integrity of the intestinal barrier,
20 promoting the translocation of distinct Gram⁺ bacteria. Bacterial translocation occurs when commensal gut microbes invade through the gut mucosa to underlying sterile tissues and organs. This phenomenon allowed Gram⁺ bacteria to mount effector pathogenic CXCR3⁺CCR6⁺ (IL-17⁺IFN γ ⁺) Th17 (abbreviated, pathogenic pTh17) and memory Th1 immune responses associated with tumor control. *E. hirae* and *Barnesiella*
25 *intestinihominis* were identified as the species acting in concert to reshape the tumor microenvironment post-CTX. The small intestine resident Gram⁺ bacteria *E. hirae* induced tumor antigen-specific, MHC class I-restricted cytotoxic IFN γ ⁺ CD8⁺ T cells (CTL), a decrease in intratumoral regulatory T cells (Treg), and led to an increase in the CTL/Treg ratio commonly associated with tumor control. The colon resident Gram-
30 *B. intestinihominis* boosted systemic polyfunctional Tc1/Th1 responses, reinstated intratumoral IFN γ producing $\gamma\delta$ T cells, and reduced $\gamma\delta$ T17 cells in the tumor microenvironment, traits associated with tumor control. These two immunogenic commensals are kept in check by intestinal NOD2 receptors, which limit bacterial accumulation (for *B. intestinihominis*) or translocation (for *E. hirae*) into secondary
35 lymphoid organs. In addition, while CTX plus cancer vaccine (B subunit of Shiga toxin fused with HPV-16 E7 antigen) no longer protected the host against the E7-expressing TC-1 tumor in combination with antibiotic treatment, oral gavages with *E. hirae* (but not

L. johnsonii nor *E. coli*) restored the accumulation of E7 tetramer-binding CD8⁺ CTLs leading to tumor rejection. In this model, *E. hirae* mediated anti-tumor effects. Finally, the immunomodulatory role of these two commensals in mice is relevant to cancer-bearing patients. Memory MHC class II-restricted Th1 immune responses against *E. hirae* or *B. intestinihominis* (and not 9 other commensals) were associated with prolonged progression-free survival in end stage lung and ovarian cancer patients who were previously treated with chemotherapy (Daillère et al., 2016). Recently, Zitvogel and others extended these findings to immune checkpoint blockers, demonstrating that distinct intestinal bacterial species belonging to *Bacteroidales* and *Burkholderiales* or *Bifidobacteriales* orders influenced the tumor microenvironment, contributing to the efficacy of anti-CTLA4 or anti-PDL-1 Abs respectively (Sivan et al., 2015; Vétizou et al., 2015). Hence, it is postulated that the intestinal microbiota ecosystem controls not only gut immune homeostasis but also the inflammatory/immune tone of secondary lymphoid organs, thereby shaping the tumor microenvironment throughout the body.

To demonstrate a causal relationship between translocated Gram⁺ bacteria and CTX-induced tumoricidal activity, Daillère et al. colonized mouse intestines with 10⁹ *E. hirae* (clone 13144 and other isolates), *L. johnsonii* or control bacteria in MCA205 sarcoma-bearing mice rendered dysbiotic by a 14 day-ATBs regimen. ATBs prevented the CTX-mediated control of tumor progression. However, oral gavage with *E. hirae* strain 13144 (EH13144) selectively restored the CTX-mediated antitumor effects while *L. johnsonii*, *E. coli* or *L. plantarum* isolates failed to do so, despite comparable gut colonization (Daillère et al., 2016). They next selected the best anticancer probiotic capable of boosting the CTX-mediated antitumor effects and tackled the mechanism by which it occurred, and tested various *E. hirae* strains to analyze their differential immunogenicity *in vivo* and their "oncomicrobiotic" (anticancer probiotic) properties. An investigation of the clonal relationship between these *E. hirae* isolates, performed by rep-PCR, revealed significant genomic diversity among the strains derived from human, mouse or environmental ecosystems (Daillère et al., 2016). Half of the isolates induced pTh17 and Th1 immune responses; only one human isolate (clone 708) induced IFN γ producing CD8⁺ T (Tc1) cells in naïve mice associated with some oncomicrobiotic properties. The only human isolate of *E. hirae* (clone EH17) capable of forming *ex vivo* biofilms in adherence assays harboured no immunogenic nor oncomicrobiotic properties (Daillère et al., 2016).

Since the publication of Daillère et al. (2016), the inventors isolated 3 novel clones of *E. hirae* (EH) endowed with high immunogenicity and derived from human stools (clone IGR7, clone IGR4 and clone IGR11) that exhibited antitumor effects.

In summary, EH13144, EH clone IGR7 and, to a lesser extent, clone IGR4 and clone IGR11, exerted a significant capacity to induce pTh17 cells in secondary lymphoid organs of CTX-treated animals, which was associated with cancer antigen-specific CTL responses and oncomicrobiotic properties, whereas the EH17 strain did not. The reasons for these differences were however unknown, and the inventors pursued their research to identify the factor(s) responsible for the immunogenic properties of the murine strain EH13144 and the human clones IGR4, IGR7 and IGR11, in order to derive new molecules or microorganisms useful in the treatment of cancer.

The inventors screened several human *E. hirae* isolates cultivated from human patients' feces and compared them with the human EH708 and the mouse EH13144. They cultivated 11 novel isolates of *E. hirae* from feces of non small cell lung cancer patients who responded to anti-PD1 Ab. Among these 11 novel isolates tested in the MCA205 tumor model together with CTX, clone IGR4, clone IGR11 to some extent and clone IGR7 were effective at synergizing with CTX, alone or best, when combined together.

They found that the mouse EH13144 shares a very high sequence homology with the human clone IGR7 (and falls into the same clade in the dendrogram of >20 EH strains), both being very special isolates with a unique immunogenicity related to the presence of a phage inserted into their genomic sequence. They identified that the immunogenicity relies on the temperate bacteriophage tail tape measure protein (TMP). Additionally, the combination of EH13144 + clone IGR4 + clone IGR7 exhibited additive antitumor effects combined with CTX.

SUMMARY OF THE INVENTION

According to a first aspect, the present invention pertains to a bacterial composition comprising bacteria selected from the group consisting of:

- (i) *Enterococcus hirae* strain 13144 (aka EHFS001) deposited on November 7, 2013 at the Collection Nationale de Cultures de Microorganismes (CNCM) under the number I-4815,
- (ii) *Enterococcus hirae* strain IGR7 deposited on August 31, 2017 at the CNCM under the number I-5224,
- (iii) *Enterococcus hirae* strain IGR11 deposited on November 27, 2017, at the CNCM under the number I-5261,
- (iv) any other bacterial strain expressing a protein with at least 65, preferably at least 80 and more preferably at least 95% identity with a fragment of at least 20, preferably at least 30 and more preferably at least 40 nucleotides from the

protein of SEQ ID No: 1 (*i.e.*, the TMP of the prophage identified as being responsible for the remarkable immunogenic properties of *Enterococcus hirae* strain 13144, CNCM I-4815), and

(v) mixtures of at least two of the strains recited in (i) to (iv).

5 The invention also relates to the use of the above bacterial composition for treating a cancer.

According to one embodiment, the composition according to the present invention is used in combination with an antineoplastic drug, for treating a cancer.

10 According to another aspect, the invention pertains to a method of increasing the immunogenicity of a bacterial strain of anticancer interest, comprising *in vitro* introducing, into said strain, a nucleotide sequence encoding the protein of SEQ ID No: 1 of a fragment thereof identified as being immunogenic, such as a fragment comprising at least the peptides of SEQ ID Nos: 13 and 14, or a sequence encoding a peptide of at least 9, preferably at least 20 amino acids comprising at least one of the
15 epitopes likely to be presented by a human HLA molecule, such as epitopes selected from the group consisting of SEQ ID No: 53 to 187

A bacterial strain which has been obtained by the above method is also part of the present invention, as well as its use in treating a cancer.

20 The present invention also relates to an immunogenic composition comprising a polypeptide comprising a sequence of at least 9 consecutive amino acids from the TMP of SEQ ID No: 1 or a polynucleotide encoding the same, for use as an anticancer vaccine.

25 According to another aspect, the present invention pertains to a cell composition comprising antigen presenting cells (APC) which have been pulsed *ex vivo* with a bacterial composition or an immunogenic composition according to the invention.

According to an embodiment, the invention pertains to an MHC multimer for isolating T-cells with high affinity for the protein of SEQ ID No: 1, wherein MHC molecules are bound to an epitope selected from the group consisting of SEQ ID No: 53-187.

30 The invention also pertains to a bacteriophage composition, wherein said bacteriophage expresses a protein having at least 80, preferably at least 90 and more preferably at least 95% identity with the protein of SEQ ID No: 1, as well as its use for treating a cancer.

35 According to one embodiment, the invention pertains to a screening method for identifying antineoplastic drugs, comprising using bacteria from the strain CNCM I-4815 for assessing the ability of drug candidates to trigger the lytic cycle of the phage comprising the protein of SEQ ID No: 1.

The present invention also pertains to a method of determining if a patient is likely to be a good responder to a treatment by chemotherapy or immune checkpoint blockade, comprising assessing the presence, in a biological sample from said patient, of a sequence having at least 80% identity with the protein of SEQ ID No: 1, wherein if
 5 such a sequence is present in the sample, the patient is likely to respond to the treatment.

The present invention also pertains to a method of determining if a patient is likely to be a good responder to a treatment by chemotherapy or immune checkpoint blockade, comprising measuring the levels of circulating CCR9+CXCR3+ CD8+ T cells during said treatment, wherein if said level is above a predetermined threshold, the
 10 patient is likely to respond to the treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Experimental setting. (A) Mice are treated with broad spectrum antibiotics (streptomycin, colistin, ampicillin and vancomycine) for 3 days before performing Fecal Microbiota Transplantation (FMT). 14 days post-FMT, MCA-205
 15 sarcoma cell lines are inoculated in the right flank of mice ($8 \cdot 10^5$ cells per mice). Chemotherapy (Cyclophosphamide, CTX - 100 mg/kg) or saline solution (NaCl) is injected weekly ip, starting 5 days post tumor inoculation for a total of 3 injections. Oral gavage with *E. hirae* strain 13144 ($1 \cdot 10^9$ bacteria) is performed the day of CTX injection as well as the day after. **(B)** Non FMT-treated SPF mice are used as control.

Figure 2. Dysbiosis by FMT BC patient 1: Effectiveness of *E. hirae* 13144 to restore CTX-mediated antitumor effects. Tumor growth curves of MCA-205 sarcoma in SPF mice **(A)** vs FMT-treated mice **(B)** injected with 3 cycles of CTX vs NaCl. **(C)** Tumor growth curves of MCA-205 sarcoma in FMT-treated mice after oral gavage with strain *E. hirae* 13144 or NaCl as control. 2 independent experiments are depicted.
 20 **(D)** Overall survival of FMT-treated, MCA-205 sarcoma bearing mice treated with CTX or NaCl after oral gavage with strain *E. hirae* 13144. A typical survival curve is depicted for six mice per group. **(E)** Concatenated data of tumor sizes at day 21 post-CTX from 2 independent experiments are shown. Anova & Student t'-test statistical analyses: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Figure 3. No dysbiosis by FMT BC patient 2: *E. hirae* 13144 ameliorates the CTX-mediated antitumor effects. Tumor growth curves of MCA-205 sarcoma in SPF mice **(A)** vs FMT-treated mice **(B)** injected with 3 cycles of CTX vs NaCl. **(C)** Tumor growth curves of MCA-205 sarcoma in FMT-treated mice after oral gavage with strain *E. hirae* 13144 or NaCl as control. 2 independent experiments are depicted.
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(D) Concatenated data of tumor sizes at day 21 post-CTX from 2 independent experiments are shown. Anova & Student t'-test statistical analyses: $*p<0.05$, $**p<0.01$, $***p<0.001$.

Figure 4. *E. hirae* IGR1, IGR10 and 10815 failed to efficiently boost

5 **CTX tumoricidal activity in a dysbiotic FMT setting.** Tumor growth curves of MCA-205 sarcoma in FMT-treated mice after oral gavage with strain *E. hirae* 13144 (A), IGR1 (B) or NaCl as control. A typical curve is depicted for six mice per group. (C) Concatenated data of tumor sizes at day 17 post-CTX from 1-2 independent experiments are shown. (D). Comparaison of various strains of *E. hirae* for their capacity to control
10 tumor growth. Identification and discovery of two novel strains EH IGR 4 and 7 which display efficacy comparable to EH 13144. EH10815 fails to work and that may be related to a mutation residing in TMP2 (see below Fig. 13B). Student t'-test statistical analyses: $*p<0.05$, $**p<0.01$, $***p<0.001$.

Figure 5. The combination of EH13144 + IGR4 + IGR7 is superior to

15 **EH13144 leading compound. (A)** Tumor surface of MCA-205 sarcoma in FMT-treated mice after oral gavage with strain *E. hirae* 13144, IGR4, IGR7, Actinomycetes spp (Ao) and injected with 3 cycles of CTX (C) vs NaCl (N). Each dot is one mouse/tumor. (B) Tumor growth curves of MCA-205 sarcoma in SPF mice of the best group, means +SEM of tumor sizes at each time point and tumor sizes at sacrifice (C: CTX). Each dot is one
20 mouse/tumor. Anova statistics indicating significant differences between CTX and the triple combination. (C) Tumor surface of MCA-205 sarcoma in FMT-treated mice after oral gavage with strains EH13144, IGR1, IGR11, Barnesiella or Akkermansia and injected with 3 cycles of CTX. Bacterial strains are used alive (V) or pasteurized (P). For pasteurization, bacteria are incubated 30 min at 70°C and frozen at -80°C at least
25 6 hours.

Figure 6. Mouse *E. hirae* 13144 mediates its efficacy only when used

alive. Tumor growth curves of MCA-205 sarcoma in FMT-treated mice after oral gavage with strain *E. hirae* 13144 alive (A), pasteurized (B) or NaCl as control. A typical curve is depicted for six mice per group. (C) Concatenated data of tumor sizes at day 17 post-
30 CTX from 3 independent experiments are shown. Student t'-test statistical analyses: $*p<0.05$, $**p<0.01$, $***p<0.001$.

Figure 7. Increased accumulation of CD8+ T cells in mLN and spleen post-oral gavages with *E. hirae* 13144. (A) Experimental setting. Mice are treated with broad spectrum antibiotics for 3 days before performing oral gavage with *E. hirae* (708

35 or 13144) and CTX ip injection. One day post-CTX, a second oral gavage with *E. hirae*

is performed. 72 hours post-CTX, CD4+ and CD8+ T cells are isolated from mesenteric lymph node (mLN). Percentage of CD8+ **(B)** and CD4+ **(C)** T cells in mLN after oral gavage with *E.hirae* 708 or 13144 and CTX treatment. **(D)** Experimental setting. Mice are treated with broad spectrum antibiotics for 3 days before performing oral gavage with *E. hirae* (708 or 13144) every three days for a total of 4 gavages. 5 days after the first oral gavage, mice are treated with CTX. One week post-CTX, CD8+ T cells are isolated from spleen. Percentage of CD8+T cells **(E)** and CCR9+CXCR3+CD8 T cells **(F)** in spleen after oral gavage with *E. hirae* 708 or 13144 and CTX treatment. Student t'-test statistical analyses: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Figure 8. Sustained accumulation of CD8+ CCR9+CXCR3+ T cells in tumor beds post oral gavage with EH13144: considering CD8+CXCR3+CCR9+ as a tumor associated-biomarker of sustained anticancer responses with OncoBax. **(A)** SPF mice were treated with ATB for 3 days and then oral gavages with EH13144 before and after CTX systemic administration, every other week for 3 weeks. Mice were sacrificed 7, 14 and 21 day after the first CTX treatment to harvest spleen, tumor draining lymph node (dLN) and tumor (MCA205) **(B)** FMT-treated mice were subjected to oral gavages with EH13144 before and after CTX systemic administration, every other week for 3 weeks. Mice were sacrificed the day of CTX treatment and 72 hrs post-CTX 1, 2, 3 (day J0, J3, J7, J10, J14, J17) to harvest dLN and tumor (MCA205). Flow cytometry analyses of CD45+ cells gating on CD8+CD3+ analyzing the percentages of CCR9+CXCR3+ double positive cells are presented. A representative experiment of 3 groups comprising 5 mice/group. Anova statistical analyses: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Figure 9. Experimental immunization protocols to elucidate the immunogenicity of EH peptides. Mice are treated with broad spectrum antibiotics for 3 days before performing oral gavage with *E. hirae* (708 or 13144) every three days for a total of 4 gavages. 5 days after the first oral gavage, mice are treated with CTX. One week post-CTX, CD8+ T cells are isolated from spleen. These CD8+ T cells are incubated with dendritic cells (DCs) pulsed with 20 µg/ml of peptides (during 1 hour) or with heat-inactivated bacteria (during 6 hours). Inactivation of bacteria consists in incubation at 65°C during 2 hours. After 24 hours of culture, ELISpot of IFN γ is performed.

Figure 10. Cross-reactivity between the two *E. hirae* 708 and 13144 species for CTL splenic reactivity. Number of IFN γ spot representing IFN γ -secreting CD8+T cells after co-culture with DCs pre-incubated with or without heat-inactivated

bacteria (708, 13144, EH17 and *L. plantarum*). The corresponding experimental setting is indicated in figure 9. Student t'-test statistical analyses: $*p<0.05$, $**p<0.01$, $***p<0.001$.

Figure 11. Effects of EH on gut inflammation and role of CD8+ T cells in this inflammation: EH-specific CTL amplified in the mLN may return into the lamina propria. (A) Experimental setting. Mice are treated with broad spectrum antibiotics for 3 days. Then, we performed oral gavage with *E. hirae* (708 or 13144) and anti-CD8 injection (200µg per mice) every three days for a total of 4 gavages/injections. 5 days after the first oral gavage, mice are treated with CTX. One week post-CTX, colon is removed for Hematoxylin Eosin Staining (HES). (B) Extent of inflammatory infiltrates in colon of mice colonized with *E. hirae* and treated with anti-CD8 ablating Abs and CTX. Student t'-test statistical analyses: $*p<0.05$, $**p<0.01$.

Figure 12. Schematic overview of the filters used to select the MHC class I binding peptides from EH. From whole bacterial sequences of *E. hirae* (708, 13144 and EH17), we selected proteins with cell wall and extracellular localization with PSORT software. Then, proteins were divided in peptides of nine amino acids. These peptides were tested for their ability to bind MHC class I H-2K^b with NetMHC software and only strong-binders were retained (IC₅₀ < 50nM).

Figure 13. Peptides from group 7 containing the TMP1 and TMP2 epitopes activate CD8+ T cells from spleen of mice immunized against *E. hirae* 13144. Number of IFNγ spot, representing IFNγ-secreting CD8+T cells, after incubation with DCs pulsed with 13 different group of peptide (Table 6). Just group n°1 (A) and group n°7 (B) are shown. (C, E) Number of IFNγ spot, representing IFNγ-secreting CD8+T cells, after incubation with DCs pulsed with 4 peptides belong to group n°7 (in table 7). The corresponding experimental setting is indicated in figure 9. (D) Sequence alignment of TMP1 and TMP2 of EH10815 compared to EH13144 at the level of immunogenic peptides. (E) *In vitro* reactivity of splenic CTL in a recall response to TMP1 and TMP2 as well as Group7 after in vivo immunization with other strains of EH. Anova statistical analyses: $*p<0.05$, $**p<0.01$, $***p<0.001$.

Figure 14. TMP epitope 1 prophage 2 specific CD8+ T cells accumulate in dLN and spleen post oral gavage with EH13144 and IGR7. Mice are treated with broad spectrum antibiotics for 3 days before performing oral gavage with *E. hirae* 13144 (A,B) or 10815 or EH17 or IGR7 (C) and CTX ip injection. The corresponding experimental setting is indicated in figure 7A for mLN and figure 7D for spleen. TMP1 specific CD8+ T cells are isolated with tetramer from mesenteric lymph node (A) and spleen (B,C). Flow cytometry analysis of TMP1 prophage 2 specific CD8+T

cells and TMP1-specific CD8⁺T among CCR9⁺ or CCR9⁻ T cells in mLN and spleen. Each dot represents one mLN or spleen. 5-6 mice/group. Anova statistical analyses: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 15. TMP epitope 1 prophage 2 specific CD8⁺ T cells accumulate in tumor beds. (A) SPF mice were treated with ATB for 3 days and then oral gavages with EH13144 before and after CTX systemic administration, every other week for 3 weeks. Mice were sacrificed 7, 14 and 21 day after the first CTX treatment to harvest tumor draining lymph node (dLN) and tumor (MCA205). **(B)** FMT-treated mice were treated with ATB for 3 days and then oral gavages with EH13144 before and after CTX systemic administration, every other week for 3 weeks. Mice were sacrificed the day of CTX treatment and 72 hrs post-CTX 1, 2, 3 (day J0, J3, J7, J10, J14, J17) to harvest dLN and tumor (MCA205). Flow cytometry analyses of TMP1-specific CD8 T cells and TMP1-specific CD8 T cells among CCR9⁺CXCR3⁺ double positive cells are presented. A representative experiment of 3 groups comprising 5 mice/group. Anova statistical analyses: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 16. Protocol of immunization with DC pulsed with TMP peptides. After DCs differentiation (with GM-CSF and IL-4 during 6 days), we incubated them with poly I:C (10 µg/ml) overnight before the addition of peptide (1 hour) or heat-inactivated bacteria (6 hours). These DCs are injected subcutaneous on right flank at day 0 and 10. One month after second injection, sarcoma (MCA205) or colon (MC38) tumor cells lines are injected subcutaneous on left flank ($8 \cdot 10^5$ and $1 \cdot 10^6$ cells per mice respectively). We constitute 4 groups, one group without DCs, one group with DCs pulsed with 20 µg/ml of irrelevant peptides (gr1) or TMP peptides or 13144 heat-inactivated bacteria. Mean tumor sizes over time (A), each mouse tumor kinetics for each group (B), and the detailed comparison in between groups at the two last time points before sacrifice for MCA205 are shown (C), each dot representing one mouse. MC38 related data are not shown.

