

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 recombinant ganciclovir sensitive TR strain human cytomegalovirus (HCMV) vector comprising: (1) a nucleic acid encoding at least one heterologous antigen; (2) an inactivating mutation in the UL82 gene; and (3) active US2, US3, US6, US7, UL97, and UL131A genes. In one example, there is disclosed 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 one aspect, the present invention refers to an immunogenic composition comprising the recombinant HCMV vector as disclosed herein and a pharmaceutically acceptable carrier. In another example, there is disclosed 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 one aspect, the present invention refers to a method of producing a pp71-deficient HCMV, the method comprising: (1) infecting a cell with the recombinant HCMV vector as disclosed herein, wherein the cell comprises an siRNA that silences the expression of DAXX; (2) incubating the cell; and (3)collecting the pp71-deficient HCMV. In another example, there is disclosed 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/IL2Ry-null (NSG) mice engrafted with human CD34+ stem cells and inoculated intraperitoneally with human fibroblasts infected with the indicated HCMV

CLAIMS

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

9. The recombinant HCMV vector of claim 8, wherein the nucleic acid encoding at least one heterologous antigen replaces all or part of the UL7 gene.
10. The recombinant HCMV vector of claim 9, wherein expression of the nucleic acid encoding at least one heterologous antigen replacing all or part of the UL7 gene is driven by the UL7 promoter.
11. The recombinant HCMV vector of claim 7, wherein the inactivating mutation in the UL38 gene is a deletion of all or part of the UL38 gene.
12. The recombinant HCMV vector of claim 11, wherein the nucleic acid encoding at least one heterologous antigen replaces all or part of the UL38 gene.
13. The recombinant HCMV vector of claim 12, wherein expression of the nucleic acid encoding at least one heterologous antigen replacing all or part of the UL38 gene is driven by the UL38 promoter.
14. The recombinant HCMV vector of claim 7, wherein the inactivating mutation in the UL45 gene is a deletion of all or part of the UL45 gene.
15. The recombinant HCMV vector of claim 14, wherein the nucleic acid encoding at least one heterologous antigen replaces all or part of the UL45 gene.
16. The recombinant HCMV vector of claim 15, wherein expression of the nucleic acid encoding at least one heterologous antigen replacing all or part of the UL45 gene is driven by the UL45 promoter.
17. The recombinant HCMV vector of claim 7, wherein the inactivating mutation in the US13 gene is a deletion of all or part of the US13 gene.

18. The recombinant HCMV vector of claim 17, wherein the nucleic acid encoding at least one heterologous antigen replaces all or part of the US13 gene.
19. The recombinant HCMV vector of claim 18, wherein expression of the nucleic acid encoding at least one heterologous antigen replacing all or part of the US13 gene is driven by the US13 promoter.
20. The recombinant HCMV vector of any one of claims 1-19, further comprising an inactivating mutation in the UL128 gene or the UL130 gene.
21. The recombinant HCMV vector of claim 20, wherein the recombinant HCMV comprises an inactivating mutation in the UL128 gene and the UL130 gene.
22. The recombinant HCMV vector of any one of claims 1-21, wherein the nucleic acid encodes at least one pathogen specific antigen or tumor antigen.
23. The recombinant HCMV vector of any one of claims 1-22, wherein expression of the nucleic acid encoding at least one heterologous antigen is driven by an endogenous promoter, and wherein the nucleic acid encoding at least one heterologous antigen is stable upon multiple passages through fibroblasts.
24. The recombinant HCMV vector of any one of claims 1-22, wherein the nucleic acid encoding at least one heterologous antigen is a nucleic acid encoding a first heterologous antigen, and wherein the recombinant HCMV vector further comprises a nucleic acid encoding a second heterologous antigen.
25. The recombinant HCMV vector of claim 24, wherein the nucleic acid encoding the first heterologous antigen replaces all or part of the UL82 gene, and wherein the nucleic acid encoding the second heterologous antigen replaces all or part of an HCMV gene selected from the group consisting of: UL7, UL45, UL78, and US13.
26. The recombinant HCMV vector of claim 25, wherein the expression of the nucleic acid encoding the first heterologous antigen is driven by the UL82 promoter, and wherein the

expression of the nucleic acid encoding the second heterologous antigen is driven by the UL7 promoter, the UL45 promoter, the UL78 promoter, or the US13 promoter.

