CLAIMS

1. A personalized cancer vaccine comprising a recombinant poxvirus encoding one or more neopeptide(s), wherein several neopeptides are expressed by the recombinant poxvirus in the form of one or more fusion(s) of 2 to 15 neopeptides, wherein said fusion does not contain any transmembrane (TM) segment.

2. The personalized cancer vaccine of claim 1, wherein said recombinant poxvirus belongs to the Orthopoxvirus genus.

3. The personalized cancer vaccine of claim 2, wherein said recombinant poxvirus belonging to the Orthopoxvirus genus is vaccinia virus.

4. The personalized cancer vaccine of claim 3, wherein said recombinant vaccinia virus is a replication-defective vaccinia virus.

5. The personalized cancer vaccine of claim 4, wherein said replication-defective vaccinia virus is a Modified Virus Ankara (MVA) virus.

6. The personalized cancer vaccine of any one of claims 1 to 5, wherein each of said one or more neopeptide(s) encoded by the recombinant poxvirus comprises at least one tumor-specific mutation.

7. The personalized cancer vaccine of claim 6, wherein at least 60% of said neopeptides comprise a missense or a frameshift mutation.

8. The personalized cancer vaccine of claim 7, wherein at least 60% of said neopeptides comprise a missense mutation.

9. The personalized cancer vaccine of any one of claims 1 to 8, wherein said one or more neopeptide(s) has a length from 16 to 90 amino acid residues, preferably from 17 to 85 amino acid residues and more preferably from 18 to 80 amino acid residues.

10. The personalized cancer vaccine of any one of claims 7 to 9, wherein said neopeptides comprising a missense mutation have a length from 18 to 29 residues and said neopeptides comprising a frameshift mutation have a length from 30 to 80 amino acid residues.

11. The personalized cancer vaccine of any one of claims 7 to 10, wherein at least 80%, preferably at least 85%, more preferably at least 90% of neopeptides having a missense mutation carry the substituted amino acid in central position.

12. The personalized cancer vaccine of any one of claims 1 to 11, wherein all neopeptides are expressed by the recombinant poxvirus in the form of one or more fusion(s) of 2 to 15 neopeptides.

13. The personalized cancer vaccine of any one of claims 1 to 12, wherein said fusion scores negatively for hydrophobicity.

14. The personalized cancer vaccine of any one of claims 1 to 12, wherein said fusion displays a hydropathy score equal or below 0.1.

15. The personalized cancer vaccine of any one of claims 1 to 14, wherein the recombinant poxvirus encodes 2 or 3 fusions, wherein:

- each fusion comprises 5 to 10 neopeptides;
- each fusion comprises linkers, preferably of 3 amino acid long, at the N-terminus of the first neopeptide, and between each neopeptide;
- each fusion comprises a signal peptide at the N terminus of said fusion;
- each fusion optionally comprises a tag at the C-terminus of said fusion; and
- each fusion:
    - displays a global negative hydrophobicity score and/or a global hydropathy score equal or below 0.1, and
    - does not comprise any potential TM segment.

16. The personalized cancer vaccine of any one of claims 1 to 15, wherein said recombinant virus is a recombinant MVA comprising one to three cassettes, each for expression of a fusion of 5 to 10 neopeptides under the transcriptional control of a

vaccinia promoter selected from the group consisting of p11k7.5, pH5R, p7.5K, and pB2R.

17. The personalized cancer vaccine of any one of claims 1 to 16, wherein said recombinant poxvirus further encodes one or more therapeutic genes, preferably selected from the group consisting of suicide and immunostimulatory genes.

18. A process for preparing the personalized cancer vaccine of any one of claims 1 to 17, comprising:

1) a step of identifying one or more neopeptide(s) suitable for being encoded by the recombinant poxvirus wherein said one or more neopeptide(s) comprise(s) at least one tumor-specific mutation(s), wherein said identification step comprises the following sub-steps a) to d) and h):

a) extracting the DNA from a tumor sample and a non-tumor sample of a subject to be treated,

b) selecting target regions, preferably the entire coding regions of the genome (exome),

c) sequencing said target regions (e.g. the exome) from said extracted DNAs,

d) identifying one or more tumor-specific mutation(s) by comparing the DNA sequences obtained from said tumor and non-tumor samples, and

h) predicting the presence of potential TM segment(s) in neoepitopes themselves and/or in neopeptide fusions comprising several neopeptides; and

2) step of generating a recombinant poxvirus encoding one or more neopeptide(s) identified in identification step 1), wherein several neopeptides are expressed by the recombinant poxvirus in the form of one or more fusion(s) of 2 to 15 neopeptides, wherein said fusion does not contain any transmembrane (TM) segment.

19. The process according to claim 18, wherein said tumor sample is a tumor biopsy, preferably comprising more than 20% of tumor cells and wherein said non-tumor sample is a biological fluid, a cytological material or a biopsy.