Figure 17. 13144 and TMP pulsed-DCs vaccination reduced tumor growth of MCA205. Tumor growth of MCA205 in mice vaccinated with DCs pulsed with gr1 peptides, TMP peptides and *E. hirae* 13144. **(A)** Mean $\pm$ SEM of tumor sizes at different kinetics. **(B)** Tumor growth kinetics for each individual group of 5-10 mice. **(C)** Tumor sizes and statistical differences at time of sacrifice. Student t'-test or ANOVA statistical analyses: * $p < 0.05$, ** $p < 0.1$, *** $p < 0.001$.

Figure 18. The presence of a sarcoma at a distant site prevents the gut inflammatory lesions induced by oral gavages with *E. hirae* 13144. (A) Experimental setting. Mice are treated with broad spectrum antibiotics for 3 days before subcutaneous injection of MCA205 tumor cells in right flank. Then we performed oral gavage with *E. hirae* (708 or 13144) every three days for a total of 4 gavages. 5 days after the first oral gavage, mice were treated with CTX. One week post-CTX, colons are removed to be embedded in PPFE for Hematoxylin, Eosin and Safran staining (HES). **(B)** Extent of inflammatory infiltrates in colons of mice bearing MCA205 tumors or not and colonized with *E. hirae* and treated with CTX. Student t'-test statistical analyses: $**p < 0.01$.

Figure 19. Comparative genomic analysis of *E. hirae* 13144 with four other *E. hirae* strains. (A) Pangenomic analysis of the five *E. hirae* genomes. **(B)** List of prophages regions in 13144 genome and homology to other phage – from the web server PHASTER (PHAge Search Tool Enhanced Release), dedicated to the identification and annotation of prophage sequences within bacterial genomes and plasmids.

Figure 20. *Enterococcus hirae* prophages comparative analysis. (A) Alignment of the sequences of several EH strains and location of the TMP sequences. A comparative analysis through a “heatmap” cluster based on a matrix of presence (black) and absence (white) of the 40.6-kb prophage **(A)** and 39.2-kb prophage **(B)** genes sequences and blast alignment parameter = 80% identity and $\geq 70\%$ coverage.

Figure 21. Homology between epitopes TMP of EH13144 and other *E. hirae* strains. (A) TMP from prophage 2 EH13144 is homologous to TMP from IGR7 (because both EH13144 and EH IGR7 sequences are identical). **(B)** Epitope TMP1 of EH13144 presents some homology with other *E. hirae* strains: 100% in IGR11 and 88.89% in IGR1 and 10815 (with 1 mutation indicated in **(C)**). Epitope TMP2 of EH13144 presents 77% homology in IGR11 and 10815 (with 2 mutations indicated in **(C)**).

Figure 22. T cell responses against commensals in blood from breast cancer patients at diagnosis priori to chemotherapy. Autologous monocytes harvested from breast cancer patients were stimulated with distinct bacterial spp. and then incubated with autologous CD4+ T cells or CD8+ T cells to monitor IFN- γ and IL-10 release. **(A)** TH1/Tc1/Tr1 immune responses to *E. hirae* 13144. Less than 20% breast cancer (BC) patients at diagnosis exhibit a blood TH1/Tc1 immune response to *E. hirae* 13144 prior to chemotherapy. **(B)** TH1/Tc1/Tr1 immune responses to *E. coli* or TCR

cross-linking. BC women display memory T cell responses against *E. coli* and respond to TCR cross-linking.

Figure 23. Localization of the predicted peptides of TMP for each HLA haplotype to be tested in humans. Localization of predicted peptides of Tmp with significant binding potential for MHC Class I alleles (threshold of 50nM) that was assessed using NetMHC software. For each peptide and allele, symbols represent the first amino acid of the identified sequence corresponding to 9 amino acid long peptides with their binding affinity to MHC Class I allele. Sequences of predicted peptides were resumed in table 9.

Figure 24. Antigenicity of TMP peptides in human PBMCs from healthy volunteers. *In vitro* stimulation assays of recall responses either indirectly, after 2 rounds of stimulations by DCs pulsed with peptides (groups or individual peptides) to educate central memory CD8⁺ T cells from PBMCs using ELISPOT to reveal IFN γ release after 24hr restimulation **(A)**. Each peptide is HLA-A2 restricted **(B)** and 6 healthy volunteers "HV" (individualized with a 6 digit number) were selected on their HLA-A2.1 genotype **(C)**. In **(B)**, we underlined the peptides which are endowed with immunogenicity in the following graphs. We determined the threshold for each HV **(D)** and calculated the number of "positive" wells (above the threshold) and the pourcentages of responders for each peptide Isited in table B **(E)**. When we represent these results in bar graphs, we observe that 5 peptides may be significantly associated with the immunogenicity of TMP in humans because they have been found to trigger Tc1 immune recall responses in at least 50% of HV **(F)**. Arrows indicate the most significant epitopes **(G)**. The list of peptides for each group and the most significant epitopes (2, 3, 9, 10, 13) are indicated in B.

Figure 25. Protocol for phage excision by mitomycin C. Each step of the technical procedure is indicated.

Figure 26. PCR detection of phage nucleic acids in the supernatants of *E. hirae*13144. Supernatants of EH13144 cultivated at various temperatures (37 or 42°C), at various concentrations of mitomycine C (0, 0.2 and 1 μ M), or active metabolite of CTX (mafostamide – 25 μ g/ml) were treated to harvest the phage proteins encoding DNA with **(B)** or without **(A)** capsid disruption. PCR was run to detect specific sequences of the prophage 1 or 2.

Figure 27. Phage Tail Length Tape Measure Protein as the unique antigenic sequence in *E. hirae* 13144.

A and C. Experimental setting. Mice (naive (C), sarcoma bearers (A)) were treated with broad spectrum antibiotics (streptomycin, colistin, ampicillin, vancomycin) for 3 days before performing oral gavage with *E. hirae* strain 13144 (1.10^9 bacteria) before and after systemic administration of cyclophosphamide (ip CTX – 100 mg/kg) or saline solution (NaCl) at day 5 and 6 respectively, once (C) or three times on a weekly basis (A). One week later (C), purified CD8⁺ T cells splenocytes were restimulated *ex vivo* in a recall assay with bone marrow-derived DC loaded with saline or distinct heat killed bacteria strains. **B.** Tumor sizes at day 25 (sacrifice) of MCA-205 sarcoma in SPF C57BL/6 mice injected with 3 cycles of CTX vs NaCl after weekly oral gavages with various strains of *E. hirae*. **D-E. Ex vivo recall assays.** After *in vivo* exposure (C), splenic CD8⁺ T cells were restimulated with dendritic cells (DCs) pulsed with heat-inactivated bacteria (65°C during 2 hours) (D) or peptides (E). IFN γ ELISpot was performed at 24 hours to enumerate IFN γ -secreting CD8⁺T cells (spots) after co-culture. Each dot represents one mouse. **F, G, H.** Flow cytometry analyses of H-2K^b/TSLARFANI tetramer binding CTL in spleens (F, H) or in tumor draining lymph nodes (G) at 72 hours post-therapy (regimen in A and C), in naive (F, H) or tumor bearers (G). The percentages of TMP1 prophage2-specific CD8⁺ T cells are depicted among splenic CD8⁺ T cells (F, left panel, G, H, top panel) or in the gate of CCR9⁺CXCR3⁺ T cells (F, right panel, G, bottom panel) or CCR9⁺ CTL (H, bottom panel). Each experiment involved one group of 10-15 mice from 2-3 independent experiments (B, D, E, F, H). A representative experiment out of 2 yielding similar results is shown in the kinetics study in G. Anova or Student t'-test statistical analyses: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 28. Prophylactic and therapeutic immunization using Phage Tail Length Tape Measure Protein against sarcomas.

A-B. Prophylactic vaccinations. TLR3 ligand exposed DC were pulsed with peptides (irrelevant groups, individual TMP1 mutated or not in position 3) or heat-inactivated bacteria before inoculation sc in the right flank of naive mice, ten days apart. One month after second injection, sarcoma (MCA205) were implanted subcutaneous in the left flank. **C-D. Therapeutic settings.** Refer to Fig. 27A for the regimen where MCA205 tumor bearers were treated with CTX and gavaged with *E. hirae* 13144 or *E. coli* genetically modified to express TMP1 (TSLARFANI), TMP1 mut2 (T Δ LARFANI), TMP1 mut3 (TSEARFANI) or EGFP sequence (as ctrl). Longitudinal tumor growth kinetics (A, C top panel) or cross-sectional tumor sizes (B, C bottom panel) of MCA205 are depicted

as means $\pm$ SEM of tumor sizes at different time points (A, C top panel) or at sacrifice (B, C bottom panel) for 12-18 animals (A), gathered from 2-3 independent experiments. **D.** Flow cytometry analyses of H-2K^b/TSLARFANI tetramer binding CTL in spleens at sacrifice. The percentages of TMP1 prophage2-specific CD8⁺ T cells are depicted among splenic CD8⁺ T cells. Student t'-test or ANOVA statistical analyses: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 29. Breadth of coverage (BOC) of the enterophage and clinical relevance of its molecular mimic GPD1-L in cancer patients.

A. BOC of the *E. hirae* and prophages genome in the MG reference catalog. 3027 metagenomes from 17 different datasets (referenced at the bottom, individual samples in columns) were screened for the presence of *E. hirae* strains and enterococcal phages genomes (featuring in rows). **B-C.** Percentages of stools with detectable *E. hirae* and/or *E. faecalis* colonies (B, top panel) and TMP in *E. hirae* and/or *E. faecalis* (B, bottom panel) colonies assessed by culturomics followed by PCR in 76 NSCLC and RCC bearing patients (cohort described in Routy et al. Science 2018) and corresponding Kaplan Meier curves indicating time to progression (C, top panel) or overall survival (C, bottom panel). Log-rank (Mantel-Cox) analysis with indicated p-value. **D.** Priming of naive CD8⁺ T cells from six HLA-A02*01 healthy volunteers with autologous monocyte-derived DC pulsed (or not) with 16 HLA-A02*01 binding TMP epitopes (Table 9, Figure 24B and Figure 37). Restimulation at day 7 with each of the 16 TMP peptides for IFN γ ELISpot assays and enumeration of positive spots. ANOVA statistical analyses: * $p < 0.05$. **E.** Blast sequence alignment of immunogenic epitopes selected in D with the publicly available NCBI BLASTP suite and TCGA data set seeking >75% homology. Only epitope 10 (KLAKFASVV, SEQ ID No: 63) obtained a significant match (KLQKFASTV, SEQ ID No: 188) and was identified in the sequence of GPD1-L protein. **F-H.** Expression levels of GPD1-L gene product among bladder (F), lung adenocarcinoma (G), 530 renal cell cancer (H, according to HLA-A02*01 typing, bottom panel) patients from the TCGA data sets segregated according to the mean and Kaplan Meier curves of survival in univariate analysis. **I-J.** Time to progression following PD-1 blockade in second line therapy in 44 stage IIIC/IV NSCLC patients (CHUM validation cohort, I) validated with a second cohort of 62 stage IIIC/IV NSCLC patients (CGFL test cohort, J). Kaplan Meier curves for time to progression; patients were stratified according to the value value of the GPD1L expression. The cutoff was defined with an optimal cutoff strategy. **K.** Pearson correlations between GPD1-L expression and tumor immune

infiltrates in all lung cancer type of TCGA assays (TCGA), lung adenocarcinoma (LUAD), lung squamous cells carcinoma (LUSC), CHUM and CGFL cohorts.

Figure 30. Molecular mimicry between enterophage TMP and the oncogenic driver PSMB4 in mouse cancers.

5 **A.** Blast sequence alignment of the only one immunogenic epitope TSLARFANI selected in 27E with the publicly available TCGA data set seeking >70% homology. Only one hit (GSLARFRNI) obtained a significant match and was identified in the sequence of PSMB4 protein. **B.** *Therapeutic settings comparing wild type versus knock in tumoral clones of MCA205.* Id. as in Figure 27A but mice were either inoculated
10 with the WT MCA205 cell line or with distinct clones harboring a knock in mutation in position 3 of TSLARFANI, and then, were treated with CTX +/- gavaged with *E.hirae* 13144 (or saline). Longitudinal tumor growth kinetics (B) or cross-sectional tumor sizes (B, right) of MCA205 are depicted as means $\pm$ SEM of tumor sizes at different time points (top and bottom) or at sacrifice (right) for 6 animals/group, in a representative experiment
15 out of 2 yielding similar conclusions. ANOVA statistical analyses: * $p < 0.05$, ** $p < 0.1$, *** $p < 0.001$. **C-D.** *In vivo excision-infection cycle of the E.hirae siphoviridae phage.* Gavage with *E.hirae* before and after CTX followed by harvesting of ileal content, for cultivation and isolation of bacterial colonies, MALDI-TOF identification and PCR using TMP specific probe sets (C). Graph depicting the proportions of colonies for each species
20 in naive and gavaged animals, with colonies harboring the TMP sequence in PCR (D). Results of 5 mice/group and >70 colonies identified and scrutinized in PCR. Also refer to Figure 39. **E.** Alignment of phage genome with *E.gallinarum* harboring TMP sequence in PCR after sequencing of this strain. **F-G.** Culture of organoid with *E.hirae* (10^8) and *E.gallinarum* (10^8) during 1 hour followed by mafosfamide treatment (25 μ g/ml). Six hours and 20 hours after mafosfamide treatment, supernatant were harvested for cultivation and isolation of bacterial colonies (F), MALDI-TOF identification and PCR using TMP specific probe sets (G). **H.** LEfSe analysis was performed on bacterial and viral species after MetaPhlAn2 analysis on localized breast cancer shotgun data, reporting the most discriminant ones (LDA score > 2) in decreasing order for neoadjuvant palbociclib
25 treatment on 10 patients from 83 included, the 73 others did not receive neoadjuvant CDK4/6 inhibitorss. Student t'-test or ANOVA statistical analyses: * $p < 0.05$, ** $p < 0.1$, *** $p < 0.001$.

Figure 31. Clading and genomic analysis of E. hirae strains.

35 **A.** Dendrogramm of the various strains based on 16S sequence similarities. **B.** Phylogenomic tree of 20 *E. hirae* isolates based on SNPs alignment.

C. Comparative genomic analysis of 13144 strain against five complete *E. hirae* genomes. From the center to the outside: GC skew, GC content, 13144, IGR7, IGR11, ATCC 9790, 708, 13344 strains. Prophages positions appear in black.

Figure 32. Pre-identification of group 7 as the only immunogenic peptide group.

Naive mice were treated with broad spectrum antibiotics (streptomycin, colistin, ampicillin, vancomycin) for 3 days before performing oral gavage with *E. hirae* strain 13144 or 708 (1.10^9 bacteria) before and after systemic administration of cyclophosphamide (ip CTX - 100 mg/kg) or saline solution (NaCl) at day 5 and 6 respectively. One week later, purified CD8⁺ T cells splenocytes were restimulated *ex vivo* in a recall assay with bone marrow-derived DC loaded with saline or distinct groups of peptides (refer to the list in Table 6). IFN γ ELISpot was performed at 24 hrs to enumerate IFN γ -secreting CD8⁺T cells (spots) after co-culture. Each dot represents one mouse. Statistical analyses revealed that only group 7 reached significant response: Anova test: *p<0.05, ** p<0.1.

Figure 33. Sequence of the TMP protein and comparative analysis of *E. hirae* 13144 prophage 2 protein sequence.

A. Whole TMP protein sequence of the 13144 prophage 2.
B. Comparative analysis through a “heatmap” clustering based on a matrix of presence (black) and absence (white) of the 13144 prophages 2 protein sequences.

Figure 34. Sequence alignment of the immunogenic epitope region within prophage 2 of *E. hirae* 13144.

The immunogenic peptide TSLARFANI from *E. hirae* 13144 was identified in experiments depicted and detailed in Figure 27. The y axis presents the sequences of 6 other *E.hirae* strains in the same region tested in our models.

Figure 35. Sub-cloning expression of part of the TMP gene in *E.coli*.

A. Amino acid sequences of TMP-FLAG, TMP-mut2-FLAG and TMP-mut3-FLAG expressed in *E. coli* DH5 α . Note that only the N-terminal part of the TMP protein, including the indicated variants of the epitope (underlined), was expressed as fusion protein with a C-terminal FLAG tag (*italics*). B. Western blot analysis demonstrating expression of EGFP and TMP-FLAG in *E. coli* strains transformed with pDL28-P23-EGFP or pDL28-P23-TMP-FLAG, respectively.

Figure 36. Sequence of the Phage Tail Length Tape Measure Protein in *E. hirae*. Genetic sequence of the whole TMP protein with binding areas for PCR primers indicated in *italics*.

Figure 37. Localization of the HLA-A02*01 binding and immunogenic epitopes in the GPD1-L protein and their affinity (refer to Figure 30A).

All the HLA-A02*01 binding and/or immunogenic epitopes found in Table 9 and depicted in Figure 29D are located in a precise region of the whole TMP protein, as indicated by the color code and the amino acid sequence position, as a function of its binding affinity to the MHC class I allele.

Figure 38. Generation of *pmsb4*-mutated MCA205 cell lines by means of the CRISPR/Cas9 technology.

A. Schematic diagrams of *Psmb4* cDNA, and the designed mutation sites. The target site of sgRNA and point mutations are indicated. **B.** Representative sequence electropherograms for the validation of *Psmb4* mutation 2 and mutation 3 introduced by CRISPR/Cas9. Mutated amino acids are highlighted in grey.

Figure 39. Identification of ileal bacterial colonies with or without treatment composed of CTX+oral gavage with 10^9 cfu *E. hirae* 13144.

PCR amplification of the TMP sequence (refer to Figure 36) in each colony growing after seeding of ileal content in aerobic conditions to isolate Gram+ bacteria. A photograph of each agarose electrophoresis gel is shown for each animal. **A** depicts the results in 5 naive mice and **B** depicts the findings after CTX+oral gavage with the phage encoding bacterium. Each vertical lane corresponds to one bacterium identified in MALDI-TOF. Initials are detailed in the lower part of panel A. The positive control (Ctl+) represents the DNA of *E. hirae* 13144.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the present text, the following general definitions are used:

Gut microbiota

The "gut microbiota" (formerly called gut flora or microflora) designates the population of microorganisms living in the intestine of any organism belonging to the animal kingdom (human, animal, insect, *etc.*). While each individual has a unique microbiota composition (60 to 80 bacterial species are shared by more than 50% of a sampled population on a total of 400-500 different bacterial species/individual), it always fulfils similar main physiological functions and has a direct impact on the individual's health:

- it contributes to the digestion of certain foods that the stomach and small intestine are not able to digest (mainly non-digestible fibers);
- it contributes to the production of some vitamins (B and K);

- it protects against aggressions from other microorganisms, maintaining the integrity of the intestinal mucosa;
- it plays an important role in the development of a proper immune system;
- 5 • a healthy, diverse and balanced gut microbiota is key to ensuring proper intestinal functioning.

Taking into account the major role gut microbiota plays in the normal functioning of the body and the different functions it accomplishes, it is nowadays considered as an “organ”. However, it is an “acquired” organ, as babies are born sterile; 10 that is, intestine colonisation starts right after birth and evolves afterwards.

The development of gut microbiota starts at birth. Sterile inside the uterus, the newborn's digestive tract is quickly colonized by microorganisms from the mother (vaginal, skin, breast, *etc.*), the environment in which the delivery takes place, the air, *etc.* From the third day, the composition of the intestinal microbiota is directly dependent 15 on how the infant is fed: breastfed babies' gut microbiota, for example, is mainly dominated by *Bifidobacteria*, compared to babies nourished with infant formulas.

The composition of the gut microbiota evolves throughout the entire life, from birth to old age, and is the result of different environmental influences. Gut microbiota's balance can be affected during the ageing process and, consequently, the 20 elderly have substantially different microbiota than younger adults.

While the general composition of the dominant intestinal microbiota is similar in most healthy people (4 main phyla, *i.e.*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*), composition at a species level is highly personalised and largely determined by the individuals' genetic, environment and diet. The 25 composition of gut microbiota may become accustomed to dietary components, either temporarily or permanently.

Dysbiosis

Although it can adapt to change and has a high resilience capacity, a loss of balance in gut microbiota composition may arise in some specific situations. This is 30 called “dysbiosis”, a disequilibrium between potentially “detrimental” and “beneficial” bacteria in the gut or any deviation to what is considered a “healthy” microbiota in terms of main bacterial groups composition and diversity. Dysbiosis may be linked to health problems such as functional bowel disorders, inflammatory bowel diseases, allergies, obesity, diabetes and also cancer. It can also be the consequence of a treatment, such 35 as a cytotoxic treatment or an antibiotic treatment.

Antineoplastic treatments

“Antineoplastic treatments” herein designate any treatment for cancer except surgery. They include chemotherapy, hormonal and biological therapies, radiotherapy and targeted therapies (such as c-KIT, EGFR or HER2/HER3 or MET or
5 ALK inhibitors...).