27. The recombinant HCMV vector of any one of claims 24-26, wherein the first heterologous antigen is a pathogen specific antigen or tumor antigen.
28. The recombinant HCMV vector of claim 27, wherein the second heterologous antigen is a pathogen specific or tumor antigen that is different from the first heterologous antigen.
29. The recombinant HCMV vector of any one of claims 24-28, wherein the expression of the nucleic acids encoding the first and second heterologous antigens are driven by endogenous promoters, and wherein the nucleic acids encoding the first and second heterologous antigens are stable upon multiple passages through fibroblasts.
30. An immunogenic composition comprising the recombinant HCMV vector of any one of claims 1-23 and a pharmaceutically acceptable carrier.
31. Use of the immunogenic composition of claim 30 in the manufacture of a medicament for inducing an immune response in a subject.
32. The use of claim 31, wherein the recombinant HCMV vector induces and maintains a long-term effector memory T cell response to the at least one heterologous antigen in the subject.
33. The immunogenic composition of claim 30 for use in inducing an immune response in a subject.
34. The immunogenic composition of claim 33, wherein the recombinant HCMV vector induces and maintains a long-term effector memory T cell response to the at least one heterologous antigen in the subject.
35. An immunogenic composition comprising the recombinant HCMV vector of any one of claims 24-29 and a pharmaceutically acceptable carrier.

36. Use of the immunogenic composition of claim 35 in the manufacture of a medicament for inducing an immune response in a subject.
37. The use of claim 36, wherein the recombinant HCMV vector induces and maintains a long-term effector memory T cell response to the first and second heterologous antigens in the subject.
38. The immunogenic composition of claim 37 for use in inducing an immune response in a subject.
39. The immunogenic composition of claim 38, wherein the recombinant HCMV vector induces and maintains a long-term effector memory T cell response to the first and second heterologous antigens in the subject.
40. An isolated polynucleotide that encodes the recombinant HCMV vector of any one of claims 1-29.
41. The polynucleotide of claim 40, wherein the polynucleotide comprises the sequence set forth in SEQ ID NO: 1.
42. An isolated cell comprising the polynucleotide of claim 40 or 41.
43. The isolated cell of claim 42, wherein the isolated cell further comprises siRNA that silences expression of the DAXX gene.
44. The isolated cell of claim 43, wherein the DAXX siRNA comprises a sense strand having a sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to the sequence set forth in SEQ ID NO: 2 and an antisense strand having a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to the sequence set forth in SEQ ID NO: 3.

45. The isolated cell of any one of claims 42-44, wherein the isolated cell is a mammalian cell.
46. The isolated cell of claim 45, wherein the isolated cell is a human cell.
47. The isolated cell of any one of claims 42-46, wherein the isolated cell is a fibroblast.
48. A method of producing a pp71-deficient HCMV, the method comprising:
 - (1) infecting a cell with the recombinant HCMV vector of any one of claims 1-29, wherein the cell comprises an siRNA that silences the expression of DAXX;
 - (2) incubating the cell; and
 - (3) collecting the pp71-deficient HCMV.
49. The method of claim 48, wherein the DAXX siRNA comprises a sense strand having a sequence 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 the sequence set forth in SEQ ID NO: 3.
50. The method of claim 48 to 49, wherein the cell is a mammalian cell.
51. The method of claim 50, wherein the cell is a human cell.
52. The method of any one of claims 48-51, wherein the cell is a fibroblast.