20. The process according to claims 18 or 19, wherein said identification step further comprises at least one of the following additional sub-steps e) to g) and i):

e) ranking potential neopeptide(s) by their levels of expression in the tumor, either at the mRNA transcriptional level, or at the protein translation level;

f) selecting non-self-expressed tumor-specific mutation(s);

g) predicting the immune potential of the neoepitope(s) comprised in said neopeptide(s);

i) ranking potential neopeptide(s) and/or neopeptide fusion(s) by their degree of hydrophobicity and in particular, selecting neopeptide(s) and/or neopeptide fusion(s) so that said neopeptide(s) and/or neopeptide fusion(s) display(s) a negative global hydrophobicity score and/or a global hydropathy score equal or below 0.1.

21. The process according to any one of claims 18 to 20, wherein the nucleic acid molecules(s) encoding said one or more neopeptides- to be inserted in the genome of the recombinant poxvirus is/are arranged in one or more expression cassette(s) under the control of suitable regulatory elements permitting expression in the subject.

22. The process according to claim 21, wherein said nucleic acid molecules(s) or expression cassette(s) encoding said one or more neopeptides is/are inserted into the genome of a parental poxvirus to generate said recombinant poxvirus.

23. The process according to claim 22, wherein said parental poxvirus comprises a fluorescent reporter gene cloned at the site of insertion that is selected for the neopeptide-encoding nucleic acid molecule(s) or expression cassette(s).

24. The process according to claim 22 or claim 23, wherein said fluorescent reporter is selected from the group consisting of GFP (Green Fluorescent Protein), eGFP (Enhanced Green Fluorescent Protein), AmCyan 1 fluorescent protein and mCherry.

25. The process according to claim 24, wherein said step of generating said recombinant poxvirus comprises a further step of cleavage by an endonuclease able to generate at least one double strand break in said fluorescent reporter nucleotide sequence but in which said endonuclease does not cleave the poxviral genome.

26. The process according to claim 25, wherein said endonuclease is selected from the group consisting of zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 nucleases and restriction enzymes with unique cleavage site within the fluorescent reporter gene.

27. The process according to any one of claims 21 to 26, wherein said step of generating the recombinant poxvirus permits to generate said recombinant poxvirus in at most 4 weeks, preferably at most 3 weeks, with a ratio of recombinant versus parental comprised between 2 to 0.05.

28. The process according to any one of claims 18 to 27, wherein said process further comprises a manufacturing step, wherein said manufacturing step comprising an amplification step in a suitable producer cell to a suitable scale, a step of recovery of the produced recombinant poxvirus from the cell culture and an optional step of purification of the recovered recombinant poxvirus.

29. The process according to claim 28, wherein said producer cell is chicken embryo fibroblasts (CEF).

30. The process according to claim 28 or 29, wherein said step of recovery the produced recombinant poxvirus comprises a lysis step wherein the producer cell membrane is disrupted preferably by using a high-speed homogenizer.

31. The process according to any one of claims 28 to 30, wherein said purification step comprises a tangential flow filtration (TFF) step.

32. The process according to any one of claims 28 to 31, wherein said manufacturing step reaches the production of at least 10^9 pfu, desirably at least $5x10^9$ pfu and preferably approximately 10^{10} pfu or more for a personalized cancer vaccine.

33. The personalized cancer vaccine of any one of claims 1 to 16, wherein said personalized cancer vaccine is a composition comprising a therapeutically effective amount of said recombinant poxvirus and a pharmaceutically acceptable vehicle.

34. The personalized cancer vaccine of claim 33, wherein said therapeutically effective amount for individual doses comprises $5x10^7$pfu to 10^9pfu.

35. The personalized cancer vaccine of any one of claims 33 or 34 for use in a subject in need thereof for treating a cancer or preventing its relapse in a subject.

36. The personalized cancer vaccine for use according to claim 35, wherein said cancer is a solid tumor.

37. The personalized cancer vaccine for use according to claim 36, wherein said cancer is brain cancer, such as for example astrocytomas, embryonal tumors, germ cell tumors, central nervous system atypical teratoid/rhabdoid tumor, craniopharyngioma, ependymoma, glioma and glioblastoma as well as head and neck cancer.

38. The personalized cancer vaccine for use according to claim 36, wherein said cancer is lung cancer, and particularly NSCLC with a specific preference for adenocarcinoma, squamous cell carcinoma and large cell carcinoma.

39. The personalized cancer vaccine for use according to claim 36, wherein said cancer is ovarian cancer.

40. The personalized cancer vaccine for use according to any one of claims 35 to 39, wherein said personalized cancer vaccine is administered to the patient by intravenous, subcutaneous, intramuscular or intratumoral injections.

41. The personalized cancer vaccine for use according to any one of claims 35 to 40, wherein said personalized cancer vaccine is administered in conjunction with one or more additional anti-cancer therapy/ies which have utility in the treatment of cancer.