Chemotherapy

“Chemotherapy” is defined herein as the treatment of cancer with one or more chemotherapeutic agents. Chemotherapeutic agents are chemical molecules which act by killing cells that divide rapidly, one of the main properties of most cancer
10 cells. Several categories of chemical agents exist:

- alkylating agents;
- spindle poisons such as mebendazole, colchicine;
- mitotic inhibitors (including taxanes (paclitaxel (Taxol ®), docetaxel (Taxotère ®)) and vinca alkaloids (e.g.: vincristine, vinblastine, vinorelbine, vindesine),
15 - cytotoxic/antitumor antibiotics: such as anthracyclines (e.g.: doxorubicin, daunorubicin, adriamycine, idarubicin, epirubicin and mitoxantrone, valrubicin), streptomycines (e.g.: actinomycin, bleomycin, mitomycin, plicamycin)
- anti-metabolites (such as pyrimidine analogues (e.g.: fluoropyrimidines analogs, 5-fluorouracil (5-FU), floxuridine (FUDR), Cytosine arabinoside (Cytarabine),
20 Gemcitabine (Gemzar ®), capecitabine; purine analogues (e.g.: azathioprine, mercaptopurine, thioguanine, fludarabine, pentostatin, cladribine, capecitabine, clofarabine); folic acid analogues (e.g.: methotrexate, folic acid, pemetrexed, aminopterin, raltitrexed, trimethoprim, pyrimethamine),
- topoisomerase inhibitors (e.g.: camptothecins: irinotecan, topotecan,
25 amsacrine, etoposide, etoposide phosphate, teniposide);
- DNA methyltransferase inhibitors: 2'-deoxy-5-azacytidine (DAC), 5-azacytidine, 5-aza-2'- deoxycytidine, 1 -[beta]-D-arabinofuranosyl-5-azacytosine, dihydro-5-azacytidine;
- vascular disrupting agents, such as flavone acetic acid derivatives, 5,6-
30 dimethylxanthenone-4- acetic acid (DMXAA) and flavone acetic acid (FAA);
- also other chemotherapeutic drugs such as aprepitant, bortezomib (Velcade ®, Millenium Pharmaceuticals), imatinib mesylate (Gleevec ®), carmustine (BCNU), lomustine (CCNU), tamoxifen, gefitinib, erlotinib, carboxyamidotriazole, efaproxiral, tirapazamine, xcytrin, thymalfasin, vinflunine.

Immune checkpoint blockers

In the present text, a “drug blocking an immune checkpoint”, or “immune checkpoint blocker (ICB)” or “immune checkpoint blockade drug” designates any drug, molecule or composition which blocks an immune checkpoint of T lymphocytes. Such a
5 drug reactivates the host immune system, and kills tumor cells indirectly by effector T lymphocytes. In particular, these terms encompass anti-CTLA-4 antibodies, anti-PD1 antibodies, anti-PD-L1 antibodies (such as Atezolizumab or Durvalumab) and anti-PD-L2 antibodies. More particularly, an ICB can be an anti-PD1 monoclonal antibody such as Nivolumab or Pembrolizumab. Other ICB include anti-Tim3, anti-BTLA, anti-VISTA,
10 anti-CD38, anti-TIGIT, anti-GITR, anti-LAG3, anti-KIR antibodies, anti-OX40 antibodies, which also inhibit immune checkpoints.

Although the currently used drugs antagonizing CTLA-4, PD1, PD-L1, PD-L2, *etc.* are monoclonal antibodies, other molecules specifically binding to these could be used for the development of future ICB such as, for example, antibody fragments or
15 specifically designed aptamers. Of course, the phrases “drug blocking an immune checkpoint”, or “immune checkpoint blocker (ICB)” or “immune checkpoint blockade drug” encompass any therapy with active molecules that antagonize and immune checkpoint such as CTLA-4, PD1, PD-L1, PD-L2, *etc.*, such as oncolytic viruses recombinant for anti-CTLA4, anti-PD1 or PDL1 Abs.

Immune-targeting antibodies for activating receptors

In the present text, a “drug activating an immunostimulatory receptor”, designates any drug, molecule or composition which activates a T or NK cell receptor reactivating the host immune system, and killing tumor cells indirectly by effector T lymphocytes. In particular, it encompasses anti-ICOS antibodies, anti-OX40 antibodies,
25 anti-CD137, anti-CD28 antibodies ...

CDK4/6 inhibitors

In the present text, a “CDK4/6 inhibitor” designates a cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitor, such as palbociclib, ribociclib, and abemaciclib. These drugs are currently used to treat patients with hormone receptor (HR)-positive, human
30 epidermal growth factor receptor 2 (HER2)-negative (HR+/HER2-) advanced breast cancer, but could also be used to treat other cancers, such as ovarian cancer and acute myeloid leukaemia.

Probiotics

“Probiotics” are micro-organisms that have claimed health benefits when

consumed. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures, such as in yogurt, soy yogurt, or as dietary supplements. Generally, probiotics help gut microbiota keep (or re-find) its balance, integrity and diversity. The effects of probiotics can be strain-dependent. Here we will use the phrase

5 “anticancer probiotics” or the neologisms “oncobax” and “oncomicrobiotics” to designate any commensal composition that restores responsiveness to chemotherapy, PD1/PD-L1 blockade or combination of anti-CTLA4+anti-PD1 or PD-L1 Ab. In the context of the present invention, a “probiotic composition” is thus not limited to food or food supplements, but it generally designates any bacterial composition comprising

10 microorganisms which are beneficial to the patients. Such probiotic compositions can hence be medicaments or drugs.

Cancer, treatment, etc.

As used herein, “cancer” means all types of cancers. In particular, the cancers can be solid or non solid cancers. Non limitative examples of cancers are

15 carcinomas or adenocarcinomas such as breast, prostate, ovary, lung, pancreas or colon cancer, sarcomas, lymphomas, melanomas, leukemias, germ cell cancers and blastomas.

The immune system plays a dual role against cancer: it prevents tumor cell outgrowth and also sculpts the immunogenicity of the tumor cells. Drugs blocking an

20 immune checkpoint can hence be used to treat virtually any type of cancer. Thus, the methods according to the invention are potentially useful for patients having a cancer selected amongst adrenal cortical cancer, anal cancer, bile duct cancer (e.g. periphilar cancer, distal bile duct cancer, intrahepatic bile duct cancer), bladder cancer, bone cancers (e.g. osteoblastoma, osteochondroma, hemangioma, chondromyxoid fibroma,

25 osteosarcoma, chondrosarcoma, fibrosarcoma, malignant fibrous histiocytoma, giant cell tumor of the bone, chordoma, lymphoma, multiple myeloma), brain and central nervous system cancers (e.g. meningioma, astocytoma, oligodendrogliomas, ependymoma, gliomas, medulloblastoma, ganglioglioma, Schwannoma, germinoma, craniopharyngioma), breast cancer (e.g. ductal carcinoma *in situ*, infiltrating ductal carcinoma, infiltrating lobular carcinoma, lobular carcinoma *in situ*, gynecomastia),

30 Castleman disease (e.g. giant lymph node hyperplasia, angiofollicular lymph node hyperplasia), cervical cancer, colorectal cancer, endometrial cancers (e.g. endometrial adenocarcinoma, adenocanthoma, papillary serous adenocarcinoma, clear cell), esophagus cancer, gallbladder cancer (mucinous adenocarcinoma, small cell carcinoma), gastrointestinal carcinoid tumors (e.g. choriocarcinoma, chorioadenoma destruens), Hodgkin's disease, non-Hodgkin's lymphoma, Kaposi's sarcoma, kidney

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cancer (e.g. renal cell cancer), laryngeal and hypopharyngeal cancer, liver cancers (e.g. hemangioma, hepatic-adenoma, focal nodular hyperplasia, hepatocellular carcinoma), lung cancers (e.g. small cell lung cancer, non-small cell lung cancer), mesothelioma, plasmacytoma, nasal cavity and paranasal sinus cancer (e.g. esthesioneuroblastoma, midline granuloma), nasopharyngeal cancer, neuroblastoma, oral cavity and oropharyngeal cancer, ovarian cancer, pancreatic cancer, penile cancer, pituitary cancer, prostate cancer, retinoblastoma, rhabdomyosarcoma (e.g. embryonal rhabdomyosarcoma, alveolar rhabdomyosarcoma, pleomorphic rhabdomyosarcoma), salivary gland cancer, skin cancer (e.g. melanoma, nonmelanoma skin cancer), stomach cancer, testicular cancers (e.g. seminoma, nonseminoma germ cell cancer), thymus cancer, thyroid cancers (e.g. follicular carcinoma, anaplastic carcinoma, poorly differentiated carcinoma, medullary thyroid carcinoma, thyroid lymphoma), vaginal cancer, vulvar cancer, and uterine cancer (e.g. uterine leiomyosarcoma). More particularly, the method according to the invention can be used for predicting and optimizing a patient's response to a medicament targeting an immune checkpoint, wherein the patient has a cancer selected from the group consisting of metastatic melanoma, non-small cell lung carcinoma (NSCLC), small cell lung cancer (SCLC), mesothelioma, bladder cancer, renal cell carcinoma, head and neck cancers, oesophageal and gastric cancers, rectal cancers, hepatocarcinoma, sarcoma, Wilm's tumor, Hodgkin lymphoma, ALK-neuroblastoma, (hormone refractory) prostate cancers and GIST.

Other definitions will be specified below, when necessary.

According to a first aspect, the present invention pertains to the use of a bacterial composition in a combined treatment for treating a cancer, wherein the bacterial composition comprises at least one bacterial strain which expresses the protein of SEQ ID No: 1 or a fragment thereof of at least 9, preferably at least 20 amino acids comprising at least one epitope selected from the group consisting of SEQ ID Nos: 13, 14, 53 to 188 and 209.

In the frame of this invention, the at least one bacterial strain present in the composition can be either a naturally occurring strain or an artificial, engineered strain obtained by gene technology or any other technique. Strains different from the *Enterococcus hirae* strain 13144 deposited on November 7, 2013 at the CNCM under the number I-4815 can be used according to the invention.

In the present text, a "bacterial composition" designates any composition comprising bacteria, especially live bacteria. The composition can comprise a pure

culture of one single strain, a mix of several cultured strains and/or a complex material such as fecal material for performing Fecal Microbiota Transplantation (FMT). The composition can be a liquid composition. Alternatively, the composition can comprise freeze dried or lyophilized materials, which can be formulated or manufactured into or as an edible or friable product, e.g. , a biscuit-like product, which can be e.g. , crushed into a powder to dissolve in a drink or to insert into a tablet or a capsule. Alternatively, the bacterial composition can be in the form of a dry lozenge or a chewing gum or equivalent. Compositions according to the present invention can also be prepared and/or formulated in a powdered form, or equivalent; these formulations can be useful for storage in e.g. , a tablet or capsule, or in an ampoule to e.g. , crack open and dissolve in a liquid for, e.g., insertion, mixing or injection into a channel of a colonoscope or a naso-enteric tube and the like; or as a powder in a bag ready to add, for example as a solution which can be infused into a nasogastric tube (or equivalent), or a colonoscope, or a gastroscope etc. Components possibly present in a bacterial composition according to the invention (apart from the bacteria) include salts, buffers, nutrients, water, pharmaceutically acceptable excipients, cryoprotectants, etc.

Using the bacterial composition in a combined treatment for treating a cancer means that the bacterial composition is used in combination with an antineoplastic drug in order to potentiate or increase the effects of this antineoplastic drug. Non-limiting example of drugs that can advantageously be administered in combination with the bacterial compositions according to the invention include chemotherapy, especially alkylating agents (e.g. cyclophosphamide), immune checkpoint blockers (e.g., drugs antagonizing CTLA-4, PD1, PD-L1 or PD-L2 etc., used alone or in combination), immune-targeting antibodies for activating receptors (e.g., anti-ICOS, anti-OX40, anti-CD137, anti-CD28 antibodies), CDK4/6 inhibitors, etc. The physician will chose, depending on the context, what drug is to be administered to the patient in combination with the bacterial composition, as well as the therapeutic protocol (*i.e.*, the sequence of administration of the antineoplastic drug(s) and the bacterial composition).

According to a preferred embodiment of the composition according to the invention, the at least one bacterial strain comprises a prophage genome encoding a protein with at least 80, preferably at least 90 and more preferably at least 95% identity with the protein of SEQ ID No: 1.

In a particular embodiment of the invention, at least one strain in the bacterial composition harbours a prophage genome with at least 80 and preferably at least 90 or 95% identity with the prophage of SEQ ID No: 2 (prophage of *E. hirae* 13144 identified as "prophage 2" in the experimental part below), so that the phage encoded by

this prophage can *in vivo* infect the other strains of the composition and/or commensal bacteria of the gut microbiota of the patient to which the composition is administered.

The present invention also pertains to a bacterial composition comprising bacteria selected from the group consisting of:

5 (i) *Enterococcus hirae* strain 13144 deposited on November 7, 2013 at the Collection Nationale de Cultures de Microorganismes (CNCM) under the number I-4815,

(ii) *Enterococcus hirae* strain IGR7 deposited on August 31, 2017 at the CNCM under the number I-5224,

10 (iii) *Enterococcus hirae* strain IGR11 deposited on November 27, 2017, at the CNCM under the number I-5261,

(iv) any other bacterial strain expressing a protein with at least 65, preferably at least 80 and more preferably at least 95% identity with a fragment of at least 20, preferably at least 30 and more preferably at least 40 nucleotides from the tail tape measure protein (TMP) of SEQ ID No: 1 (called "the TMP of prophage 2" in the
15 experimental part below), and

(v) mixtures of at least two of the strains recited in (i) to (iv).

According to one embodiment, this bacterial composition is used for treating a cancer.

20 The bacterial strains present in the composition according to the invention can belong to any bacterial family. Of course, since the bacteria are administered to a patient suffering from cancer, non pathogenic bacteria will be preferentially used. Hence, bacteria other than *Enterococcus hirae* strains can also be used according to the present invention.

25 As already mentioned, the bacterial composition is typically administered to a patient in need thereof in combination with an antineoplastic drug.

According to one embodiment, the bacterial composition of the invention further comprises *Enterococcus hirae* strain IGR4, deposited on November 27, 2017, at the CNCM under the number I-5260.

30 According to one embodiment, illustrated in the experimental part below, the composition comprises *Enterococcus hirae* strain 13144 (CNCM I-4815), *Enterococcus hirae* strain IGR7 (CNCM I-5224) and *Enterococcus hirae* strain IGR4 (CNCM I-5260).

35 According to a particular embodiment of the invention, the bacterial composition is used in combination with an antineoplastic drug capable of triggering the lytic cycle of a phage encoded by the prophage. Non-limitative examples of such a drug

are mitomycin C, as illustrated in Example 8 below, as well as CDK4/6 inhibitors, as illustrated in Example 16.

According to one embodiment, the bacterial composition comprises at least one strain harbouring a prophage genome with at least 80 and preferably at least 95% identity with the prophage of SEQ ID No: 2, so that the phage encoded by this prophage can *in vivo* infect other strains of the composition and/or, possibly, other bacteria already present in the patient's gut microbiota.

Another aspect of the present invention is a method of increasing the immunogenicity of a bacterial strain of anticancer probiotic interest, by *in vitro* introducing into said bacterial strain a nucleotide sequence encoding the TMP of SEQ ID No: 1 of a fragment thereof comprising at least the peptides of SEQ ID Nos: 13 and 14, or a sequence encoding a peptide of at least 9, preferably at least 20 amino acids comprising at least one epitope selected from the group consisting of SEQ ID No: 53 to 187 or a sequence encoding a peptide of at least 9, preferably at least 20 amino acids comprising at least one epitope of SEQ ID No: 209. The peptides of SEQ ID Nos: 13 and 14 correspond to immunogenic epitopes binding to mouse H-2Kb, but they might also be immunogenic in humans harbouring appropriate HLA class I haplotypes capable of binding some of these sequences or broader sequences, shared across species. The sequences of SEQ ID No: 53 to 187 have been identified *in silico* as being epitopes from the TMP of SEQ ID No: 1 which are bound by human HLA molecules (see Table 9 below). SEQ ID No: 209 (KLX₁KFASX₂V with X₁=A or Q and X₂=V or T) corresponds to the TMP1 HLA-A*0201-restricted immunogenic epitope of sequence KLAKFASVV (SEQ ID No: 63), the human HLA-A*0201-restricted epitope from GPD1L of sequence KLQKFASTV (SEQ ID No: 188), as well as two hybrids of these two epitopes: KLAKFASTV (SEQ ID No: 210) and KLQKFASVV (SEQ ID No: 211). Advantageously, the bacterial strain of anticancer probiotic interest is transduced with a sequence comprising several epitopes, which can be presented by different HLA haplotypes, to that the resulting bacterial strain is immunogenic in patients of different HLA haplotypes.

According to one embodiment, the nucleotide sequence encodes at least KMVEILEEI (SEQ ID No: 55), RLLKYDVG (SEQ ID No: 56), LLGIYQSYV (SEQ ID No: 62), KLAKFASVV (SEQ ID No: 63) or ILVAITTTI (SEQ ID No: 66), which are HLA-A0201-restricted epitopes, the immunogenicity of which has been experimentally confirmed in humans (see Example 7 and Fig. 24B below). In a particular embodiment, the nucleotide sequence encodes at least KLAKFASVV (SEQ ID No: 63), KLQKFASTV (SEQ ID No: 188) or any other epitope of sequence KLX₁KFASX₂V with X₁=A or Q and X₂=V or T (SEQ ID No: 209).

According to one embodiment, the bacterial strain is transduced with a nucleic acid (for example, a plasmid) encoding a protein having 80%, 90% or 95%, preferably 97.5% and more preferably 98,7% identity with the TMP of SEQ ID No : 1, or encoding a fragment of said protein including at least one epitope as above-described.

- 5 The skilled artisan will chose appropriate sequences (promoter *etc.*) so that the resulting bacterial strain expresses the TMP or fragment thereof.

According to one embodiment, bacteria of the strain are infected with a bacteriophage encoding a TMP with at least 80%, preferably at least 90% and more preferably at least 95% identity with the TMP of SEQ ID No: 1.

- 10 Again, any bacterial strain of interest can be used in this method, provided it is not highly pathogenic. In particular, bacterial strains already known for their probiotic interest can be engineered by *in vitro* infection with a bacteriophage or by transduction with a plasmid encoding the TMP or fragment thereof. Non-limitative examples of such bacteria include *Akkermansia muciniphila*, *Ruminococcaceae*, *Faecalibacterium*,
15 *Clostridium ramosum*, *Clostridium XVIII*, *Alistipes* species (*A. onderdonkii*, *A. finegoldii*, *A. shahii*), *Eubacterium* species, *Bacteroidales* species, *Methanobrevibacter smithii*, *Lactobacillus johnsonii*, *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Bacteroides salyersiae*, *Burkholderia cepacia*, *Burkholderia cenocepacia*, *Barnesiella intestinihominis*, *Erysipeloclostridia* and *Erysipelotrichaceae*, *Colinsella intestinalis*,
20 *Collinsella takakaei*, *Eggerthella lenta* /*Coriobacteriaceae*, *Bifidobacteria* (*longum*, *breve*, *termophilus*, *adolescentis*...) and *E. coli*. More particularly, *Escherichia coli*, *Enterococcus gallinarum*, *Enterococcus faecalis* and *Enterococcus hirae* can advantageously be used as starting material to obtain a bacterial strain with increased immunogenicity through a method as above-described.

- 25 According to a particular embodiment of the above method, the bacteriophage has a genome comprising a nucleotide sequence of the SEQ ID Nos:2 or a sequence having at least 90% or at least 95% identity thereto. According to a preferred embodiment, the bacteriophage has a genome identical to the prophage of 39.2 kb present in *Enterococcus hirae* 13144 (CNCM I-4815).

- 30 According to a particular embodiment, the present invention pertains to a bacterial strain which has been obtained by a method as above-described. In particular, the invention pertains to a non-naturally occurring bacterial strain, obtained by *in vitro* infection of a probiotic strain with a phage encoding the TMP protein defined above, wherein the probiotic strain is not known to be infected by this bacteriophage in nature.
- 35 According to another embodiment, the invention pertains to a non-naturally occurring bacterial strain obtained by transduction with a nucleic acid encoding the TMP protein defined above or a fragment thereof, by gene editing or by any other technology.

The bacterial strains according to the invention, engineered to have increased immunogenicity and improved anticancer properties, are advantageously used in the treatment of cancer.

5 These engineered bacterial strains, in particular those that express a protein, polypeptide or peptide comprising an epitope selected amongst SEQ ID Nos: 63, 188 and 209, can efficiently be used to treat cancer in a HLA-A*0201 patient.

10 As already mentioned, the bacterial compositions of the invention, including those comprising an engineered bacterial strain as above-described, are advantageously used in combination with an antineoplastic drug capable of triggering the lytic cycle of a phage encoded by a prophage present in bacteria and/or with an immune checkpoint blocker.

15 The present invention also relates to an immunogenic composition comprising a polypeptide comprising a sequence of at least 9 consecutive amino acids from the TMP of SEQ ID No: 1 or a sequence of SEQ ID No: 209 or a polynucleotide encoding the same. Such a composition can advantageously be used as an anticancer vaccine, either as a nucleotide sequence (mRNA or cDNA) or as a peptide stretch of at least 9 amino acids, for example between 9 aa (short peptides directly binding to the proper MHC class I groove) and 20 to 30 amino acids (long peptides cross presented by DC in their MHC class I and II molecules). Dendritic cells or artificial antigen presenting
20 cells presenting this polypeptide or recombinant for this cDNA could serve as a platform to prime and amplify naïve or effector memory T lymphocytes from a tumor or blood from a patient or a healthy volunteer.

25 According to a particular embodiment of the immunogenic composition according to the invention, the sequence of at least 9 consecutive amino acids from the TMP is a peptide which has been identified as likely to be presented by a MHC I human molecule, such as the peptides of SEQ ID No: 53-187. Specific immunogenic compositions according to the invention comprise the peptidic sequences of SEQ ID Nos: 55, 56, 62, 63 or 66, which have been demonstrated to be immunogenic in HLA-A2 individuals (example 7). According to another specific embodiment, the immunogenic
30 composition comprises a peptide comprising a sequence of SEQ ID No: 63, SEQ ID No: 188 or SEQ ID No: 209 or a polynucleotide encoding the same. Such an immunogenic composition is particularly useful as an anticancer vaccine in a HLA-A*0201 patient, especially if this patient has a tumor exhibiting a high GPD1L expression level (measured at the mRNA or protein level).

