

UL38, UL45, or US13 such that the viral gene promoter drives expression of the heterologous antigen gene.

Also disclosed herein is a method of producing an HCMV lacking a functional pp71 protein (encoded by the UL82 gene). The method involves infecting a cell with the HCMV lacking a functional pp71 protein, wherein the cell contains an siRNA that silences the DAXX gene. In other embodiments, the method involves infecting a cell with the HCMV lacking a functional pp71 protein, wherein expression of the DAXX gene in the cell is downregulated at the protein or RNA level by other techniques known in the art, for example by RNA interference (e.g., microRNA targeting and short hairpin RNA (shRNA) targeting), ribozyme cleavage, regulated expression by a conditional or inducible promoter, expression of DAXX binding proteins, or targeting DAXX or DAXX protein complexes for ubiquitination and degradation. Using these methods, the HCMV is produced efficiently without complementation. The cell can be any cell, including a human fibroblast.

In one aspect, the present invention refers to a recombinant human cytomegalovirus (HCMV) comprising (1) at least one heterologous antigen; (2) an inactivating mutation in the UL82 gene; and (3) active US2, US3, US6, US7, and UL131A genes; wherein the recombinant HCMV is derived from the TR strain of HCMV; and wherein the recombinant HCMV is ganciclovir-sensitive. In another aspect, the present invention refers to an immunogenic composition comprising the recombinant HCMV as disclosed herein and a pharmaceutically acceptable carrier. In yet another aspect, the present invention refers to use of the immunogenic composition as disclosed herein in the manufacture of a medicament for inducing an immune response in a subject. In a further aspect, the present invention refers to an immunogenic composition comprising the recombinant HCMV as disclosed herein and a pharmaceutically acceptable carrier. In yet another aspect, the present invention refers to use the immunogenic composition as disclosed herein in the manufacture of a medicament for inducing an immune response in a subject. In another aspect, the present invention refers to an isolated polynucleotide that encodes the recombinant HCMV as disclosed herein. In one aspect, the present invention refers to an isolated cell comprising the polynucleotide. In another aspect, the present invention refers to a method of producing a pp71-deficient HCMV, the method comprising (1) infecting a cell with a pp71-deficient HCMV, wherein the cell comprises an siRNA that silences the expression of DAXX; (2) incubating the cell; and (3) collecting the pp71-deficient HCMV.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Some of the drawings herein are better understood when presented in color, which is not available in patent application publications. However, Applicants consider the color drawings to be part of the original disclosure and reserve the right to present color versions of the drawings herein in later proceedings.

Figures 1A and 1B collectively show that HCMV TR is superior in establishing latency and in reactivating from latency (+G-CSF) compared to other HCMV strains. **Figure 1A** is a map of the genome organization of the HCMV clones used in **Figure 1B**. HCMV genomes are flanked by terminal repeats (TRL and TRS as indicated) and internal repeats (IRS) that separate the unique long (UL) and unique short (US) regions. The location of the BAC cassette in each construct is indicated by the region indicated as B. The US region of HCMV TR lacks US2-7 due to insertion of the BAC-cassette. TRΔ4 lacks the genes UL128-UL150 in addition to lacking US2-7. The UL131A gene is deficient in AD169 but repaired in AD169 BAD UL131A (Wang and Schenk, 2005 *infra*). Toledo has an inversion of the UL133-128 region with a deletion in UL128 (Murphy *et al.*, 2003 *infra*). **Figure 1B** is a plot summarizing the results of NOD/SCID/IL2Rγ-null (NSG) mice engrafted with human CD34+ stem cells and inoculated intraperitoneally with human fibroblasts infected with the indicated HCMV

CLAIMS

1. A recombinant human cytomegalovirus (HCMV) comprising:
(1) at least one heterologous antigen;
(2) an inactivating mutation in the UL82 gene; and
(3) active US2, US3, US6, US7, and UL131A genes;
wherein the recombinant HCMV is derived from the TR strain of HCMV; and
wherein the recombinant HCMV is ganciclovir-sensitive.
2. The recombinant HCMV of claim 1, further comprising an active UL97 gene.
3. The recombinant HCMV of claim 1 or 2, wherein the active US2, US3, US6, and US7 genes are derived from the AD169 strain of HCMV.
4. The recombinant HCMV of any one of claims 1-3, wherein expression of the at least one heterologous antigen is driven by the UL82 promoter, the UL7 promoter, the UL45 promoter, the UL78 promoter, or the US13 promoter.
5. The recombinant HCMV of any one of claims 1-4, wherein the inactivating mutation in the UL82 gene is a deletion of all or part of the UL82 gene.
6. The recombinant HCMV of claim 5, wherein the at least one heterologous antigen replaces all or part of the UL82 gene.
7. The recombinant HCMV of claim 6, wherein expression of the at least one heterologous antigen replacing all or part of the UL82 gene is driven by the UL82 promoter.
8. The recombinant HCMV of any one of claims 1-7, further comprising an inactivating mutation in a HCMV gene selected from the group consisting of: UL7, UL38, UL45, and US13.

