

Claims

1. A recombinant virulence attenuated Gram-negative bacterial strain selected from the group consisting of the genera *Yersinia*, *Escherichia*, *Salmonella*, and *Pseudomonas*, which comprises a nucleotide molecule comprising a nucleotide sequence encoding a heterologous protein fused in frame to the 3' end of a nucleotide sequence encoding a delivery signal from a bacterial effector protein, wherein the delivery signal from a bacterial effector protein is a bacterial T3SS effector protein of the Gram-negative bacterial strain comprising a bacterial T3SS effector protein or a N-terminal fragment thereof comprising a chaperone binding site, wherein the nucleotide sequence encoding the delivery signal from a bacterial effector protein is operably linked to a promoter, and wherein the heterologous protein is a protein involved in induction or regulation of a type I IFN response selected from the group consisting of the RIG-I-like receptor (RLR) family, other CARD domain containing proteins involved in antiviral signaling and type I IFN induction, and cyclic dinucleotide generating enzymes such as cyclic-di-AMP, cyclic-di-GMP and cyclic-di-GAMP cyclases selected from the group consisting of WspR, DncV, DisA and DisA-like, CdaA, CdaS and cGAS, leading to stimulation of STING.
2. The recombinant virulence attenuated Gram-negative bacterial strain of claim 1, wherein the protein involved in induction or regulation of a type I IFN response is selected from the group consisting of RIG1, MDA5, MAVS/IPS-1, WspR, DncV, DisA and DisA-like, CdaA, and cGAS or a fragment thereof.
3. The recombinant virulence attenuated Gram-negative bacterial strain of claim 1 or claim 2, wherein the recombinant virulence attenuated Gram-negative bacterial strain further comprises a deletion of a chromosomal gene coding for an endogenous protein essential for growth and an endogenous virulence plasmid which comprises a nucleotide sequence comprising a gene coding for said endogenous protein essential for growth operably linked to a promoter.
4. The recombinant virulence attenuated Gram-negative bacterial strain of claim 3, wherein said virulence attenuated recombinant Gram-negative bacterial strain is deficient in producing at least one bacterial effector protein.

5. The recombinant virulence attenuated Gram-negative bacterial strain of claim 3 or claim 4, wherein the gene coding for an endogenous protein essential for growth is selected from a gene coding for an enzyme essential for amino acid production, a gene coding for an enzyme involved in peptidoglycan biosynthesis, a gene coding for an enzyme involved in LPS biosynthesis, a gene coding for an enzyme involved in nucleotide synthesis and a gene coding for a translation initiation factor.
6. The recombinant virulence attenuated Gram-negative bacterial strain of any one of claims 3-5, wherein the gene coding for an endogenous enzyme essential for growth is a gene coding for an enzyme essential for amino acid production, wherein the enzyme essential for amino acid production is aspartate-beta-semialdehyde dehydrogenase (asd).
7. The recombinant virulence attenuated Gram-negative bacterial strain of any one of claims 1-6, wherein the recombinant virulence attenuated Gram-negative bacterial strain is a *Yersinia* strain.
8. The recombinant virulence attenuated Gram-negative bacterial strain of any one of claims 3-7, wherein the gene coding for an endogenous enzyme essential for growth located on the endogenous virulence plasmid comprises its endogenous promoter and its endogenous transcriptional terminator.
9. The recombinant virulence attenuated Gram-negative bacterial strain of claim 8, wherein the gene coding for the endogenous enzyme essential for growth, its endogenous promoter and its endogenous transcriptional terminator are located 122 bp upstream of the start of orf155 (SycO) on the endogenous virulence plasmid.
10. The recombinant virulence attenuated Gram-negative bacterial strain of any one of claims 1-9, wherein the recombinant virulence attenuated Gram-negative bacterial strain further comprises a modulation within a RNA thermosensor region upstream of a gene coding for an endogenous AraC-type DNA binding protein selected from the group consisting of VirF, LcrF, YbtA, Rns, MxiE, AraC, XylS, ExsA, PerA, MmsR, RhaS, TcpN, HrpX, HrpB, GadX, HilC, HilD, MarA, CafR, FapR and InvF.
11. The recombinant virulence attenuated Gram-negative bacterial strain of claim 10, wherein the modulation within a RNA thermosensor region upstream of a gene coding

for an endogenous AraC-type DNA binding protein comprises a deletion which removes a RNA hairpin structure or parts thereof upstream of the gene coding for an endogenous AraC-type DNA binding protein.

12. The recombinant virulence attenuated Gram-negative bacterial strain of claim 10 or claim 11, wherein the AraC-type DNA binding protein is VirF.
13. The recombinant virulence attenuated Gram-negative bacterial strain of any one of claims 1-12, wherein the recombinant virulence attenuated Gram-negative bacterial strain is *Yersinia enterocolitica*.
14. Use of the recombinant virulence attenuated Gram-negative bacterial strain of any one of claims 1-13, in the manufacture of a medicament for use in a method of treating cancer in a subject, whereby the medicament is to be administered to the subject in an amount sufficient to treat the subject.