35 According to a particular immunogenic composition according to the invention, the polypeptide is a short polypeptide (9-, 10- or 11-mer). When such short peptides are subcutaneously injected, they bind directly to MHC molecules of every cells

present at the site of injection. According to this embodiment of the invention, a cocktail of peptides, comprising several TMP epitopes, can advantageously be used. It is to be noted that when several epitopes specific for the same HLA molecule are used together, the epitopes are in competition for the binding to the corresponding HLA molecule.

5 Contrarily, by using a mix of different HLA-restricted epitopes (such as HLA-A*0201, HLA-A*2402, HLA-B*0702 or others), there will be no competition for HLA binding. Another advantage of such a cocktail of peptides is that it will be efficacious in a broader range of patients *i.e.*, in individuals expressing any of the HLA molecules corresponding to the epitopes present in the cocktail.

10 According to another particular immunogenic composition according to the invention, the polypeptide is a long polypeptide of 20 to 50 amino acids, preferably 25 to 40 amino acids encompassing at least one 9-10 TMP immunogenic stretch selected amongst SEQ ID No: 53 to 187, flanked by 10-20 amino acids, which will be *in vivo* internalized and processed by antigen presenting cells. These cells will then present
15 fragments thereof, thereby triggering an immunogenic response against TMP epitopes. Such long polypeptides are advantageously able to be cross-presented by local DC into not only MHC class I but also MHC class II molecules of the host. For example, chimeric polypeptide polypeptides, *i.e.*, polypeptides comprising a concatenation of epitopes of the TMP, possibly separated by peptidic linkers, can be used. Of course, for the same
20 reasons as mentioned in the above paragraph, a long polypeptide advantageously comprises different HLA-restricted epitopes. Such chimeric polypeptide polypeptides can also comprise epitopes from antigens different from the TMP, for example epitopes from tumor antigens.

The immunogenic compositions according to the invention also
25 advantageously comprise appropriate adjuvants. The skilled artisan will chose, depending on the type of peptide and the type of application, the most appropriate adjuvant. Non-limitative adjuvant which can be used according to the present invention include Montanide, Flt3L, cyclophosphamide, DC and TLR or STING agonists.

According to one embodiment, the immunogenic composition comprises
30 a polynucleotide encoding a polypeptide comprising a sequence of at least 9 consecutive amino acids from the TMP of SEQ ID No: 1. Such a composition can advantageously be used as an anticancer vaccine. Examples of polynucleotide compositions according to the invention include naked DNA, mRNAs encoding TMP, RNA loaded nanoparticles and recombinant viruses (such as lentiviruses, oncolytic viruses adenoviruses,
35 poxviruses *etc.*). Of course, the considerations mentioned above regarding the advantages of mixing epitopes specific for different HLA molecules in polypeptide compositions apply when designing the polynucleotides to be included in the

compositions according to the invention. Alternatively, the epitopes can be selected and personalized according to the HLA haplotype of the patient (Table 9).

According to another aspect, the present invention pertains to a cell composition comprising antigen presenting cells (APC) which have been pulsed *ex vivo* with a bacterial composition as above described or with an immunogenic composition according to the invention. Such a cell composition can advantageously be used for cell therapy of cancer patients.

According to another of its aspects, the present invention relates to a MHC multimer, for isolating T-cells with high affinity for the TMP of SEQ ID No: 1. In such a MHC multimer, MHC molecules are preferably bound to a peptide selected from the group consisting of SEQ ID No: 53 to 187, for example to at least one peptide selected amongst those of SEQ ID Nos: 55, 56, 62, 63 and 66, or to at least one peptide of SEQ ID No: 188 or 209. MHC multimers according to the invention range in size from dimers to octamers (e.g.: tetra, penta, hexamers) or use even higher quantities of MHC per multimer (e.g., dextramers). Particular MHC multimers according to the invention are HLA-A*0201/KLAKFASVV multimers, HLA-A*0201/KLQKFASTV multimers, HLA-A*0201/KLQKFASVV multimers and HLA-A*0201/KLAKFASTV multimers (e.g., tetramers or dextramers).

Another cell composition according to the present invention comprises CD4⁺ or CD8⁺ T cells specific for the TMP of SEQ ID No: 1. Cells comprised in such a composition can be obtained either by cell sorting (using, for example, a MHC multimer as above-described) followed by *ex vivo* expansion, or by transduction of T lymphocytes with a cDNA encoding a TCR with a high avidity for TMP. Examples of protocols that can be used to obtain such compositions are disclosed in examples 10 and 11 below.

As already mentioned, the immunogenic compositions and the cell compositions described above can be used for treating a cancer. In such a treatment, they can be used alone or combined with peptidic or nucleotidic vaccines as described above and/or combined with antineoplastic treatments such as chemotherapy, for example an alkylating agent such as cyclophosphamide, or immune checkpoint blockers, especially anti-PD1/PD-L1/PD-L2 antibodies.

The present invention also pertains to the use of a bacteriophage expressing a protein having at least 90, preferably at least 95-98.7% identity with the TMP of SEQ ID No: 1 for treating a cancer. Such a bacteriophage is preferably formulated in a pharmaceutically acceptable composition, which can be administered *per os* or intratumorally.

According to a particular embodiment of the bacteriophage composition of the invention, the bacteriophage has a genome comprising a nucleotide sequence of SEQ ID NOs: 2 or a sequence having at least 90% or 95% identity thereto.

When treating a cancer patient, a bacteriophage composition according to the invention can advantageously be administered in combination with a drug blocking an immune checkpoint, for example in combination with anti-PD1/PD-L1/PD-L2 antibodies.

As explained in the experimental part below, the inventors found that the HLA-0201-restricted epitope KLAKFASVV (SEQ ID No: 63), shares a 78% sequence homology with an epitope (KLQKFASTV, SEQ ID No: 188) of the glycerol-3 phosphate dehydrogenase 1 like protein (GPD1L, gene encoded on 3p22.3), and it is conceivable that cross-reactivities between TMP phage specific TCR and self tissues or tumor tissues overexpressing GPD1L account for the anticancer effectiveness of the phage delivered in the context of the invention. The bacterial compositions, the immunogenic compositions, the cell compositions and the bacteriophage compositions of the invention are thus particularly useful for treating a tumor overexpressing GPD1L, *i.e.*, having mRNA levels of GPD1L superior to levels expressed in paired normal tissues (for example lung cancer versus surrounding "healthy" lung parenchyma). Tumors overexpressing GPD1L would indeed be considered as electively eligible for an oral therapy with *E.hirae* 13144 or *EH IGR7* or *EH IGR11* or a combination of all the 3 strains, or with any other bacterium recombinant for the TMP of SEQ ID No: 1 or a fragment thereof comprising SEQ ID No: 63, with or without intradermal boosts with TMP phage-related HLA restricted-peptides or nucleic acid or other vaccine modality. This applies to lung cancers, melanoma, tumors of the digestive tract, bladder cancer, RCC and breast cancer or any tumor expressing foetal antigens.

Another aspect of the present invention is a screening method for identifying antineoplastic drugs, comprising assessing the ability of drug candidates to trigger the lytic cycle of the phage comprising the TMP of SEQ ID No: 1 when they are incubated with bacteria from the strain CNCM I-4815 (or any other bacterial strain harbouring a prophage genome with at least 80 and preferably at least 90% or 95% identity with the prophage of SEQ ID No: 2). When performing this method, the skilled artisan can use different concentrations of the drug candidates and measure the phage excision at several time points of incubation.

The present invention also pertains to theranostic methods for determining if a patient is likely to be a good responder to a treatment by chemotherapy or immune checkpoint blockade, all based on the results disclosed below showing the importance of the phage TMP protein expressed by the strain CNCM I-4815 in the

patient's response to the treatment. These methods are particularly useful to determine if a patient having a cancer such as NSCLC, RCC, bladder cancer, pancreas cancer, colorectal cancer and breast cancer is likely to be a good responder to a treatment by chemotherapy or immune checkpoint blockade. More particularly, such a method can advantageously be used for a patient at diagnosis of a NSCLC or RCC prior to immunotherapy with an ICB.

According to one embodiment of this aspect of the invention, the theranostic method of the invention comprises assessing the presence, in a biological sample from said patient, of a sequence having at least 80%, 90% or 95% identity with the TMP of SEQ ID No: 1. According to this method, the patient is considered as likely to respond to the treatment if such a sequence is present in the sample. Non-limitative examples of biological samples which can be used for performing this method are the tumor genome, intratumoral bacterial load, fecal phages, fecal bacteria containing the phages, feces samples, bronchoalveolar samples, buccal samples and sputum.

More specifically, this method can be performed by PCR amplification of specific sequences and comprise the following steps:

- (i) cultivating a stool sample from the patient in aerobic conditions in a permissive medium to allow isolation of enterococci colonies,
- (ii) performing a PCR on several cultivable isolated colonies with a pair of primers specific for a fragment of SEQ ID No: 1, and
- (iii) detecting the amplified fragment.

More precisely, in step (ii), the PCR can be done within each *E. gallinarum*, *E. hirae* and *E. faecalis* isolated stool colony, or at least in 3 to 5 of them. For example, the PCR can be performed with the primers of SEQ ID Nos: 191 and 192, generating an amplicon of 1026 bp.

The present invention also pertains to a method of determining if a patient is likely to be a good responder to a treatment by chemotherapy or immune checkpoint blockade, comprising measuring, for example by flow cytometry or TMP tetramer-binding T lymphocytes, the levels of circulating CCR9⁺ CXCR3⁺ CD8⁺ T cells during said treatment, wherein if said level is above a predetermined threshold, the patient is likely to respond to the treatment.

According to a particular embodiment, a MHC multimer as above-described is used to assess the presence of T cells specific to the protein of SEQ ID No: 1, in a biological sample from a cancer patient. The presence of such T cells in the sample indicates that the patient is likely to respond to the treatment. More particularly, when this method is performed for assessing whether a HLA-A*0201 patient is likely to

be a good responder to a treatment by chemotherapy or immune checkpoint blockade, a HLA-A*0201/SEQ ID No: 209 multimer can be used.

According to another embodiment, the theranostic method of the invention comprises assessing the level of GPD1L mRNA or the level of GPD1L protein in the tumor, wherein if said level is above a predetermined threshold, the patient is likely to respond to the treatment.

Of course, the physician or skilled technician can combine the above methods to refine the prognosis that a patient will respond to the treatment, for example by assessing both the GPD1L expression level in the tumor and the presence of T cells specific for KLAKFASVV (SEQ ID No: 63), or the GPD1L expression level and the presence of a sequence having a high percentage of identity with the TMP of SEQ ID No: 1.

If, when performing a theranostic method as above-described, the patient is not identified as likely to respond to a treatment by chemotherapy or immune checkpoint blockade, a probiotic treatment with a bacterial composition according to the present invention can advantageously be administered to the patient to increase his/her chances to respond to the treatment. Another preconditioning treatment to increase the patient's response is a vaccination with an immunogenic composition as above-described.

Other characteristics of the invention will also become apparent in the course of the description which follows of the biological assays which have been performed in the framework of the invention and which provide it with the required experimental support, without limiting its scope.

EXAMPLES

Some of the experiments illustrating the present invention are described two times in the following examples, showing the continued research effort made by the inventors in the past years, which led to a more precise knowledge of the mechanism explaining the effects of *E. hirae* strains 13144, IGR7 and IGR11 and to the design of newly engineered strains with anti-tumor properties.

Materials and Methods

For each experiment illustrated in Figures 1 to 26, the materials and methods used to perform the experiment are detailed in the figure legend.

The following table summarizes the *E. hirae* strains referred to in examples 1 to 11 below.

Example	Strain	Origin	Deposit number
1 - 9	13144	Murine – CTX-treated	CNCM I-4815
1, 5, 9	IGR 1	stools of lung cancer patients	
1, 9	IGR 4		CNCM I-5260
1, 3, 5, 9	IGR 7		CNCM I-5224
1, 9	IGR 10		
1, 5, 9	IGR 11		CNCM I-5261
2, 3, 5, 6	708	Human - Unknown	
2, 3, 5	EH17 (13344)	Human - Blood	
1, 3, 5	10815 (ATCC 9790)	Type strain CIP 53.48 ^T	ATCC 9790
9	5348	Human - Unknown	
	7030	Human – Liver abscess	
	12607	Environmental – RiskManche project	
	13150	Environmental - Water	
	13152	Environmental - Water	
	13153	Environmental - Water	
	13155	Environmental – RiskManche project	
	13161	Environmental - Cockle	
	13343	Conservation liquid of kidney	
	13346	Human - Urine	
	13347	Blood culture	

Table 1: Strains used in this study. Strains called "IGR" were isolated by U1015 INSERM at Gustave Roussy, Villejuif, in 2017, anonymously from non small cell lung cancer patients at diagnosis about to be treated with nivolumab as a second line therapy. 708 comes from INRA but was originally sent by a spanish collaborator more than 10 years ago. All the other strains come from Dr Vincent Cattoir, director of the reference center for commensal and pathogenic enteroccci, CHU de Rennes – Hôpital Ponchaillou-Service de Bactériologie-Hygiène hospitalière-2 rue Henri Le Guilloux – 35033 RENNES Cedex.

The following materials and methods were also used for performing the experiments reported in Examples 12 to 16 below.

Cell culture, reagents and tumor cell lines

MCA-205 WT were cultured at 37°C with 5% CO₂ in RPMI 1640 containing 10% FCS, 2 mM L-glutamine, 100 UI/ml penicillin/streptomycin, 1 mM sodium

pyruvate and MEM non-essential amino acids (henceforth referred to as complete RPMI1640). All reagents were purchased from Gibco-Invitrogen (Carlsbad, CA, USA).

Mice

All animal experiments were carried out in compliance with French and European laws and regulations. The local institutional board approved all mouse experiments (permission numbers: 2016-109-7450). Experiments were performed in accordance with Government and institutional guidelines and regulations. Female C57BL/6 were purchased from Harlan (France). Mice were used at an age between 7 and 12 weeks of age. All mouse experiments were performed at the animal facility in Gustave Roussy Cancer Campus where animals were housed in specific pathogen-free conditions.

Antibiotic treatments

Mice were treated during 3 days with an antibiotic solution (ATB) containing ampicillin (1 mg/ml), streptomycin (5 mg/ml), colistin (1 mg/ml) (Sigma-Aldrich) and vancomycin (0.25 mg/ml) added in the sterile drinking water of mice. Antibiotic activity was confirmed by cultivating fecal pellets resuspended in BHI+15% glycerol at 0.1 g/ml on COS (BD Columbia Agar with 5% Sheep Blood) plates for 48h at 37°C in aerobic and anaerobic conditions. In the context of bacterial transplantation experiments, mice received 3 days of ATB before undergoing bacterial transplantation the next day by oral gavage using animal feeding needles.

Tumor challenge and treatment

Syngeneic C57BL/6 mice were implanted with 0.8×10^6 MCA-205 WT sarcoma cells subcutaneously and treated intraperitoneally (i.p.) when tumors reached 20 to 35 mm² in size with CTX (100mg/kg). Depending on the experimental setting, mice were injected once or 3 times at 1-week interval. Tumor size was routinely monitored every 3 days by means of a caliper.

Gut colonization with dedicated commensal species

Enterococcus hirae 13144 were originally isolated from spleens of SPF mice treated with CTX in our laboratory. *E. hirae* 708 was provided by INRA (P. Langella), while *E. hirae* 13344, ATCC9790 were provided by Prof. Cattoir, CHU de Caen, France. *L. plantarum* was provided by Prof. Ivo Gomperts Boneca, Institut Pasteur strain repository, France. All *E.hirae* IGR strains were isolated from NSCLC patient stools in our laboratory, according to patient informed consent and local IRB approval (study Oncobiotics). All bacteria were grown on COS plates for 24h-48h at 37°C in aerobic

conditions. Colonization of ATBs pre-treated mice was performed by oral gavage with 100 µl of suspension containing 1×10^9 bacteria. For bacterial gavage: suspensions of 10^8 CFU/mL were obtained using a fluorescence spectrophotometer (Eppendorf) at an optical density of 600 nm in PBS. Depending on the experimental setting, 2 or 6 bacterial gava-
5 gages were performed for each mouse: the first, the same day as CTX injection, and then 24h after the injection of CTX. The efficacy of colonization was confirmed by culturing the feces 48h post-gavage. Fecal pellets were harvested and resuspended in BHI+15% glycerol at 0.1 g/ml. Serial dilutions of feces were plated onto COS plates and incubated for 48h at 37°C in aerobic and anaerobic conditions. After 48h, the
10 identification of specific bacteria was accomplished using a Matrix-Assisted Laser Desorption/ Ionisation Time of Flight (MALDI-TOF) mass spectrometer (Andromas, Beckman Coulter, France).

Culture and propagation of bone marrow-derived dendritic cells

Bone marrow-derived dendritic cells (BM-DCs) were generated by
15 flushing bone marrow precursors from the femurs and tibia of female C57Bl/6 WT mice aged of 8 to 12 weeks. Bones were collected in sterile PBS, washed in alcohol and Iscove's medium (IMDM, Sigma-Aldrich) baths, extremities of bones were cut and flushed using a 26G needle. After red blood cell lysis, cells were cultured in IMDM supplemented with 10% of FCS + 2mM L-glutamine + 100 UI/ml penicillin/streptomycin
20 + 50µM 2-mercaptoethanol (Sigma-Aldrich) (referred herein as complete IMDM medium) at 0.5×10^6 /ml and treated with 40ng/ml of GM-CSF (supernatant of GM-CSF transfected-cells J558) and 10 ng/ml of recombinant interleukin-4 (IL-4) for BM-DCs (from Peprotech). Cells were split at day 3 and used in experiments on day 7 or 8.

Test of memory TC1 immune response and H2-Kb restricted-peptides on splenic CD8+ T cells by ELISpot IFN γ

25

IFN- γ ELISPOT assay was performed in 96-well PVDF bottomed sterile plates (Millipore MSIP S4510) by using a IFN- γ ELISPOT kit (Cell sciences, Newburyport, Etats-Unis) according to the manufacturer's instructions. After PVDF membrane activation with ethanol 35%, plates were coated overnight with capture
30 antibody to IFN- γ and washed before incubation of blocking buffer during 2h. BM-DC cells (1×10^5 /well) were infected with heat-inactivated (2h at 65°C) bacterial strains (*E. hirae* 13144, *E. hirae* 708, *E. hirae* 13344 and *L. plantarum*) at a multiplicity of infection 1:10 (MOI) or pulsed with peptides (20µg/ml) and were added together with CD8+ T cells (2×10^5 /well) and incubated for 20 h at 37°C. Cells were then removed and plates were
35 developed with a detection antibody to IFN- γ (biotinylated) during 1h30 and streptavidin-

alkaline phosphatase during 1h. Finally, substrate of streptavidin (BCIP/NBT buffer) were incubated 5-20 min. Spots were counted by using CTL Immunospot Analyzer (Germany).

Mice vaccination

After DCs differentiation, these DCs were activated with poly I:C (10µg/ml, Invivogen) overnight before infection with heat-inactivated (2h at 65°C) bacterial strains (MOI 10) or pulsed with peptides (20µg/ml, peptide 2.0). After 6 hours of incubation with bacteria or 1 hour of incubation with peptides, BM-DCs were washed 3 times with PBS before subcutaneous injection in the right flank of mice (1.5×10^5 cells per mice). Mice were vaccinated twice at 10 days apart and challenged 4 weeks after the second vaccination with the minimal tumorigenic dose of MCA-205 tumor cells in left flank.

Flow cytometry analyses

In experiments without tumor, spleens were harvested 7 days after the injection of CTX. In tumor growth experiments, spleens, tumors and tumor draining lymph node were harvested at different time points, 7, 14 and 21 days after the first injection of CTX into mice bearing MCA-205 tumors. Excised tumors were cut into small pieces and digested in RPMI medium containing Liberase TM at 25 µg/mL and DNase1 at 150 UI/mL (Roche) for 30 minutes at 37°C and then crushed and filtered twice using 100 and 40µm cell strainers (BD). Lymph nodes and spleen were crushed in RPMI medium and subsequently filtered through a 70 µm cell strainer. Two million splenocytes, tumor cells or lymph node cells were pre-incubated with purified antimouse CD16/CD32 (clone 93; eBioscience) for 15 minutes at 4°C, before membrane staining. Dead cells were excluded using the Live/Dead Fixable Yellow dead cell stain kit (Life Technologies). Antimouse antibodies for CD3 (145-2C11), CD4 (GK1.5), CD8 (eBioH35-17.2), CXCR3 (CXCR3-173), CCR9 (CW-1.2), and TMP specific tetramer (BD, BioLegend, eBioscience and Cliniscience) were used. Stained samples were acquired on Canto II 7 colors cytometer (BD) and analyses were performed with FlowJo software (Tree Star, Ashland, OR, USA).

Human T cell responses to HLA-A02*01 restricted-TMP epitopes

Cytapheresis cones were collected from healthy volunteers (EFS, *Etablissement français du sang*) and peripheral blood mononuclear cells (PBMC) were separated using a Ficoll Hypaque gradient. We selected only donors with HLA-A02*01 haplotype determined by flow cytometry with anti-HLA-A2 antibodies. PBMC were washed and resuspended in the separation medium (PBS, 1mM EDTA, 2% human AB+ serum) for magnetic bead separation. CD14+ monocytic cells (human CD14 MicroBeads, Miltenyi) were enriched from 75×10^6 PBMC and cultured at 0.5×10^6 /ml

in IMDM supplemented with 10% human AB+ serum, 1% of 2 mmol/L glutamine (GIBCO Invitrogen), 1000 IU/ml GM-CSF and 1000 IU/ml IL-4 (Miltenyi). Cells were split at day 3 and used in experiments on day 6 or 7. Monocytes were seeded in 96-well plates at 1×10^5 cells/well either alone or in the presence of peptides (20 µg/ml), and incubated for 2 hour at 37°C, 5% CO₂. The remaining autologous PBMC fractions were enriched for CD8+ T cells (CD8+ T Cell Isolation Kit, human, Miltenyi). The enriched CD8+ T cells were washed, counted and resuspended at 1×10^5 cells/well in RPMI-1640 supplemented with 10% human AB+ serum, 1% 2 mMol/L glutamine, 1% penicillin/streptomycin (GIBCO Invitrogen) and 50 U/mL IL-2 (Proleukin). Monocyte-peptide/ T cell co-cultures were incubated for one week at 37°C, 5% CO₂ (medium was changed every 2 days). Then, the pools of cells were seeded in 96-well ELISPOT plates at 2×10^5 cells/well and restimulated with or without peptides (20 µg/ml) or anti-CD3/anti-CD28 coated beads (1 µL/mL, Dynabeads T-Activator, Invitrogen) as a positive control for 20h at 37°C. IFN-γ ELISPOT assay were performed in 96-well PVDF bottomed sterile plates (Millipore MSIP S4510) by using a IFN-γ ELISPOT kit (Cell sciences, Newburyport, Etats-Unis) according to the manufacturer's instructions.