9. The recombinant HCMV of claim 8, wherein the inactivating mutation in the UL7 gene is a deletion of all or part of the UL7 gene.
10. The recombinant HCMV of claim 9, wherein the at least one heterologous antigen replaces all or part of the UL7 gene.
11. The recombinant HCMV of claim 10, wherein expression of the at least one heterologous antigen replacing all or part of the UL7 gene is driven by the UL7 promoter.
12. The recombinant HCMV of claim 8, wherein the inactivating mutation in the UL38 gene is a deletion of all or part of the UL38 gene.
13. The recombinant HCMV of claim 12, wherein the at least one heterologous antigen replaces all or part of the UL38 gene.
14. The recombinant HCMV of claim 13, wherein expression of the at least one heterologous antigen replacing all or part of the UL38 gene is driven by the UL38 promoter.
15. The recombinant HCMV of claim 8, wherein the inactivating mutation in the UL45 gene is a deletion of all or part of the UL45 gene.
16. The recombinant HCMV of claim 15, wherein the at least one heterologous antigen replaces all or part of the UL45 gene.
17. The recombinant HCMV of claim 16, wherein expression of the at least one heterologous antigen replacing all or part of the UL45 gene is driven by the UL45 promoter.
18. The recombinant HCMV of claim 8, wherein the inactivating mutation in the US13 gene is a deletion of all or part of the US13 gene.

19. The recombinant HCMV of claim 18, wherein the at least one heterologous antigen replaces all or part of the US13 gene.
20. The recombinant HCMV of claim 19, wherein expression of the at least one heterologous antigen replacing all or part of the US13 gene is driven by the US13 promoter.
21. The recombinant HCMV of any one of claims 1-20, further comprising an inactivating mutation in the UL128 gene or the UL130 gene.
22. The recombinant HCMV of claim 21, wherein the recombinant HCMV comprises an inactivating mutation in the UL128 gene and the UL130 gene.
23. The recombinant HCMV of any one of claims 1-22, wherein the at least one heterologous antigen is a pathogen specific antigen or tumor antigen.
24. The recombinant HCMV of any one of claims 1-23, wherein the nucleic acid sequences encoding the recombinant HCMV genome and the at least one heterologous antigen are stable upon multiple passages through fibroblasts.
25. The recombinant HCMV of any one of claims 1-23, further comprising a second heterologous antigen.
26. The recombinant HCMV of claim 25, wherein the first heterologous antigen replaces all or part of the UL82 gene, and wherein the second heterologous antigen replaces all or part of an HCMV gene selected from the group consisting of: UL7, UL45, UL78, and US13.
27. The recombinant HCMV of claim 26, wherein the expression of the first heterologous antigen is driven by the UL82 promoter, and wherein the expression of the second heterologous

antigen is driven by the UL7 promoter, the UL45 promoter, the UL78 promoter, or the US13 promoter.

28. The recombinant HCMV of any one of claims 25-27, wherein the first heterologous antigen is a pathogen specific antigen or tumor antigen.

29. The recombinant HCMV of claim 28, wherein the second heterologous antigen is a pathogen specific or tumor antigen that is different from the first heterologous antigen.

30. The recombinant HCMV of any one of claims 25-29, wherein the nucleic acid sequences encoding the recombinant HCMV genome and the first and second heterologous antigens are stable upon multiple passages through fibroblasts.

31. An immunogenic composition comprising the recombinant HCMV of any one of claims 1-24 and a pharmaceutically acceptable carrier.

32. Use of the immunogenic composition of claim 31 in the manufacture of a medicament for inducing an immune response in a subject.

33. The use of claim 32, wherein the recombinant HCMV induces and maintains a long-term effector memory T cell response to the at least one heterologous antigen in the subject.

34. The immunogenic composition of claim 31 for use in inducing an immune response in a subject.

35. The immunogenic composition of claim 34, wherein the recombinant HCMV induces and maintains a long-term effector memory T cell response to the at least one heterologous antigen in the subject.

36. An immunogenic composition comprising the recombinant HCMV of any one of claims 25-30 and a pharmaceutically acceptable carrier.
37. Use of the immunogenic composition of claim 36 in the manufacture of a medicament for inducing an immune response in a subject.
38. The use of claim 37, wherein the recombinant HCMV induces and maintains a long-term effector memory T cell response to the first and second heterologous antigens in the subject.
39. The immunogenic composition of claim 36 for use in inducing an immune response in a subject.
40. The immunogenic composition of claim 39, wherein the recombinant HCMV induces and maintains a long-term effector memory T cell response to the first and second heterologous antigens in the subject.
41. An isolated polynucleotide that encodes the recombinant HCMV of claims 1-30.
42. The polynucleotide of claim 41, wherein the polynucleotide comprises a sequence with at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 1
43. An isolated cell comprising the polynucleotide of claim 41 or 42.
44. The isolated cell of claim 43, wherein the isolated cell further comprises siRNA that silences expression of the DAXX gene.
45. The isolated cell of claim 44, wherein the DAXX siRNA comprises a sense strand that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to SEQ ID NO: 2 and an antisense strand that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to SEQ ID NO: 3.

46. The isolated cell of any one of claims 43-45, wherein the isolated cell is a mammalian cell.
47. The isolated cell of claim 46, wherein the isolated cell is a human cell.
48. The isolated cell of any one of claims 43-47, wherein the isolated cell is a fibroblast.
49. A method of producing a pp71-deficient HCMV, the method comprising:
- (1) infecting a cell with a pp71-deficient HCMV, wherein the cell comprises an siRNA that silences the expression of DAXX;
 - (2) incubating the cell; and
 - (3) collecting the pp71-deficient HCMV.
50. The method of claim 49, wherein the pp71-deficient HCMV has an inactivating mutation in the UL82 gene.
51. The method of claim 50, wherein the inactivating mutation in the UL82 gene is a deletion of all or part of the UL82 gene.
52. The method of any one of claims 49-51, wherein the DAXX siRNA comprises a sense strand that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to SEQ ID NO: 2 and an antisense strand that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to SEQ ID NO: 3.
53. The method of any one of claims 49-52, wherein the cell is a mammalian cell.
54. The method of claim 53, wherein the cell is a human cell.
55. The method of any one of claims 49-54, wherein the cell is a fibroblast.