Stool detection of phage TMP sequence by PCR

We cultivated the stools (of patients) or ileal material (mice) after several dilutions in aerobic conditions and permissive medium to allow isolation of enterococci colonies (according to a procedure described in (Samb-Ba et al., 2014)). We performed a PCR of the TMP sequence in each single cultivable *Enterococcus* colony. One colony was put in 100 µl of nuclease-free water to release the bacterial DNA and PCR was performed with 5 µl of DNA, 12.5 µl of PCR master mix (Thermoscientific), 5 µl of nuclease-free water and 1.25 µl of each TMP primers (20 µM). PCR products were separated on 1.5% agarose gel containing ethidium bromide and revealed by UV exposition. The sequence of primers are: forward 5'-ACTGCAGCCGTAAAATGGGA-3' (SEQ ID No: 191) and reverse 5'-TCCGTATCGTTTGCCAGCTT-3' (SEQ ID No: 192) (amplicon 1026 bp).

Generation of TMP-expressing E. coli

A DNA fragment containing the P23 promoter sequence was generated by annealing two complementary primers (5'-CAATAAAAAATCAGACCTAAGACTGATGACAAAAAGAGCAAATTTTGATAAAATAGTATTAGAATTAAATTAAGGGAGGCCAAATATAG-3' (SEQ ID No: 193) and 5'-GATCCTATATTTGGCCTCCCTTTTAAATTTAATTCTAATACTATTTTATCAAAATTTGCTCTTTTGTGCATCAGTCTTAGGTCTGATTTTATTGCATG-3' (SEQ ID No: 194)).

The sequence was then inserted into SphI/BamHI-digested vector pDL278 (Addgene 46882, gift from Gary Dunny (LeBlanc et al., 1992)) to generate vector pDL278-P23. A part of the TMP gene (N-terminal 1185 nucleotides of TMP, including the epitope TSLARFANI (SEQ ID No: 13), fused to a C-terminal FLAG-tag) was amplified from

5 *E. hirae* 13144 genomic DNA (5'-TCCGGATCCATGGCACAAAGTAAAACAGTCAAAGCG-3', (SEQ ID No: 195) 5'-CAGGAATTCTTACTTGTCGTCATCGTCTTTGTAGTCACGTAGTAACTATCACGTAATCGAACTTC-3' (SEQ ID No: 196)) and inserted into BamHI/EcoRI-digested vector pDL278-P23 to generate vector pDL278-P23-TMP-FLAG. Mutations in the epitope were

10 introduced using the QuikChange Lightning Kit (Agilent). Primers 5'-AACGAGCTAAGGCAGTAGCAGCTGTATCTGCAGAC-3' (SEQ ID No: 197) and 5'-GTCTGCAGATACAGCTGCTACTGCCTTAGCTCGTT-3' (SEQ ID No: 198) were used to mutate position 2 (S to A, pDL278-P23-TMP-mut2-FLAG), primers 5'-ATTAGCAAAACGAGCGAAGGAAGTAGCAGCTGTATCTG-3' (SEQ ID No: 199) and 5'-

15 CAGATACAGCTGCTACTTCCTTCGCTCGTTTTGCTAAT-3' (SEQ ID No: 200) were used to mutate position 3 (L to F, pDL278-P23-TMP-mut3-FLAG). To generate the control plasmid pDL278-P23-EGFP, EGFP was amplified from pCIB1(deltaNLS)-pmGFP (Addgene 28240, gift from Chandra Tucker (Kennedy et al., 2010)) using primers 5'-CTTGGATCCATGGTGAGCAAGGGCGAG-3' (SEQ ID No: 201) and 5'-

20 CAGGAATTCCTACATAATTACACACTTTGTC-3' (SEQ ID No: 202) and inserted into BamHI/EcoRI-digested vector pDL278-P23. Plasmids were transformed into chemically competent *E. coli* DH5α (NEB) and the presence of plasmids with the correct insert was verified by sequence analysis (5'-CCCAGTCACGACGTTGTAAAACG-3' (SEQ ID No: 203) and 5'-GAGCGGATAACAATTCACACAGG-3' (SEQ ID No: 204)). Expression of

25 EGFP and TMP-FLAG in *E. coli* was verified by western blot analysis using antibodies targeting GFP (Cell Signaling, 2956) or FLAG (Sigma-Aldrich, F7425), respectively.

CRISPR/Cas9-mediated mutations of mouse *Psmb4* in MCA205 cells

Wild type MCA205 cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and was maintained in RPMI-1640

30 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% FBS (Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 µg/ml streptomycin (Thermo Fisher Scientific, Inc.) at 37°C. For the CRISPR knock in mutations, we designed the gRNA (sequence AGATATTGCGAAACGAGCC (SEQ ID No: 205)) by using the CRISPR design tool developed by the Zhang lab (<http://crispr.mit.edu/>). Oligonucleotides

35 containing the designed sequence were synthesized (Sigma) and ligated into the pX458 backbone (Addgene #48138, (Ran et al., 2013)) containing the Cas9 gene (human

codon-optimised and fused with 2A-GFP allowing for selection) under a CBh promoter and the cloned sgRNA under a U6 promoter. Homology templates (sequence attached) containing the mutation sites were synthesized by Invitrogen GeneArt Gene Synthesis (Thermo Fisher Scientific, Inc.). The cloned pX458 plasmid and synthesized homology arms were cotransfected into MCA205 cells by means of lipofectamine 3000 (Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. Forty-eight hours after transfection, GFP-positive cells were sorted to 96-well plates as single cells before surviving clones were expanded in duplicated conditions, one for frozen storage at -80 and the other for genomic DNA extraction. The targeted region in genomic DNA from clones was further amplified by PCR using the Phusion® High-Fidelity PCR Master Mix (New England BioLabs; Ipswich, MA, USA) and primers 5'CTCAGGGACCCTTTTCACGA 3' (SEQ ID No: 206) and 5'CCCACTCCCTGTTCTACACA 3' (SEQ ID No: 207), and purified with the Monarch® DNA Gel Extraction Kit (New England BioLabs) before being sent to Eurofins Genomics GmbH (BERSBERG GERMANY) for sequencing with the primer 5'GGACCCTTTTCACGATTCAGG 3' (SEQ ID No: 208). According to sequence results, positive clones were expanded and subjected to DNA extraction for validating sequences. Transfected single cell clones that did not harbor the designed mutations were used as "WT" clones.

20 **Genome sequencing and analysis**

The whole genome sequence of 5 *E. hirae* (13144, 708, 13152, 13344 and EH-17) strains was determined with PacBio technology (GATC Biotech, Konstanz, Germany). Genomic DNA was isolated from 15 other *E. hirae* isolates using the Quick-DNA fungal/bacterial miniprep kit (Zymo Research, Irvine, CA) according to the manufacturer's recommendations. After DNA shearing, the DNA libraries were prepared using the NEBNext Ultra DNA library prep kit for Illumina (New England Biolabs, Ipswich, MA) and sequenced as paired-end reads (2 x 300 bp) using an Illumina MiSeq platform and the MiSeq reagent kit version 3. The Illumina reads were trimmed using Trimmomatic (Bolger et al., 2014), quality filtered with the Fastx-toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) and assembled using SPAdes (Bankevich et al., 2012). Protein sequences were predicted using prokka v1.11 software (Seemann, 2014). Prophage regions were detected using PHAST software. Predicted proteins were annotated using BLASTp against the National Center for Biotechnology Information (NCBI) non-redundant (NR) database.

Phylogenomic and comparative genomics

Single nucleotide polymorphism between 20 *E. hirae* genomes was investigated using the parsnp program (Treangen et al., 2014) and strain 13144 genome sequence as a reference. Phylogenetic analysis was performed by considering the 47,303 polymorphic sites retained in the core genome of the 20 genomes. Maximum likelihood phylogeny was constructed using Fasttree (Guindon et al., 2010). Phylogenetic tree was visualized using figtree (<http://tree.bio.ed.ac.uk/software/figtree/>). Complete Proteome sequences of 20 *E. hirae* strains were compared using by BlastP and pairwise alignments using ClustalW. We clustered the *E. hirae* homologous genes using orthoMCL (Li et al., 2003) on the translated protein sequences of all predicted genes with a conservative parameter value of 70% amino acid sequence identity and 50% sequence coverage. The determination of the different unique core genomes was based on the homology clusters found by orthoMCL.

Statistical analyses

Data analyses and representations were performed either with Prism 6 (GraphPad, San Diego, CA, USA). Tumor size differences were calculated either using Anova or dedicated software (<https://kroemerlab.shinyapps.io/TumGrowth/>). Briefly, tumor growth was subjected to a linear mixed effect modeling applied to log pre-processed tumor surfaces. P-values were calculated by testing jointly whether both tumor growth slopes and intercepts (on a log scale) were different between treatment groups of interests. Survival probabilities were estimated using the Kaplan-Meier method, best cutoffs for continuous variables were chosen using the median value or an optimal cutoff approach. Survival curves were evaluated using the log-rank test. All reported tests are two-tailed and were considered significant at P-values <0.05. The normalized gene expression values (FPKM-UQ) and the corresponding clinical data were downloaded from the TCGA data portal. For the survival analysis patients were grouped by the median expression of GPD1L across per cancer type datasets and by predicted HLA-alleles (Charoentong et al., 2017). Hazard ratio (HR) and 95% confidence interval (CI) for overall survival (OS) and progression-free survival (PFS) were calculated with Cox regression analysis. The tumor-infiltrating immune cell compositions were determined using CIBERTSORT (absolute mode) (Newman et al., 2015) and were compared with gene expression using Pearson correlation.

Example 1: Different strains of *E. hirae* exhibit distinct immunogenic potential and antitumor effects

We first tested our *E. hirae* isolates in a more physiological setting of dysbiosis than the one caused by the 14 day-administration of a broad spectrum antibiotics. We indeed transferred feces (FMT) from two breast cancer patients exhibiting or not a deviated repertoire of the gut microbiome (and a distinct prognosis) into ATB-treated recipients and compensated their dysbiosis with different strains of adjunctive *E. hirae* (Figure 1) attempting to restore full blown efficacy of CTX (as shown in eubiotic mice). While the FMT from a dysbiotic BC patient conferred resistance to CTX compared with eubiotic littermates (Figures 2A-B), live EH13144 was very effective at restoring the efficacy (Figures 2C-E). In a second example of FMT from a different BC patient (exhibiting no overt dysbiosis (Figures 3A-B), the effects mediated by live EH13144 were also significant (Figure 3C-D). However, in a similar setting, other strains of *E. hirae* (IGR1 and IGR11), cultivated from stools of lung cancer patients were not immunogenic (Figure 4 A-C), although as effective as EH13144 to induce IL-1 β and IL-12 production by BM-DC (not shown).

In addition, EH10815 did not mediate antitumor effects while *E. hirae* (IGR4 and IGR7), cultivated from stools of lung cancer patients were also immunogenic, as observed with 13144 (Figure 4 D). Moreover, the combination of EH13144, IGR4 and IGR7 is superior to EH13144 leading compound (Figure 5).

Interestingly, pasteurized EH13144 was far less effective than its live counterpart and failed to restore CTX tumoricidal activity in a dysbiotic FMT (Figure 6).

We conclude that EH13144 have unique properties to boost CTX immune and tumoricidal activity against MCA205 sarcoma in not only ATB- but also FMT-induced patients' dysbiosis.

Example 2: *E. hirae* 13144 amplifies CD8⁺Tc1 cells in the mesenteric lymph nodes and spleens cross-reactive with EH708

EH13144 plays an adjuvant role with cyclophosphamide to boost antitumor immune responses and anticancer effects in a T cell- and IFN γ -dependent manner (Daillère et al., 2016). We dissected the mechanisms of the immunogenicity of EH13144 by analyzing the dynamics of the T cell immune responses in secondary lymphoid organs from the mesenteric lymph node (mLN) and the spleen (Figure 7A and 7D). EH13144 as well as *E. hirae* 708 were both capable of inducing the accumulation

of CD8⁺ T cells in both, mLN after 1 week and spleen after 2 weeks (Figures 7B and 7E). However, while EH13144 induces an efficient anti-tumor immune response, *E. hirae* 708 fails to do so (Fig. 1D of Daillère et al., 2016). EH13144 also amplified the CD4⁺ T cell pool in the mLN (Figure 7C). Moreover, EH13144 induce accumulation of CD8⁺ CCR9⁺ CXCR3⁺ in spleen (Figure 7F) and in the tumor beds (Figure 8).

To analyze the specificity of this CD8⁺ T cell expansion, we challenged splenic CD8⁺ T cells with bone marrow-derived dendritic cells (BM-DC) infected with various EH isolates (13144, EH708 and EH17 or *L. plantarum*). A commensal-specific memory CD8⁺ T cell response was monitored by an ELISPOT assay at 24 hrs by the enumeration of IFN γ positive spots (Figure 9), pathognomonic of a Tc1 immune response. Interestingly, only EH708 and EH13144 could induce memory Tc1 immune responses with a cross-protection inbetween themselves (Figure 10) while EH17 failed to do so, corroborating the poor protective antitumor effects of EH17 reported by Daillère et al. (Daillère et al., 2016).

We scrutinized colon mucosae for CD8⁺ T cell infiltrates by monitoring intestinal inflammatory lesions in the presence or absence of neutralizing anti-CD8 antibodies in immunohistochemistry (Figure 11A). Hematoxylin eosin stained colon mucosae were more infiltrated after oral gavages with EH13144 or EH708 than without commensals post-CTX but these infiltrates were drastically reduced when animals were pretreated by ip administrations of neutralizing anti-CD8 Abs (Figure 11B). Of note, these CD8⁺ T cells primed in mLN or spleen have a tendency to home to the lamina propria in the absence of a distant tumor but not if a sarcoma deposit is introduced subcutaneously (Figure 18).

Altogether, these findings suggest that oral gavages with EH13144 or EH708 in the context of cyclophosphamide trigger not only a splenic Th1 (Daillère et al., 2016) but also a systemic Tc1 immune response recognizing both EH708 and EH13144 (but not EH17) sequences with the potential to traffic back to the colon if no tumor deposit exist or to tumor beds when they develop.

Example 3: Screening EH specific H-2K^b restricted peptides: *E. hirae* 13144 genome contains immunogenic peptide sequences in the TMP phage protein

Whole genome differential analyses were performed on three *E. hirae* strains (13144, 708 and EH17) to identify potential 9-mer MHC class I-binding epitopes. The search focused on subcellular localization (enrichment for cell wall and extracellular

proteins, via PSORT software), and on binding affinity to H2-K^b (<50 nM binding affinity, NetMHC software) (Figure 12, Tables 2-5).

Hirae	sequence	names of the proteins	SEQ ID No:
13144	SAFPYEQEL	C3 family ADP-ribosyltransferase	3
13144	YNYSKSYV	hypothetical protein	4
13144	VSFSHYRPG	hypothetical protein	5
13144	VTFLGYNAF	cell surface protein	6
13144	TVYTFHVNI	cell surface protein	7
13144	TSYSPLFLL	cell surface protein (putative)	8
13144	TNYIYPNIL	2',3'-cyclic-nucleotide 2'-phosphodiesterase	9
13144	VVPILFLGL	FmtB protein	10
13144	KNYKAYVEL	hypothetical protein	11
13144	SAMKYGIPL	hypothetical protein	12
13144	TSLARFANI	Phage tail length tape-measure protein	13
13144	AMIEFIQGL	Phage tail length tape-measure protein	14
13144	VAITFGGPL	Phage tail length tape-measure protein	15
13144	VSTNHYGLL	hypothetical protein	16
13144	VMFGLFITI	cell surface protein precursor	17
13144	TVFSLVSLL	Chitinase	18
13144	SIYNLEKPL	IgA1 protease	19
13144	YTIIRYGNL	IgA1 protease	20
13144	SNGLLYTPM	IgA1 protease	21
13144	NNYHYVGGL	IgA1 protease	22
13144	SMFLNCNNL	hypothetical protein	23
13144	IAFQGYSSL	hypothetical protein	24
13144	QVTNFFNMF	hypothetical protein	25
13144	IMLGLFMTM	cell surface protein precursor	26

Table 2: H-2b restricted-EH13144 peptides not present in the other strains

5

Hirae	sequence	names of the proteins	SEQ ID No:
708	INAKFSSQL	Membrane proteins related to metalloendopeptidases	27
708	YIYNHYKDM	Membrane proteins related to metalloendopeptidases	28
708	YVYGKSRTM	Membrane proteins related to metalloendopeptidases	29
708	IAFLSYKLF	cell surface protein precursor	30
708	IMYEYMYPV	hypothetical protein	31
708	SSMEYFLKV	Phage tail length tape-measure protein	32
708	ISFFQENQL	Collagen adhesin	33
708	TNLLFMTSL	extracellular protein	34
708	KIFSIFMLL	Phosphatidylinositol-specific phospholipase C	35
708	LNIFKFNR	Chitinase	36

708	MTYDYRGGF	Chitinase	37
708	PSYMFRTSF	Chitinase	38
708	QSYTYYMTA	cell wall surface anchor family protein	39
708	ITFSHYEPT	cell wall surface anchor family protein	40

Table 3: H-2b restricted-EH708 peptides not present in the other strains

Hirae	sequence	names of the proteins	SEQ ID No:
EH17	MSFTFFSST	hypothetical protein	41
EH17	IAFQNFVNL	Chitinase	42
EH17	SMFIAFQNF	Chitinase	43
EH17	LNVDYGNRI	Chitinase	44
EH17	AGICFFTGV	Peptidoglycan N-acetylglucosamine deacetylase	45
EH17	VEYTYFPTL	Membrane proteins related to metalloendopeptidases	46
EH17	AAYVFEMNF	Membrane proteins related to metalloendopeptidases	47
EH17	EMYRKLSTL	Membrane proteins related to metalloendopeptidases	48
EH17	YNYGYKSVL	enhancin family protein	49
EH17	VIHELNSL	bacteriocin immunity protein	50

Table 4: H-2b restricted-EH17 peptides not present in the other strains

Hirae	sequence	names of the proteins	SEQ ID No:
708-13144	TNYVKLRPL	hypothetical protein	51
708-13144	QAVNHFTGI	Portal protein phage associated	52

5 **Table 5:** H-2b restricted-EH13144/EH708 common peptides

CD8⁺ T cell splenocytes isolated from CTX- treated mice were restimulated with pooled 9-mer peptides (group1-13, Table 6) to identify potentially immunogenic epitopes *in vivo*. Using the same protocol as the one outlined in Figure 9, we found that the immunogenicity of 13144 relied upon group 7 associated peptides (Figures 13B) contrary to other group of peptides (data not shown) such as group 1 (Figure 13A). Next, we split the group 7 into the four H-2K^b peptides (Table 7). Immunogenicity of *E. hirae* 13144 could reside within the Phage Tail Length Tape Measure Protein (Tnp) (Table 7). Indeed, we unveiled that only TMP1 and TMP2 peptides were highly recognized by memory EH13144 (but not 708 and EH17) - induced Tc1 cells (Figure 13C). Of note, the strain EH10815, which harbored the two same prophages as EH13144 but mutations in position 3 in TMP1 and in positions 2 and 7 of TMP2 (Figure 13D), does not induce recall responses (Figure 13E) and might explain why this bacterium failed to mediate significant antitumor effects. In contrast, results

obtained with EH clone IGR7 which shares a very high sequence homology with EH13144, induce recall responses (Figure 13E).

Group	n°	Hirae	sequence	names of the proteins	SEQ ID No:
1	1	708	INAKFSSQL	Membrane proteins related to metalloendopeptidases	27
	2	708	YIYNHYKDM	Membrane proteins related to metalloendopeptidases	28
	3	708	YVYGKSRTM	Membrane proteins related to metalloendopeptidases	29
	4	708	IAFLSYKLF	cell surface protein precursor	30
2	5	708	IMYEYMPV	hypothetical protein	31
	6	708	SSMEYFLKV	Phage tail length tape-measure protein	32
	7	708	ISFFQENQL	Collagen adhesin	33
	8	708	TNLLFMTSL	extracellular protein	34
3	9	708	KIFSIFMLL	Phosphatidylinositol-specific phospholipase C	35
	10	708	LNIFKFNRF	Chitinase	36
	11	708	MTYDYRGGF	Chitinase	37
	12	708	PSYMFRTSF	Chitinase	38
4	13	708	QSYTYMTA	cell wall surface anchor family protein	39
	14	708	ITFSHYEPT	cell wall surface anchor family protein	40
	15	13144	SAFPYEQEL	C3 family ADP-ribosyltransferase	3
	16	13144	YNYSKSYPV	hypothetical protein	4
5	17	13144	VSFSHYRPG	hypothetical protein	5
	18	13144	VTFLGYNAF	cell surface protein	6
	19	13144	TVYTFHVNI	cell surface protein	7
	20	13144	TSYSPLFLL	cell surface protein (putative)	8
6	21	13144	TNYIYPNIL	2',3'-cyclic-nucleotide 2'-phosphodiesterase	9
	22	13144	VVPILFLGL	FmtB protein	10
	23	13144	KNYKAYVEL	hypothetical protein	11
	24	13144	SAMKYGIPL	hypothetical protein	12
7	25	13144	TSLARFANI	Phage tail length tape-measure protein	13
	26	13144	AMIEFIQGL	Phage tail length tape-measure protein	14
	27	13144	VAITFGGPL	Phage tail length tape-measure protein	15
	28	13144	VSTNHYGLL	hypothetical protein	16
8	29	13144	VMFGLFITI	cell surface protein precursor	17
	30	13144	TVFSLVSLL	Chitinase	18
	31	13144	SIYNLEKPL	IgA1 protease	19
	32	13144	YTIIRYGNL	IgA1 protease	20
9	33	13144	SNGLLYTPM	IgA1 protease	21
	34	13144	NNYHYVGGL	IgA1 protease	22
	35	13144	SMFLNCNNL	hypothetical protein	23
	36	13144	IAFQGYSSL	hypothetical protein	24

10	37	13144	QVTNFFNMF	hypothetical protein	25
	38	13144	IMLGLFMTM	cell surface protein precursor	26
	39	EH17	MSFTFFSST	hypothetical protein	41
	40	EH17	IAFQNFVNL	Chitinase	42
11	41	EH17	SMFIAFQNF	Chitinase	43
	42	EH17	LNVDYGNRI	Chitinase	44
	43	EH17	AGICFFTGV	Peptidoglycan N-acetylglucosamine deacetylase	45
	44	EH17	VEYTYFPTL	Membrane proteins related to metalloendopeptidases	46
12	45	EH17	AAYVFEMNF	Membrane proteins related to metalloendopeptidases	47
	46	EH17	EMYRKLSTL	Membrane proteins related to metalloendopeptidases	48
	47	EH17	YNYGYKSVL	enhancin family protein	49
	48	EH17	VIHELNSL	bacteriocin immunity protein	50
13	49	708-13144	TNYVKLRPL	hypothetical protein	51
	50	708-13144	QAVNHFTGI	Portal protein phage associated	52

Table 6: H-2b restricted *E. hirae* peptides by groups used in *in vivo* experiments

Group	n°	Hirae	sequence	names of the proteins	SEQ ID No:
7	1	13144	TSLARFANI	Phage tail length tape-measure protein	13
	2	13144	AMIEFIQGL	Phage tail length tape-measure protein	14
	3	13144	VAITFGGPL	Phage tail length tape-measure protein	15
	4	13144	VSTNHYGLL	hypothetical protein	16

Table 7: Proteins corresponding to the immunogenic EH peptide sequences

5 The manufacturing of TMP1 specific tetramers allowed us to monitor TMP1-specific T cells in the mLN (Figure 14A) and the spleen (Figure 14B-C) in mice without tumor and post-oral gavages with EH13144, EH17, 10815 or IGR7 and CTX treatment. We observed that only T cells from the gut (CCR9+ T cells) are TMP1-specific T cells for EH13144 and IGR7 (Figure 14). Moreover, in tumor bearing mice, TMP-specific tetramer stainings in tumor draining lymph node and tumor show that TMP epitope 1 prophage 2 specific CD8+ T cells accumulate in tumor beds and are contained in the CCR9+CXCR3+ CTLs (Figure 15).

10 Temperate bacteriophages are bacterial viruses that transfer virulence, antimicrobial resistance genes, and immunogenic sequences to new bacterial hosts via transduction (Weinbauer, 2004). The Phage Tail Length Tape Measure Protein (Tmp) is

highly conserved in a large number of phages and prophages, most importantly in *Siphoviridae* family of phages, containing a variable number of tandem repeats with highly conserved tryptophan and phenylalanine aminoacids at fixed positions. Phages belonging to the *Siphoviridae* family contain several motifs in their Tmp (Belcaid et al., 2011; Piuri and Hatfull, 2006), among which peptidoglycan hydrolases facilitating their infectivity of surrounding bacteria and containing rescuscitation–promoting factors (Rpfs). Rpfs have not only been implicated in the reactivation of dormant bacteria but also modulate innate responses to *Mycobacterium tuberculosis* (Russell-Goldman et al., 2008) as well as cognate long-term immune responses (Commandeur et al., 2011). In fact, *M. tuberculosis* Rpfs T cell epitopes were reported to be key immunogens in the human immune responses to *M. tuberculosis* (Commandeur et al., 2011).

Example 4: Immunization with *E. hirae* 13144 Tmp peptides or live bacteria confer protection against tumor challenge

We performed a subcutaneous vaccination using $1.5 \cdot 10^5$ BM-DC activated with poly I:C followed by exposition to TMP peptides or live *E. hirae* 13144 to immunize twice, 10 days apart, naïve C57BL/6 animals against MCA205 sarcomas or syngeneic MC38 colon cancers (Figure 16). While unpulsed BM-DC or BM-DC exposed to Group 1 peptides were not efficient at mediating protection against a lethal challenge with MCA205, BM-DC exposed to TMP1-TMP2 markedly conveyed protective effects against MCA205 (Figure 17) as efficiently as live bacteria.

We hypothesized that oral gavages with *E. hirae* 13144 trigger an immune response in the mLN and spleen that can either traffick back to intestinal mucosae or in the tumor microenvironment. To link gut and tumor immunosurveillance, we compared the relative immune infiltrates induced by sequential oral gavages with *E. hirae* 13144 in naïve versus tumor bearing mice. Indeed, we found that *E. hirae* 13144-induced CD8⁺ T cell-dependent colon inflammatory lesions (Figure 11) were markedly reduced in tumor bearers, supporting the competition between the gut and tumor microenvironment during oral administration of the bacteria (Figure 18).

Example 5: *E. hirae* 13144 genome encodes two 40.6-kb and 39.2-kb prophage sequences of the *Siphoviridae* family

Strain EH13144 and four other *E. hirae* genomes (708, 10815, 13152 and EH17) were annotated using Prokka program. The homology relationships between genes of each different strain were assessed using BLASTP program and Roary

software with >80% amino acid identity cutoff. Core genome alignment of the five *E. hirae* genomes was performed using PRANK program. Prophage regions were predicted using PHASTER online program.

Comparative analysis of *E. hirae* strains yielded a pangenome of 12,748 genes. The core genome (the set of genes shared by all strains) was composed of 2,036 orthologous genes (59%) and the accessory genome (the set of genes present in some but not all the species) was composed of 570 orthologous genes and 923 unique genes (unique genes to individual species). Strain EH13144 encoded 196 unique genes while strains 13144 and 708 shared 27 orthologous proteins (Figure 19A).

A particularity in the genome of *E. hirae* 13144 is that it encodes two intact prophages regions (40.6-kb and a 39.2-kb) showing sequence homology to *Enterococcus* phage phiEf11 and *Staphylococcus* phage CNP_x, respectively (Figure 19B). The 40.6-kb prophage encoded 57 genes, including 12 shared between the five genomes, 16 shared between strains 13144 and 708 and 8 unique to *E. hirae* 13144 (Figure 20A). The 39.2-kb prophage encoded 65 genes, including 7 shared between the five genomes, 3 shared between strains 13144 and 708 and 22 unique to *E. hirae* 13144 (Figure 20B). Genomic structures of both prophages suggested that they belonged to the *Siphoviridae* family. Indeed, the two prophages harbor genes encoding capsid, portal and tail structure, a characteristic of *Siphoviridae* phages. The two prophages encoded a tail tape measure protein (TMP) with a 38% amino acid homology. The TMP of the 40.6-kb prophage presented sequence homology with *E. hirae*, *Enterococcus villorum* and *Enterococcus faecium* prophage TMPs with 98.8%, 96.7 %, and 97.7% amino acid identity, respectively. The TMP of the 39.2-kb prophage showed sequence homology with only EH clone IGR7 (CNCM I-5224) with 100% homology (Figure 21, Table 8) and to a lesser extent with *E. faecalis* prophage TMP with 89.2% amino acid identity. Despite sequence homologies with other phage genes, the whole two prophages were uniquely encoded in the strain 13144. Finally, both TMP1 and TMP2 peptides are part of the same phage tail tape measure protein (TMP) gene of the second prophage (SEQ ID No: 2). TMP1 of 13144 presented 100% homology with IGR7 and IGR11 but 88.89% homology with 10815 and IGR1 (1 mutation in position 3). TMP2 of 13144 presented 100% homology only with IGR7 and 77.78% homology with 10815 and IGR11 (2 mutations in position 2 and 7 or 2 and 4 respectively) (Figure 21, Table 8).

Strains	Anti-tumor effect	Homology with			
		Epitope TMP1 (Prophage n°2)	Epitope TMP2 (Prophage n°2)	TMP Protein (prophage n°1)	TMP Protein (prophage n°2)
13144	Yes	-	-	-	-
708	No	No homology	No homology	98,72%	No homology
EH17 (13344)	No	No homology	No homology	25,52%	No homology
10815 (ATCC9790)	No	88,8889% (1 mutation)	77,778% (2 mutations)	24,03%	66,38%
IGR 1	No	88,8889% (1 mutation)	No homology	99,04%	74,77%
IGR 4	Yes	No homology	No homology	99,15%	No homology
IGR 7	Yes	100%	100%	100%	100%
IGR 10	No	No homology	No homology	No homology	No homology
IGR 11	Yes	100%	77,778% (2 mutations)	No homology	66,52%

Table 8: summary of the characteristics of the strains

Example 6: *E. hirae* 13144 bacterial and phage sequences have been occasionally found in the MG reference catalog containing >3,000 metagenomes in humans

A total of 3027 metagenomes from 17 different datasets were screened (Figure 29A). They were - for the large majority - gut microbiomes (stools) but also oral cavity, skin, vagina, and sputum. Most of the datasets are publicly available covering all continents, including several non-westernized populations. We also included some of Nicola Segata's unpublished data (most notably of 25 paired mother/infant subjects followed longitudinally). We assessed the breadth of coverage (BOC) of the *E. hirae* genome and its phages in each of the sample. The BOC measures the fraction of the genome that is covered by the reads in the metagenomes. A BOC of 1.0 means that the whole genome of the reference strain is found in the metagenome. Based on this score, we could confidently conclude that *E. hirae* is present for sure in two samples. One is from a mongolian subject, the other from a Swedish one (BOC of 0.9 and 0.75 respectively). There are other 5 samples with a BOC between 0.1 and 0.37 that are probably indicative of *E. hirae* presence. Below a BOC of 0.1, we fail to characterize the presence of a *E. hirae* strain. As for the phages sequences, it seems that the mongolian subject positive for *E. hirae* also has prophage 2 from 13144 of SEQ ID No: 2, whereas the Swedish one has prophage 2 from 708. The phages seem to be present in more samples than the bacterium genome. One interesting case is the presence at 0.66 BOC of the Phage 13144 in three samples from an infant at 1, 3, and 7 days of life

(samples CA_C10006IS2084FE_t1M15, CA_C10006IS2087FE_t2M15, CA_C10006IS2091FE_t3M15). These three samples do not have *E. hirae*, but seem to have another strain from another *Enterococcus* species. This might suggest that this phage is not specific of *E. hirae* only.

5 **Example 7: *E. hirae* 13144 is immunogenic not only in mice but also in humans (normal volunteers and breast cancer patients): defining TMP-specific peptides of high immunogenicity in humans**

Since *E. hirae* 13144 is a mouse strain but its sequence has been recovered in study alignment with human stool metagenomics, we postulated that humans (normal volunteers or breast cancer patients) could develop immune responses against some of the epitopes presented by this strain, as reported in (Daillère et al., 2016). Figure 22A shows that 12 to 19% of CD4 and CD8+ T cells can display memory IFN γ -geared T cell responses to *E. hirae* 13144, suggesting that indeed, this EH strain can be recognized by human cells. Figure 22A also highlights that 32% of BC patients have CD4+ T cells exhibiting no reactivity to monocytes pulsed with EH13144 while 52% developed IL-10 producing memory CD4+ T cell responses. This is in sharp contrast with the reactivity of the memory CD4+ TH1 cells to *E. coli* and TCR cross-linking (Figure 22B). The predicted peptides of Tmp to be tested in human PBMC or TILs are listed in Table 9 below, and their localization has been described in Figure 23. Are indicated their binding potential for MHC class I alleles (threshold set at <50nM, Y axis) assessed using NetMHC software. For each peptide and allele, symbols represent the first amino acid of the identified sequence corresponding to 9 amino acid long (mer) peptides. Next, we tested all HLA-A2-restricted Tmp epitopes on PBMC by *in vitro* stimulation assays of recall responses (Figure 24A) from six HLA-A2+ healthy volunteers (HV) (Figure 24C) and found that 5 peptides (indicated in Figure 24B) exhibited significant reactivities at least in 50% of HV (Figure 24 D-E). Arrows indicate the most significant epitopes (Figure 24F-G).

Start	Stop	HLA	Peptide	Affinity(nM)	SEQ ID No :
968	976	HLA-A0101	YTDYSNQLK	35,98	53
357	365	HLA-A0201	AMIEFIQGL	4,88	54
1462	1470	HLA-A0201	KMVEILEEI	7,8	55
1397	1405	HLA-A0201	RLLKYDVG	11,55	56
765	773	HLA-A0201	TLVGVTFAI	16,94	57
1374	1382	HLA-A0201	AMQNLVAAV	17,32	58

793	801	HLA-A0201	AIMAIANGV	20,56	59
862	870	HLA-A0201	AMSMNMEEV	24,83	60
504	512	HLA-A0201	KVFGKMTSV	26,84	61
1130	1138	HLA-A0201	LLGIYQSYV	29,4	62
631	639	HLA-A0201	KLAKFASVV	29,89	63
1176	1184	HLA-A0201	KLWANMSKA	30,99	64
692	700	HLA-A0201	MLSNPITAI	32,68	65
700	708	HLA-A0201	ILVAITTTI	36,32	66
491	499	HLA-A0201	KMAALAASA	46,27	67
691	699	HLA-A0201	AMLSNPITA	49,58	68
473	481	HLA-A0201	NMAEAFASA	49,85	69
835	843	HLA-A0301	TMFSDSALK	20,82	70
573	581	HLA-A0301	LLTRFTTLK	23,32	71
727	735	HLA-A0301	KTAFSGIVK	32,3	72
515	523	HLA-A0301	KTISTMFEK	47,18	73
505	513	HLA-A2402	VFGKMTSVF	40,33	74
904	912	HLA-A2403	AYFNHTLDL	4,83	75
714	722	HLA-A2403	AWKSNFMNI	5,04	76
427	435	HLA-A2403	RYGTTESQL	5,97	77
1136	1144	HLA-A2403	SYVNNGASI	10,38	78
556	564	HLA-A2403	KYKSNLAGL	13,47	79
505	513	HLA-A2403	VFGKMTSVF	14,88	80
729	737	HLA-A2403	AFSGIVKSF	19,19	81
967	975	HLA-A2403	VYTDYSNQL	23,45	82
1074	1082	HLA-A2403	AFQNQITQL	45,36	83
639	647	HLA-A3101	VINPIGSLR	19,59	84
1389	1397	HLA-A3101	KAKIKSPSR	23,3	85
515	523	HLA-A3101	KTISTMFEK	24,29	86
667	675	HLA-A3101	ASKAGGGFR	27,79	87
675	683	HLA-A3101	RTFAATGIR	28,02	88
611	619	HLA-A3101	GNKVTNFFR	35,72	89
1437	1445	HLA-A3101	ITGSRLIKR	39,31	90
401	409	HLA-A3301	DVFEGAVKR	33,42	91
476	484	HLA-A6801	EAFASADPK	6,97	92
401	409	HLA-A6801	DVFEGAVKR	7,96	93
835	843	HLA-A6801	TMFSDSALK	14,72	94
94	102	HLA-A6801	ESAFTGVKK	15,34	95

1419	1427	HLA-A6801	TSVAVQSAK	15,68	96
175	183	HLA-A6801	DTAATSLAR	15,91	97
358	366	HLA-A6801	MIEFIQGLK	17,45	98
1148	1156	HLA-A6801	MALLAGMLR	20,5	99
675	683	HLA-A6801	RTFAATGIR	28,54	100
730	738	HLA-A6801	FSGIVKSFK	29,62	101
252	260	HLA-A6801	EAGGSAFSR	29,95	102
1350	1358	HLA-A6801	TSVGSNMAK	31,53	103
515	523	HLA-A6801	KTISTMFEK	38,32	104
968	976	HLA-A6801	YTDYSNQLK	42,02	105
1463	1471	HLA-A6801	MVEILEEIR	42,29	106
639	647	HLA-A6801	VINPIGSLR	42,9	107
333	341	HLA-A6801	EDFANVTGR	43,39	108
773	781	HLA-A6801	IAGFVDGLR	47,53	109
1458	1466	HLA-B0702	TPMGKMVEI	24,41	110
572	580	HLA-B0801	NLLTRFTTL	4,7	111
1441	1449	HLA-B0801	RLIKRSNAI	16,31	112
182	190	HLA-B2705	ARFANITQM	30,73	113
674	682	HLA-B2705	FRTFAATGI	30,97	114
780	788	HLA-B2705	LRAIITVGK	47,66	115
182	190	HLA-B2720	ARFANITQM	14,78	116
1146	1154	HLA-B2720	QQMALLAGM	32,65	117
1441	1449	HLA-B2720	RLIKRSNAI	42,54	118
353	361	HLA-B3501	NPSQAMIEF	7,99	119
946	954	HLA-B3501	FANASTEYM	9,08	120
250	258	HLA-B3501	EAEAGGSAF	25,17	121
492	500	HLA-B3501	MAALAASAG	32,95	122
240	248	HLA-B3501	FAAALSSVG	33,38	123
495	503	HLA-B3501	LAASAGPVL	42,72	124
89	97	HLA-B3501	AAVKWESAF	48,3	125
1182	1190	HLA-B3901	SKADIVNTL	26,44	126
539	547	HLA-B3901	IKNGSSSAL	36,65	127
1018	1026	HLA-B3901	NKLNNNQAL	47,23	128
902	910	HLA-B4001	VEAYFNHTL	6,41	129
385	393	HLA-B4001	TEVRLRDSL	30,39	130
1092	1100	HLA-B4001	SELEQGAQL	39,74	131
385	393	HLA-B4002	TEVRLRDSL	42,24	132

143	151	HLA-B4402	AEAAGQLGI	18,68	133
752	760	HLA-B5801	KGLGNIFKW	6,09	134
797	805	HLA-B5801	IANGVKGLW	6,74	135
523	531	HLA-B5801	KAGNIDSKW	9,31	136
351	359	HLA-B5801	KSNPSQAMI	12,06	137
619	627	HLA-B5801	RSFSASLQL	13,45	138
395	403	HLA-B5801	RAANASDVF	37,43	139
898	906	HLA-B5801	STAGVEAYF	41,32	140
693	701	HLA-B5801	LSNPITAIL	42,57	141
627	635	HLA-B5801	LSNSKLAKF	48,23	142
946	954	HLA-C0303	FANASTEYM	3,56	143
350	358	HLA-C0303	FKSNPSQAM	4,75	144
495	503	HLA-C0303	LAASAGPVL	5,93	145
324	332	HLA-C0303	EASKASGSL	6,31	146
445	453	HLA-C0303	VAITFGGPL	9,21	147
459	467	HLA-C0303	SAISAAKPM	9,9	148
412	420	HLA-C0303	EAFNENTAL	10,33	149
1328	1336	HLA-C0303	SANNAGREL	10,61	150
652	660	HLA-C0303	AAGKSGTVL	11,56	151
844	852	HLA-C0303	KAACKSTEEL	12,11	152
635	643	HLA-C0303	FASVVINPI	12,38	153
677	685	HLA-C0303	FAATGIRSI	13,26	154
395	403	HLA-C0303	RAANASDVF	15,87	155
1142	1150	HLA-C0303	ASIDQQMAL	19,24	156
463	471	HLA-C0303	AAKPMIEAL	21,86	157
1284	1292	HLA-C0303	NAKQKGAEL	23,31	158
789	797	HLA-C0303	TAVNAIMAI	23,51	159
480	488	HLA-C0303	SADPKTQEF	23,9	160
35	43	HLA-C0303	NASDIPSNL	25,71	161
173	181	HLA-C0303	SADTAATSL	26,05	162
478	486	HLA-C0303	FASADPKTQ	31,85	163
250	258	HLA-C0303	EAEAGGSFA	31,9	164
553	561	HLA-C0303	FVSKYKSNL	33,06	165
89	97	HLA-C0303	AAVKWESAF	36,44	166
685	693	HLA-C0303	IASLTGAML	40,14	167
651	659	HLA-C0303	SAAGKSGTV	41,97	168
449	457	HLA-C0303	FGGPLVAAL	44,33	169

539	547	HLA-C0303	IKNGSSSAL	45,59	170
480	488	HLA-C0501	SADPKTQEF	18,54	171
107	115	HLA-C0501	MVDSNGKVI	25,04	172
173	181	HLA-C0501	SADTAATSL	29,14	173
580	588	HLA-C0501	LKDTIVGLF	33,18	174
182	190	HLA-C0602	ARFANITQM	34,48	175
182	190	HLA-C0701	ARFANITQM	28,58	176
677	685	HLA-C1203	FAATGIRSI	7,86	177
635	643	HLA-C1203	FASVVINPI	15,83	178
1012	1020	HLA-C1203	FVEAGVNKL	40,2	179
946	954	HLA-C1203	FANASTEYM	41,29	180
1475	1483	HLA-C1203	VVMDTGQVV	45,03	181
504	512	HLA-C1203	KVFGKMTSV	47,93	182
904	912	HLA-C1402	AYFNHTLDL	7,99	183
1136	1144	HLA-C1402	SYVNNGASI	15,64	184
967	975	HLA-C1402	VYTDYSNQL	25,25	185
617	625	HLA-C1402	FFRSFSASL	31,08	186
619	627	HLA-C1502	RSFSASLQL	14,65	187

Table 9: Predicted peptides of Tmp for each HLA haplotype

Example 8: Phage excision in EH13144 *in vitro*

Using a classical procedure to excise a phage and harvest it in the supernatants of the *E. hirae* (Figure 25) (Duerkop et al., 2014), we could observe prophage 1 and 2 excision after incubation of EH13144 with mitomycin C (1 µg/ml) but not CTX (Figure 26A), at different temperatures (37°C and 42°C) regardless of expansion phase of the bacterium. After capsid lysis of the supernatants (augmenting the sensitivity of phage DNA detection), prophages DNA were visualized in spontaneous conditions, suggesting that phages can also be released without excision stresses (Figure 26B).

Example 9: identification of novel strains harboring the gene encoding the TMP of SEQ ID No: 1

The genomes of twenty different *E. hirae* strains (Table 10 below) were sequenced and aligned. Remarkably, 3 prophage regions, of which 2 regions are intact, were identified in the genome of the strain IGR7, which proved immunogenic (see example 1 above), and it was found that this genome encode the same TMP as the TMP

- of SEQ ID No: 1 encoded by *E. hirae* 13144 (Table 9). This strain was deposited at the CNCM on 12 Oct 2017, under the number I-5224. Moreover, IGR11 which proved efficient to mediate some tumoricidal activity with CTX have also the same TMP1 epitope encoded by 13144 (Table 9). This strain was deposited at the CNCM on November 27, 2017, at the CNCM under the number I-5261.

Strain	N° of scaffolds	Length	GC%	N° of CDS
5348	4	2773687	36.84	2541
7030	2	2839464	36.72	2665
12607	3	2981483	36.62	2662
13150	3	2881518	36.76	2692
13153	1	2732232	36.84	2479
13155	9	2956728	36.56	2688
13161	2	2814115	36.90	2696
13343	2	2748768	37.01	2555
13346	3	2758418	36.98	2511
13347	3	2876247	36.71	2668
IGR1	2	2874823	36.73	2681
IGR4	3	2958768	36.71	2649
IGR7	2	2705237	36.84	2543
IGR10	2	2921736	36.70	2589
IGR11	3	2836600	36.91	2600
13144	1	2735684	36.96	2542
13152	1	2835470	36.92	2486
13344	1	2711996	37.12	2472
ATCC9790	1	2827741	36.94	2670
708	1	2951512	36.89	2613

Table 10

Example 10: Cell sorting and expansion of TIL or blood T cells specific for the TMP

- Several protocols can be used to obtain TILs or T cells specific for the TMP of SEQ ID No: 1.

Protocol 1: Immunomagnetic cell sorting and expansion of T cell sorted populations

- HLA-A*0201/TMP monomers (20µg/ml) are incubated for 1 h at room temperature with $6.7 \cdot 10^6$ streptavidin-coated beads (Dynabeads M-280 streptavidin,

DYNAL, Compiègne, France) and washed in PBS/0.1% BSA. $5 \cdot 10^6$ PBMC are rotated for 4 h at 4 °C with monomer-coated beads (Bodinier et al., 2000). After ten washes, bead coated cells are expanded using a polyclonal T cell stimulation protocol (Jotereau et al., 1991). Subsequently, cells are incubated with sheep anti-mouse IgG coated

5 Dynabeads (Dynal Biotec, Compiègne, France) at a 1:1 ratio for 4 h at 4 °C with gentle rotation. The cell/bead suspension are incubated in culture medium in 6-well plates overnight at 37°C to allow beads to detach. After overnight incubation, beads are extracted by the magnet, and sorted lymphocytes are transferred on feeder cells, as previously described (Jotereau et al., 1991). Briefly, 2000 bead-coated T cells/well are

10 distributed in 96-well plates mixed with irradiated feeder cells [LAZ EBV-B cells ($2 \cdot 10^4$ /well) and allogeneic PBMC (10^5 /well)], in 150 µl of culture medium supplemented with IL-2 (150 U/ml) and PHA (15µg/ml).

Protocol 2

Tumor samples from cancer specimens are cultured with cytokines (IL-2,

15 IL-15, and IL-21) to expand TILs. After 10 days of culture, TILs are stimulated with an anti-CD3 antibody (OKT3) and irradiated allogeneic peripheral blood mononuclear cells (as described in Meng et al., 2016).

Example 11: Obtention of T cells specific for the TMP by Transduction of T lymphocytes

20 Autologous or allogeneic T cells are placed in an expanding phase using anti-CD3/CD28 coated beads or low dose IL-2 (and IL-7, IL-15, IL-21) before being exposed to retroviral vectors or lentiviral vectors engineered to express the beta chain of the high avidity TCR encoding cDNA (for the TMP prophage 2 epitope corresponding to HLA-A2 or other haplotypes) and/or the alpha chain of the high avidity TCR encoding

25 cDNA (for the TMP prophage 2 epitope corresponding to HLA-A2 or other haplotypes) and the CDR3 region of this TCR encoding cDNA (for the TMP prophage 2 epitope corresponding to HLA-A2 or other haplotypes). Polyclonal T cells are then cloned and tested for their specificity (IFNγ or TNFα release upon exposure to TMP epitopes) in 96 well plates. Cytokine-producing T cells are then selected and reexpanded with T cell

30 growth factors (IL-2; IL-7, IL-15, IL-21) on a weekly basis for two to three weeks until expansion to 10^{10} to 10^{12} cells in GMP cell factories.

Discussion

Our findings revealed that the antigenicity of mouse *EH 13144* and the

newly cloned human EH IGR7 (CNCM I-5224) or EH IGR11 (both 100% homologous in their sequence) relies on the Phage Tail Length Tape Measure Protein (Tmp) of the *Siphoviridae* phages (lactococcal bacteriophage tail tape measure protein TP901 family) mainly in the 39.2 -kb prophage encoded 65 genes, including 7 shared between the five
5 genomes, 3 shared between strains 13144 and 708 and 22 unique to *E. hirae* 13144. Transfer of genetic materials inbetween enterococci species have been reported (Mazaheri Nezhad Fard et al., 2010, 2011). Of note, phages that infect Gram positive bacteria often contain peptidoglycan-hydrolysing motifs corresponding to peptidase and transglycosylase activities localized within tape measure proteins of *Siphoviridae* phages
10 (Piuri and Hatfull, 2006). We will now attempt to restore immunogenicity of non-immunogenic *E. hirae* strains (EH17, 10815) by transducing these strains with bacteriophages belonging to the family of *Siphoviridae*, or with genetically modified plasmids encoding the whole Phage Tail Length Tape Measure Protein (Tmp) or epitopes selected from TMP prophage 2, and with negative controls (proteins or epitopes
15 mutated in the MHC binding groove). Of note, *M. tuberculosis* RpfS T cell antigens were reported to be important targets in the human immune responses to *M. tuberculosis* (Commandeur et al., 2011).

These mouse data have a clinical relevance. Based on *in silico* prediction
20 (sequence alignment of the several strains of *E. hirae*, protein subcellular localization and algorithms of prediction of MHC binding affinities), a list of candidate epitopes harbouring a putative immunogenicity has been established in the first step of the selection (Figure 23). In a second step, *in vitro* stimulation assays using peripheral blood mononuclear cells from HLA-A0201+ normal volunteers with 4 groups of 4 nine mer-
25 TMP specific epitopes followed by a recall response using single 9 mer epitopes performed in 6 healthy volunteers revealed that 5 epitopes could be considered immunogenic (Figure 24). Third step, a bioinformatic screening of the normal human proteome and gene expression in >350 tumors cell lines investigating sequence homologies between these 5 human immunogenic TMP epitopes and such proteomes
30 concluded that one of this HLA-0201 epitope KLAKFASVV (SEQ ID No: 63), shares a 78% sequence homology with the glycerol-3 phosphate dehydrogenase 1 like protein (GPD1L, gene encoded on 3p22.3). GPD1L is a cytosolic protein, associated at the plasma membrane with a sodium channel, voltage-gated, type V alpha subunit (SCN5A). GPD1L is a hypoxia-associated protein negatively regulated by miR-210, overexpressed
35 in many tissues (brain, during embryo- and foeto-genesis), and contributes to the

proteosomal degradation of hypoxia inducible factor 1-alpha (HIF-1 α). Indeed, MiR-210 represses levels of the glycerol-3-phosphate dehydrogenase 1-like (GPD1L) enzyme, contributing to suppression of prolyl hydroxylase (PHD) activity. Under normal physiological conditions, PHD hydroxylates prolines in hypoxia inducible factor 1-alpha (HIF-1 α), leading to its degradation by the proteasome. When PHD activity is suppressed due to downregulation of GPD1L by miR-210, HIF-1 α is not degraded by the proteasome and translocates to the nucleus where it forms a heterodimer with hypoxia-inducible factor 1-beta (HIF-1 β); dimerization of HIF-1 α and HIF-1 β activates transcriptional responses that contribute to cancer metastasis (Costales et al., 2017). Hence, higher mRNA levels and/or protein expressions of GPD1L in head and neck squamous cell carcinoma compared with healthy surrounding tissues was an independent prognostic parameter and a favorable predictor of longer time to local recurrence and distant metastases in multivariate Cox regression analyses (Feng et al., 2014).

Since the binding affinity for HLA-A0201 of this phage peptide is similar to the one of normal tissue peptide, the phage peptide could be viewed as an immunogenic molecular mimick of the normal peptide. It is therefore conceivable that cross-reactivities between TMP phage specific TCR and self tissues or tumor tissues (overexpressing GPD1L), mainly foetal tissues or stem cells, could account for the anticancer effectiveness of the phage delivered in the context of this EH bacterium. Of note, tumor cell lines can express variable levels of GPD1L protein, as appears from gene expression atlas available on the web.

HLA-A0201 restricted-epitope TMP 2 (KMVEILEEI, SEQ ID No: 55), epitope 3 (RLKDYDVG, SEQ ID No: 56), epitope 9 (LLGIYQSYV, SEQ ID No: 62), epitope 10 (KLAKFASVV, SEQ ID No: 63, homologous to sequences from GPD1L) and epitope TMP 13 (ILVAITTTI, SEQ ID No: 66) have been found so far (Figure 24), enabling tetramer manufacturing, T cell capture in tumor bearers (human patients or mice) and structural and functional characterization. Reactivity of *E. hirae*-specific CTL clones from tumor infiltrating lymphocytes can be tested against a variety of syngeneic tumor cell lines to bring up the proof of principle of molecular mimicry between microbial and tumor antigens.

This asset accounts for the quasi-unique antigenicity of *E. hirae* 13144 exploitable for cancer vaccines and T cell transfer and prompts to the use of this phage to infect other bacterial strains for use as anticancer probiotics or OncoBax in

combination with any drug identical or different from cyclophosphamide capable of enabling the niching of the dedicated species and/or stressing the excision of this phage. For instance, cyclophosphamide could allow the overrepresentation of *E. hirae* over its competitor *E. gallinarum* while the competition between these two strains promoted phage excision *per se*.

Example 12: Phage Tail Length Tape Measure Protein as the unique antigenic sequence in *E. hirae* 13144.

Unleashing immune responses against tumor-associated antigens through chemotherapy, radiotherapy, targeted therapies or immune checkpoint inhibitors has become the mainstay of successful cancer treatments (Galluzzi et al., 2015; Sharma and Allison, 2015). The recent discovery that the gut microbiota determines the cancer-immune set point, thus influencing the clinical outcome of anticancer therapies, has rekindled the concept that microbes or their products modulate not only intestinal but also systemic immunity (Zitvogel et al., 2018). Indeed, memory IFN γ producing CD4⁺ (TH1) and CD8⁺ (TC1) T cell responses directed against *Enterococcus hirae*, *Bacteroides fragilis*, and *Akkermansia muciniphila* are associated with favorable clinical outcome in cancer patients (Daillère et al., 2016; Rong et al., 2017; Routy et al., 2018; Vétizou et al., 2015), suggesting that pre-existing microbe-specific T cells may contribute to anticancer immune responses. However, the question how microbes may affect the development of systemic autoimmune disease or local intestinal chronic inflammation has not been resolved (Rose, 2017). The theory of molecular mimicry posits that T cells elicited by bacteria or viruses may accidentally recognize autoantigens as they 'escaped' from self-tolerance inducing mechanisms (such as clonal deletion or inactivation). While MHC class I and class II binding epitopes from bacterial genomes have been identified to mediate immunogenicity *in vitro* or *in vivo* (Chai et al., 2017; Perez-Muñoz et al., 2015; Rubio-Godoy et al., 2002; Vujanovic et al., 2007; Yang et al., 2014), very few reports have unarguably demonstrated the functional relevance of microbe-specific CD4⁺ or CD8⁺ T lymphocytes for immune responses against normal or neoplastic tissues (Balachandran et al., 2017; Bradley et al., 2017; Ji et al., 2010).

Cyclophosphamide (CTX) induces the translocation of *E. hirae* from the gut lumen to the mesenteric and splenic immune tissues, thereby eliciting specific CD4⁺ and CD8⁺ T lymphocytes producing IL-17 and IFN γ , correlating with therapeutically effective anticancer immune responses (Daillère et al., 2016; Viaud et al., 2013). Broad-spectrum antibiotics abolished the therapeutic efficacy of CTX unless *E. hirae* was supplied by oral gavage (Daillère et al., 2016). When comparing a panel of *E. hirae*

strains (Table 11, Figure 31A) for their capacity to restore the antibiotic-perturbed anticancer effects of CTX, we found that only a few *E. hirae* isolates (such as 13144, IGR7, and IGR11) were efficient (Figure 27A-B, (Daillère et al., 2016)). Given that the therapeutic efficacy of the combination of CTX and *E. hirae* 13144 is abrogated by the depletion of CD8⁺ T cells or the neutralization of IFN γ (Daillère et al., 2016), we screened the differential capacity of *E. hirae* strains to elicit memory TC1 immune responses after a prime-boost exposure of the host (Figure 27C), and ex vivo restimulation of splenic CD8⁺ T cells with various *E. hirae* strains loaded onto dendritic cells (DC). While *E. hirae* 13144 triggered specific TC1 immune responses (that were not cross-reactive against irrelevant *Enterococci*), *E. hirae* 708 (a prototypic inefficient strain) failed to do so (Figure 27D). Pan genomic analysis of twenty *E. hirae* strains yielded a core genome of 1,677 orthologous genes (59%) and the accessory genome was composed of 946 and 477 orthologous and unique genes respectively (Figure 31B). This phylogenomic analysis showed that the strain 13144 is 100% homologous to strain IGR7 with 86 orthologous unique genes (Figure 31B).

Species	Samples	Origin	Cancer	Patient outcome
<i>Enterococcus hirae</i> 13144		Murine – CTX-treated		
<i>Enterococcus hirae</i> 708		Human - Unknown		
<i>Enterococcus hirae</i> 13344		Human - Blood		
<i>Enterococcus hirae</i> ATCC9790		Type strain CIP 53.48T		
<i>Enterococcus hirae</i> IGR1	MAT-HE	Human (stool)	Lung	Responder
<i>Enterococcus hirae</i> IGR2	AND-CL	Human (stool)	Lung	Responder
<i>Enterococcus hirae</i> IGR3	BLO-VA	Human (stool)	Lung	Responder
<i>Enterococcus hirae</i> IGR4	SAI-GE	Human (stool)	Lung	Complete Responder (HR selon RECIST)
<i>Enterococcus hirae</i> IGR5	CAR-RO	Human (stool)	Lung	Responder
<i>Enterococcus hirae</i> IGR6	BOU-MO	Human (stool)	Lung	Responder
<i>Enterococcus hirae</i> IGR7	BOU-MO	Human (stool)	Lung	Responder
<i>Enterococcus hirae</i> IGR8	AND-CL	Human (stool)	Lung	Responder
<i>Enterococcus hirae</i> IGR9	AND-CL	Human (stool)	Lung	Responder
<i>Enterococcus hirae</i> IGR10	ADO-EL	Human (stool)	Lung	Responder
<i>Enterococcus hirae</i> IGR11	ADO-EL	Human (stool)	Lung	Responder
<i>Enterococcus hirae</i> IGR12	TROJA	Human (stool)	Kidney	Responder
<i>Enterococcus hirae</i> IGR13	LOUNO	Human (stool)	Kidney	Responder
<i>Enterococcus hirae</i> IGR14	BOLAR	Human (stool)	CRC	Responder
<i>Enterococcus hirae</i> IGR15	NAIFE	Human (stool)	CRC	Responder
<i>Enterococcus hirae</i> IGR16	GIRAL	Human (stool)	CRC	Responder
<i>Enterococcus hirae</i> IGR17	LEZPH	Human (Tumor juice)	Melanoma	Responder
<i>Enterococcus hirae</i> IGR18	PRIMI	Human (Tumor juice)	Melanoma	Responder
<i>Enterococcus hirae</i> IGR19	NONRO	Human (Tumor juice)	Melanoma	NA
<i>Enterococcus hirae</i> IGR20	Lyon N°1(CAR)	Human (Tumor juice)	Melanoma	NA
<i>Enterococcus hirae</i> IGR21	PRUPA	Human (Skin swab)		

Table 11: Description of *E. hirae* strains

Next, we performed sequence alignment of bacterial genes encoding cell wall and secreted proteins (PSORT software) for immunogenic (13144) versus non-immunogenic (708 and 13344) bacterial strains followed by the identification of

nonapeptides with strong binding affinities for the MHC class I H-2K^b protein (<50 nM binding affinity, NetMHC software) (Table 6 in Example 3). Subsequently, we recovered splenic CD8⁺ T cells from mice that had been repeatedly exposed to *E. hirae* 13144 and CTX (Figure 27C). These T cells were restimulated *in vitro* with pools of potentially immunogenic nonapeptides from *E. hirae* 13144 (Table 6) to measure IFN γ production (Figure 32), then splitting the most efficient group 7 into individual peptides (Figure 27E). This approach led to the identification of one dominant epitope (one-letter amino acid code: TSLARFANI (SEQ ID No: 25), abbreviation TMP1) in position 197 to 187 of the amino acid sequence of the phage tail length tape measure protein (TMP) from a prophage of 39.2kb (1506 aa) of *E. hirae* 13144 (Figure 33A). Indeed, a particular genomic trait of *E. hirae* 13144 is that it encodes two intact prophages regions (40.6-kb and a 39.2-kb) showing weak sequence homologies with the most common *Enterococcus* phages phiEf11 vB_EfaS_IME197 (14% and 11% of shared genes respectively) (Figure 31C, Table 12). Comparative analysis of the 39.2 -kb prophage of *E. hirae* 13144 with 19 other sequenced *E. hirae* genomes showed 100% protein identity with IGR7 prophage proteins. The 39.2 -kb prophage encodes 65 genes, including 1 shared between all genomes and 38 unique to *E. hirae* 13144 and IGR7 (Figure 33B), encoding capsid, portal and tail structure, a characteristic of *Siphoviridae* phages. Importantly, the TMP1 epitope of the 39.2-kb prophage from *E. hirae* 13144, *E. hirae* IGR7 and *E. hirae* IGR11 showed 100% sequence homology (Figure 34). As a matter of fact, *E. hirae* IGR7 and *E. hirae* IGR11 were as efficient as *E. hirae* 13144 in reducing the growth of MCA205 fibrosarcomas treated with CTX (Fig. 27B). In contrast, no homologies (observed in *E. hirae* 708 and 13344) and a mutation in position 3 of the TSLARFANI peptide (L→F observed in *E. hirae* ATCC9790, Figure 34) correlated with a reduced anticancer effect of these *E. hirae* strains (Figure 27B and (Daillère et al., 2016)). Elispot assays designed to detect peptide-specific IFN γ -producing T cells revealed that mice gavaged with *E. hirae* 13144 (or strain *E. hirae* IGR7 and IGR11) mounted a TC1 response against TMP1 (but not the control peptides TMP2 and TMP3), while *E. hirae* strains devoid of anticancer activity and lacking TMP1 (strains 708, 13344, ATCC9790) were unable to do so (Figure 27E). We used a fluorescent H-2K^b/TSLARFANI tetrameric complex to detect the frequency and distribution of TMP1-specific cytotoxic T lymphocytes (CTLs) in naive and MCA205 fibrosarcoma bearing C57BL/6 mice. Up to 1% of splenic CD8⁺ T recognized the TMP1 peptide at day 12 following a CTX/*E. hirae* 13144 regimen (Figure 27F, left panel) with a 3-5 fold enrichment of TMP1-specific CTLs in the TC1 subset harboring the ileal chemokine receptor CCR9 in spleens from naive (Figure 27F, right panel) and tumor bearers, as well as in tumor draining lymph nodes (Figure 27G). Of note, H-2K^b/TSLARFANI tetramers also revealed a high proportion of

splenic TMP1-specific CTLs after immunization with *E. hirae* strains *IGR7* or *IGR11* but not with *ATCC9790* nor *E. hirae* 13344 (Figure 27H).

Region	Region Length	Completeness	Score	# Total Proteins	Region Position	Most Common Phage	GC %
1	40.6Kb	intact	150	58	481066-521729	PHAGE_Enterophages11_NC_013696(9)	33.79%
2	39.2Kb	intact	140	59	2123983-2163272	PHAGE_Enterophages197_NC_028671(6)	34.95%

Table 12: Seeking prophage sequences in *E. hirae* 13144 genomes

5 **Example 13: Prophylactic and therapeutic immunization using Phage Tail Length Tape Measure Protein in a mouse model**

To explore the capacity of TMP1-specific H-2K^b restricted TC1 cells to control the growth of MCA205 cancers, we subcutaneously (s.c.) immunized naive C57BL/6 mice with dendritic cells (DC) loaded with heat-inactivated *E. hirae* (positive control), the naturally occurring TSLARFANI peptide from 13144, *IGR7* or *IGR11*, its L→F mutant from *E. hirae* *ATCC9790* ('mut3', Figure 34) or other non-immunogenic bacterial peptides (group 1, Figure 32). In this prophylactic setting, DC pulsed with TSLARFANI (but not mut3 TSEARFANI) were as efficient as the whole *E. hirae* extract in preventing or restraining tumor outgrowth (Figure 28A-B). Next, we explored whether the TMP1 peptide would be able to confer immunogenicity to the usually inefficient bacterium *Escherichia coli* strain DH5α in the therapeutic setting, in which antibiotic treatment is followed by gavage with different bacterial strains and CTX-based chemotherapy (Fig. 27A, (Daillère et al., 2016)). *E. coli* engineered to express the TSLARFANI (SEQ ID No: 13) peptide (Figure 35) was as efficient as *E. hirae* 13144 in restraining MCA205 tumor growth (Figure 28C) and eliciting the generation of tetramer binding CTL in the spleen (Figure 28D). In contrast, *E. coli* expressing an irrelevant sequence (encoding mouse EGFP protein) or a mutant peptide bearing a S→A exchange in the anchor position 2 ('mut2', TALARFANI) or the 'mut3' TMP1 peptide failed to induce such a cancer-protective immune response (Figure 28C-D).

25 **Example 14: Clinical relevance of the enterophage's effects in cancer patients**

We next explored the possible pathophysiological relevance of these findings. We screened a total of 3027 adult and mother-infant metagenomes (mostly from human stools but also from various mucosae) from 17 publicly available datasets to assess the breadth of coverage (BOC) of the *E. hirae* genome and its phages (Figure

29A). *E. hirae* was present with 100% confidence in 13 samples from disparate geography, age and datasets. In other ~40 cases, strains closely related to *E. hirae* were detectable. In 90% of the samples in which *E. hirae* was found, one of the three phages (from *E. hirae* 13144, 708 or 13344) sequence were inserted in the genome in a mutually exclusive fashion (Figure 29A). However, the *E. hirae* 13144 phage was detectable in many samples lacking the presence of the *E. hirae* core genome, suggesting that other bacteria than *E. hirae* can host this phage as well. We could detect the presence of the phage 13144 at 0.66 BOC in three mother-infant paired stool specimens and in the infant at 1, 3, and 7 days after birth. Contrasting with metagenomics analyses that are unable to detect low-abundant species, culturomics followed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) provides a technology for detecting even rare *E. hirae* colonies in the stool of healthy individuals (Samb-Ba et al., 2014) or cancer patients (Routy et al., 2018). PCR analyses of each single cultivatable enterococcal colony (up to 5 per species and individual, representing colonies from *E. hirae*, *E. gallinarum*, *E. durans*, *E. faecium*, *E. faecalis*, *E. casseliflavus* or *E. avium*) from 76 cancer patients led to the detection of the TMP sequence encompassing the TMP1 peptide (Figure 36) in 34% of the patients, mostly inside *E. faecalis* (Figure 29B). Renal and lung cancer patients with detectable fecal TMP at diagnosis exhibited a prolonged overall survival after therapy with immune checkpoint inhibitors (Figure 29C). Therefore, we screened sixteen TMP-derived nonapeptides predicted to bind the human MHC class I HLA-A02*01 with high affinity (SEQ ID Nos: 54-69) for their ability to prime naive CD8⁺ T cells from six healthy volunteers *in vitro*. We found 6 out of 16 epitopes capable of triggering significant peptide-specific IFN γ release that were located in two distinct regions of the TMP protein (504-708 and 1397-1462, Figure 29D, Figure 37, Figure 24B, Table 9). Using the NCBI BLASTP suite, we searched the human peptidome associated with significant expression levels in human cancer biopsies (TCGA database) for a high degree of homology with these 6 HLA-A02*01-restricted immunogenic nonapeptides. We found that only peptide KLAKFASVV (631-639, SEQ ID No: 63) shared an 78% homology (7 out of nine 9 positions, with identical amino acids at the MHC anchoring positions 2 and 9) with a peptide contained in the protein glycerol-3-phosphate dehydrogenase 1-like GPD1-L (Figure 29E). GPD1-L reportedly counteracts the oncogenic HIF1 α -dependent adaptation to hypoxia and is associated with favorable prognosis in head and neck squamous cell carcinomas (Feng et al., 2014; Kelly et al., 2011; Liu et al., 2014). To our knowledge, no mutation in the GPD-1L gene has been reported in cancers. The TCGA transcriptomics database unveiled that high expression of GPD1-L is associated with improved overall survival in bladder, lung adenocarcinoma

and in a large cohort of >500 kidney cancers (with a trend towards a worse prognosis in tumors with low GPD-1L that do not express HLA-02*01) (Figure 29F-H).

Moreover, high expression of GPD1-L mRNA by tumors at diagnosis was associated with improved progression-free survival in two independent cohorts of non-small cell lung cancer (NSCLC) patients treated with anti-PD1/PDL-1 Abs (Figure 29I, J). Interestingly, expression levels of GPD-1L correlated with the cytotoxic lymphocytes, myeloid dendritic cells, neutrophils and endothelial cells gene signature in TCGA dataset of lung cancer (LUAD, LUSC and TCGA) and in the two cohorts of NSCLC patients (CHUM and CGFL) (Figure 29K).

10 **Example 15: Molecular mimicry between enterophage TMP and the oncogenic driver PSMB4 in mouse cancers**

In order to identify the mechanism by which TMP1 exerted its anticancer activity in the mouse model (MCA205 tumors in C57/B6 mice, Fig 27-28), we investigated whether there exist any H-2Kb-restricted mouse tumor antigens with high homology to the TMP1 peptide (TSLARFANI, SEQ ID No: 13). Using the NCBI BLASTP suite we found that the peptide (GSLARFRNI, SEQ ID No: 189) belonging to the proteasome subunit beta type-4 (PSMB4) between amino acid positions 76-84 shared an 78% homology (7 out of 9 amino acid, with identical amino acids at the MHC anchoring positions 2 and 9) (Figure 30A). We queried the potential neoepitopes of MCA205 but found no significant homology that would explain our results, hence our focus on the non-mutated PSMB4 peptide. PSMB4 is an oncogenic driver involved in proliferation and invasion (Lee et al., 2014) in a variety of malignancies such as glioblastoma (Cheng et al., 2018), melanoma (Zhang et al., 2017) and breast cancers (Wang et al., 2018), associated with dismal prognosis (Cheng et al., 2018; Lee et al., 2014; Wang et al., 2018). CRISPR/Cas9-mediated genomic knock-in of the PSMB4 sequence replacing GSLARFRNI (SEQ ID No: 189) by GSEARFRNI (SEQ ID No: 190) (with an L→F exchange in position 3 equivalent to mut 3 of TSLARFANI) in MCA205 cells (Figure 38) slowed down spontaneous tumor growth kinetics but drastically blunted the anticancer effects of *E. hirae* 13144 while not interfering with the CTX treatment alone (Figure 30B). These results support the idea that the TSLARFANI TMP1 peptide encoded by *E. hirae* 13144 indeed induces a therapy-relevant response against the PSMB4-derived GSLARFRNI peptide.

Example 16: Capacity of *E. hirae* 13144 to contagiously disseminate the phage TMP sequence

Temperate bacteriophages are bacterial viruses that transfer virulence, antimicrobial resistance genes, and immunogenic sequences to new bacterial hosts (Weinbauer, 2004). The TMP protein, which contains a variable number of tandem repeats with highly conserved tryptophan and phenylalanine residues at fixed positions is encoded by the genome of *Siphoviridae* phages (Belcaid et al., 2011; Piuri and Hatfull, 2006). To analyze the capacity of *E. hirae* 13144 to contagiously disseminate the phage TMP sequence, we performed culturomic analyses of the ileal content from C57BL/6 mice subjected to oral gavage with *E. hirae* 13144 and systemic CTX therapy (Figure 30C). We tested 7 to 18 bacterial colonies from each animal to discover that, out of 76 colonies, *E. gallinarum* was the only by-stander Enterococci transduced by the *E. hirae*-born phage *in vivo*, as confirmed by sequencing of the phage genome (Figure 30D, E and Figure 39). Of note, none of the 90 colonies (mostly of *E. gallinarum*) isolated from naive mice harbored the TMP sequence (Figure 39). Moreover, CTX plays a role in eliminating the *E. gallinarum*, allowing the niching and/or colonization of *E. hirae* in the small intestine, as shown *in vitro* in enteroid systems or in PCR of the ileal content (Figure 30G and not shown). Importantly, incubation of small intestine enteroids with a balanced 1:1 ratio of *E. hirae* or *E. gallinarum* harbouring or not the phage respectively, lead to the transmission of the infectious/lytic phage to *E. gallinarum* by *E. hirae* in 9% of colonies at 6 hrs and 26% at 20 hrs (Figure 30G).

In contrast to cyclophosphamide, CDK4/6 inhibitors (such as palbociclib) appear to trigger phage excision in breast cancer patients. Indeed, shot gun metagenomics analyses of patients stools harvested prior to surgery in 10 patients treated by 3 week- palbociclib, versus 73 non treated patients revealed a drastic enrichment and over-representation of phages sequences (from *Lactococcus*, *Salmonella*, *Sodalis*, *Escherichia*, enterobacteria) as shown in the LefSe diagramm (Figure 30H).

This observation suggests that the peptide encoding phage exhibiting this molecular mimicry with cancer antigens is infectious, in line with its detection in *E. faecalis* in humans.

To the best of our knowledge, these results represent the first demonstration that an enterococcal phage codes for an MHC class I-restricted antigen, TMP1, that induces a memory TC1 immune response, which then cross-reacts with cancer antigens, following three major lines of evidence. First, naturally occurring ('mut3') or artificial mutations ('mut2' or 'mut3') introduced into the MHC class I-binding TMP1

epitope suppressed the tumor-prophylactic and therapeutic potential of the phage-bearing *E. hirae* strains. Second, transfer of the TMP1-encoding gene into *E. coli* conferred immunogenic capacity to this proteobacterium, which acquired the same antitumor properties as TMP1-expressing *E. hirae*. Third, when cancer cells were

5 genetically modified to remove the TMP1-crossreactive peptide within the PSMB4 protein, they formed tumors that could no longer be controlled upon oral gavage with TMP1-expressing *E. hirae*.

Discussion 2

Phages are among the most abundant biological entities on earth. Their

10 numbers have been estimated to reach as high as 10^{31} particles with the potential of 10^{25} phage infections occurring every second (Pedulla et al., 2003; Wommack and Colwell, 2000). The antigenicity of the 'enterophage' studied here resides in hot spots of the TMP protein. Beyond their structural role in determining the length of the phage tail, TMP encoded by phages from the *Siphoviridae* family contains several functional domains,

15 one of which has peptidoglycan hydrolase activity, facilitating efficient infection of bacteria, and another one with lysozyme activity, acting as resuscitation-promoting factor, Rpf (Duerkop et al., 2014). Rpfs have not only been implicated in the reactivation of dormant bacteria but also modulate innate responses to *Mycobacterium tuberculosis* (Russell-Goldman et al., 2008) and cognate long-term immune responses (Commandeur

20 et al., 2011). In fact, T cell epitopes contained in *M. tuberculosis* Rpfs are key for the human immune response against this pathogen (Commandeur et al., 2011). Beyond their specific antigenic properties, phages convey broad adjuvanticity to DNA vaccines through filamentous bacteriophage coat protein III domain I (Cuesta et al., 2006; Larsen et al., 2008). Thus, the perspective opens that bacteriophages may enrich the

25 therapeutic armamentarium for stimulating anticancer immune responses.

Abbreviations used in this text:

ATB: Antibiotic

30 **BHI:** Brain heart infusion

BM-DC: Bone marrow dendritic cell

cDNA: complementary deoxyribonucleic acid

CGFL: Centre Georges François Leclerc

CHUM: Centre Hospitalier Universitaire de Montréal

35 **CTL:** Cytotoxic Tcell

Ctrl: Control

CTX: Cyclophosphomide

- DC:** Dendritic cell
DNA: Deoxyribonucleic acid
GM-CSF: Granulocyte-macrophage colony-stimulating factor
GPD1-L: Glycerol -3 -phosphate dehydrogenase 1 -like
- 5 **IFN γ :** Interferon gamma
IMDM: Iscove's Modified Dulbecco's Medium
Ip: Intraperitoneal
MALDI-TOF: Matrix-assisted laser desorption ionization time-of-flight
- 10 **MHC:** Major histocompatibility complex
NaCl: Sodium chloride
NSCLC: Non-small cell lung cancer
PBMC: Peripheral blood mononuclear cell
PCR: Polymerase chain reaction
- 15 **PSMB4:** Proteasome subunit beta type-4
PVDF: Polyvinylidene difluoride
RCC: Renal cell carcinoma
Rpf: Resuscitation-promoting factor
RPMI: Roswell Park Memorial Institute
- 20 **Sc:** Subcutaneous
SEM: Standard error of mean
Tc1: T cytotoxic cell type 1
Th1: T helper cell type 1
TLR3: Toll-like receptor 3
- 25 **TMP:** Tape-measure protein

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CLAIMS

1. A bacterial composition comprising at least one naturally occurring or engineered bacterial strain for use in treating a cancer, in combination with an antineoplastic drug, wherein said at least one bacterial strain expresses the protein of SEQ ID No: 1 or a fragment thereof of at least 9, preferably at least 20 amino acids comprising at least one epitope selected from the group consisting of SEQ ID Nos: 13, 14, 53 to 188 and 209, with the proviso that said at least one bacterial strain is different from the *Enterococcus hirae* strain 13144 deposited on November 7, 2013 at the CNCM under the number I-4815.
2. The bacterial composition of claim 1, for the use of claim 1, wherein said at least one bacterial strain comprises a prophage genome encoding a protein with at least 80, preferably at least 90 and more preferably at least 95% identity with the protein of SEQ ID No: 1.
3. The bacterial composition of claim 1 or claim 2, for the use of claim 1, wherein said composition comprises at least one strain harbouring a prophage genome with at least 80 and preferably at least 95% identity with the prophage of SEQ ID No: 2, so that the phage encoded by this prophage can *in vivo* infect the other strains of the composition and/or commensal bacteria of the gut microbiota of a subject in need thereof.
4. The bacterial composition of any of claims 1 to 3, for the use of claim 1, wherein said composition comprises bacteria selected from the group consisting of:
- (i) *Enterococcus hirae* strain CNCM I-4815,
 - (ii) *Enterococcus hirae* strain IGR7 deposited on August 31, 2017 at the CNCM under the number I-5224,
 - (iii) *Enterococcus hirae* strain IGR11 deposited on November 27, 2017, at the CNCM under the number I-5261,
 - (iv) any other bacterial strain expressing a protein with at least 65, preferably at least 80 and more preferably at least 95% identity with a fragment of at least 20, preferably at least 30 and more preferably at least 40 nucleotides from the protein of SEQ ID No: 1, and
 - (v) mixtures of at least two of the strains recited in (i) to (iv), preferably a mixture of the strains recited in (i) and (ii).
5. The bacterial composition of any of claims 1 to 4, for the use of claim 1, wherein said composition further comprises *Enterococcus hirae* strain IGR4 deposited on November 27, 2017, at the CNCM under the number I-5260.

6. A method of increasing the immunogenicity of a bacterial strain, comprising *in vitro* introducing, into said strain, a nucleotide sequence encoding the protein of SEQ ID No: 1 or a fragment thereof comprising at least the peptides of SEQ ID Nos: 13 and 14, or a sequence encoding a peptide of at least 9, preferably at least
5 20 amino acids comprising at least one epitope selected from the group consisting of SEQ ID No: 53 to 187, or a sequence encoding a peptide of at least 9, preferably at least 20 amino acids comprising at least one epitope of SEQ ID No: 209.

7. The method of claim 6, comprising *in vitro* infecting bacteria of said strain with a bacteriophage encoding a protein with at least 80, preferably at least 90 and
10 more preferably at least 95% identity with the protein of SEQ ID No: 1.

8. The method of claim 7, wherein said bacteriophage has a genome comprising a nucleotide sequence of SEQ ID NOs: 2 or a sequence having at least 90% or at least 95% identity thereto.

9. A bacterial composition comprising an engineered bacterial strain
15 which has been obtained by the method according to any of claims 6 to 8, for use in treating a cancer.

10. The bacterial composition of claim 9, for the use of claim 9, wherein said bacterial strain is selected from the group consisting of *Escherichia coli*, *Enterococcus gallinarum*, *Enterococcus faecalis* and *Enterococcus hirae*.

20 11. The bacterial composition of claim 9 or claim 10, for the use of claim 9, for use in treating a cancer in a HLA-A*0201 patient.

12. A bacterial composition selected from the group consisting of a bacterial composition comprising the *Enterococcus hirae* CNCM I-4815 and the bacterial composition of claims 1 to 5 and 9 to 11, for use in treating a cancer, wherein said
25 bacterial composition is used in combination with an antineoplastic drug capable of triggering the lytic cycle of a phage encoded by a prophage present in at least some bacteria of the composition.

13. The bacterial composition of claims 12, for the use of claim 12, wherein said bacterial composition is used in combination with a CDK4/6 inhibitor.

30 14. The bacterial composition of claims 12, for the use of claim 12 or 13, wherein said bacterial composition is used in combination with an immune checkpoint blocker.

15. An immunogenic composition comprising a polypeptide comprising a sequence of at least 9 consecutive amino acids from the protein of SEQ ID
35 No: 1 or a sequence of SEQ ID No: 209, or a polynucleotide encoding the same, for use as an anticancer vaccine.

16. The immunogenic composition of claim 15, for the use of claim 15, wherein the sequence of at least 9 consecutive amino acids from the protein of SEQ ID No: 1 is selected from the group consisting of SEQ ID No: 53-187.

17. The immunogenic composition of claim 15 or claim 16, which
5 comprises a peptide comprising sequence selected from the group consisting of SEQ ID No: 63, SEQ ID No: 188 and SEQ ID No: 209 or a polynucleotide encoding the same, for use as an anticancer vaccine in a HLA-A*0201 patient.

18. A cell composition comprising antigen presenting cells (APC)
10 which have been pulsed *ex vivo* with a bacterial composition according to any of claims 1 to 5 and 9 to 12 or with an immunogenic composition according to any of claim 15 to 17.

19. An MHC multimer for isolating T-cells with high affinity for the protein of SEQ ID No: 1, characterized in that MHC molecules are bound to an epitope selected from the group consisting of SEQ ID No: 53-188 and SEQ ID No: 209.

20. The MHC multimer of claim 19, which is a HLA-A*0201 multimer loaded with an antigenic peptide comprising SEQ ID No: 63, SEQ ID No: 188 or another sequence of SEQ ID No: 209.

21. A bacteriophage composition for use in treating a cancer, wherein said bacteriophage expresses a protein having at least 80, preferably at least 90 and
20 more preferably at least 95% identity with the protein of SEQ ID No: 1.

22. The bacteriophage composition of claim 21, for the use of claim 20, wherein said bacteriophage has a genome comprising a nucleotide sequence of SEQ ID No: 2 or a sequence having at least 90% or at least 95% identity thereto.

23. The immunogenic composition of any of claims 15 to 17, or the cell
25 composition of claim 18, or T cells isolated with the MHC multimer of claim 19 or claim 20, or the bacteriophage composition of claim 21 or claim 22, for use in treating a cancer, wherein said composition is administered in combination with a drug blocking an immune checkpoint.

24. The bacterial composition of any of claims 1 to 5 and 9 to 12, or
30 the immunogenic composition of any of claims 15 to 17, or the cell composition of claim 18, or T cells isolated with the MHC multimer of claim 19 or claim 20, or the bacteriophage composition of claim 21 or claim 22, for use in treating a tumor expressing mRNA levels of GPD1L superior to levels expressed in paired normal tissues.

25. A screening method for identifying antineoplastic drugs,
35 comprising using bacteria from the strain CNCM I-4815 or any other bacterial strain harbouring a prophage genome with at least 80 and preferably at least 90% or 95%

identity with the prophage of SEQ ID No: 2, for assessing the ability of drug candidates to trigger the lytic cycle of the phage comprising the protein of SEQ ID No: 1.

26. A method of determining if a patient having a cancer is likely to be a good responder to a treatment by chemotherapy or immune checkpoint blockade, comprising assessing the presence, in a biological sample from said patient, of a sequence having at least 80% identity with the protein of SEQ ID No: 1, wherein if such a sequence is present in the sample, the patient is likely to respond to the treatment.

27. The method of claim 27, comprising the steps of

- (i) cultivating a stool sample from said patient in aerobic conditions in a permissive medium to allow isolation of enterococci colonies,
- (ii) performing a PCR in several cultivable isolated colonies with a pair of primers specific for a fragment of SEQ ID No: 1, and
- (iii) detecting the amplified fragment.

28. A method of determining if a patient is likely to be a good responder to a treatment by chemotherapy or immune checkpoint blockade, comprising measuring the levels of circulating CCR9⁺ CXCR3⁺ CD8⁺ T cells at diagnosis and/or during said treatment, wherein if said level is above a predetermined threshold, the patient is likely to respond to the treatment.

29. The method of claim 28, comprising using the MHC multimer of claim 19 or claim 20 to assess the presence, in a biological sample from the patient, of T cells specific to the protein of SEQ ID No: 1, wherein if such T cells are present in the sample, the patient is likely to respond to the treatment.

30. The method of claim 29, wherein the patient is HLA-A*0201 and the MHC multimer is according to claim 20.

31. The method of any of claims 26 to 30, further comprising assessing the level of GPD1L mRNA or the level of GPD1L protein in the tumor, wherein if said level is above a predetermined threshold, the patient is likely to respond to the treatment.

32. A method of determining if a patient having a tumor is likely to be a good responder to a treatment by immune checkpoint blockade, comprising assessing the level of GPD1L mRNA or the level of GPD1L protein in the tumor, wherein if said level is above a predetermined threshold, the patient is likely to respond to the treatment.

33. The method of any of claims 26 to 32, wherein if the patient is not identified as likely to be a good responder to a treatment by chemotherapy or immune checkpoint blockade, a bacterial composition according to any of claims 1 to 5 and 9 to 12 or an immunogenic composition according to any of claims 15 to 17 is administered to the patient to increase his/her response to the treatment by chemotherapy or immune checkpoint blockade.