

[0007] This is different from other technologies in the field which express a single scFV but do not express a library. Moreover, other technologies are designed to re-direct vaccinia infection through the scFV for gene therapy and are not used for antibody discovery. Additionally, the current technology differs from the previous technology by using EEV instead of the IMV, and also by using different fusion proteins (e.g., A56R).

SUMMARY

[0007a] In one aspect, there is provided a fusion protein comprising an immunoglobulin heavy chain variable region domain fused to the N-terminus of (a) a polypeptide segment consisting of amino acids 117 to 421 of SEQ ID NO: 11, which is the CH1 domain of human IgG1 fused to the stalk region, the transmembrane domain, and the intracellular domain of the vaccinia virus A56R protein, or (b) a polypeptide segment consisting of amino acids 117 to 653 of SEQ ID NO: 30, which is the full constant region of human IgG1 fused to the stalk region, the transmembrane domain, and the intracellular domain of a vaccinia virus A56R protein; wherein the fusion protein can be expressed on the surface of a vaccinia extracellular enveloped virus (EEV) virus particle, and wherein the fusion protein when combined with a light chain variable domain can form a functional antibody or antigen binding fragment thereof.

[0008] In certain embodiments, the disclosure is directed to fusion protein comprising (a) a first polypeptide segment comprising a heavy chain CHI domain and (b) a second polypeptide segment comprising the transmembrane domain of a vaccinia extracellular enveloped virus (EEV)-specific membrane protein.

[0009] In some embodiments, the fusion protein further comprising a third polypeptide segment comprising an immunoglobulin heavy chain variable region or fragment thereof. In another embodiment, the vaccinia EEV-specific membrane protein is A56R.

[0009a] In one aspect, there is provided a polynucleotide encoding the fusion protein as described herein.

[0010] In certain embodiments, the disclosure is directed to a polynucleotide encoding a fusion protein comprising (a) a first polypeptide segment comprising the human heavy chain CHI domain and (b) a second polypeptide segment comprising the transmembrane domain of a vaccinia extracellular enveloped virus (EEV)-specific membrane protein.

[0011a] In one aspect, there is provided a recombinant vaccinia virus comprising the polynucleotide as described herein.

[0012] In certain embodiments, the disclosure is directed to a recombinant vaccinia virus comprising a polynucleotide encoding a fusion protein comprising (a) a first polypeptide segment comprising the human heavy chain CHI domain and (b) a second polypeptide segment comprising the transmembrane domain of a vaccinia extracellular enveloped virus (EEV)-specific membrane protein. In another aspect, there is provided a host cell infected with the recombinant vaccinia virus as described herein.

[0012a] In one aspect, there is provided recombinant vaccinia library comprising a library of polynucleotides encoding a plurality of immunoglobulin fusion polypeptides, each immunoglobulin fusion polypeptide comprising a heavy chain variable domain fused to a CHI constant domain fused to a polypeptide segment comprising the transmembrane domain of an EEV-specific membrane protein, wherein the library of polynucleotides is constructed in a vaccinia virus vector, and wherein each polynucleotide in the library comprises the following elements:

(a) a first polynucleotide encoding a first polypeptide segment comprising a heavy chain IgG CHI domain;

(b) a second polynucleotide encoding a second polypeptide segment comprising the stalk region, the transmembrane domain, and the intracellular domain of vaccinia virus A56R EEV-specific membrane protein, situated downstream of the first polynucleotide encoding the first polypeptide segment; and

(c) a third polynucleotide encoding an immunoglobulin heavy chain variable region or fragment thereof situated upstream of the first polynucleotide encoding the first polypeptide segment; wherein the immunoglobulin fusion protein can be expressed on the surface of a vaccinia EEV particle, and wherein the fusion protein can combine with an immunoglobulin light chain to form an antigen-binding domain of an immunoglobulin molecule.

[0013] In another embodiment, the disclosure is directed to recombinant vaccinia library comprising a first library of polynucleotides constructed in a vaccinia virus vector encoding a plurality of immunoglobulin fusion polypeptides, wherein the vaccinia virus vector comprises (a) a first polynucleotide encoding a first polypeptide segment comprising a heavy chain CHI domain (b) a second polynucleotide encoding a second polypeptide segment comprising the transmembrane domain of a vaccinia virus EEV-specific membrane protein situated downstream of the CHI domain, and (c) a third polynucleotide encoding an immunoglobulin heavy chain variable region or fragment thereof situated upstream of the CHI domain. In one embodiment, the first library further comprising a signal peptide for facilitating expression of the fusion polypeptides on the surface of EEV. In another embodiment, the EEV-specific membrane protein is A56R. In another embodiment, the vaccinia EEV-specific membrane protein is A56R. In another embodiment, the second polypeptide segment further comprises the extracellular domain of the EEV-specific membrane protein, or a portion thereof. In another embodiment, the second polypeptide segment further comprises the intracellular domain of the EEV-specific membrane protein, or a portion thereof. In certain embodiments, the fusion protein comprises amino acids of SEQ ID NO: 11 which correspond to the polypeptide sequence amino acids 108 to 314 of A56R from Western

7. The method of any one of claims 1 to 4, wherein the second polypeptide segment comprises the transmembrane domain of the vaccinia EEV-specific membrane protein A33R, A34R, A56R, A36R, F13L, or B5R.
8. The method of claim 7, wherein the vaccinia EEV-specific membrane protein is A56R.
9. The method of claim 8, wherein the second polypeptide segment comprises the stalk region, the transmembrane domain, and the intracellular domain of the A56R protein.
10. The method of claim 9, wherein the third polypeptide segment comprises amino acids 215 to 421 of SEQ ID NO: 11 or amino acids 447 to 653 of SEQ ID NO: 30.
11. The method of any one of claims 1 to 4, wherein the one or more polynucleotides encoding an immunoglobulin light chain of (b) are each contained on a vaccinia virus vector.
12. The method of any one of claims 1 to 4, wherein the one or more polynucleotides encoding an immunoglobulin light chain of (b) are contained on a plasmid vector.
13. A fusion protein comprising an immunoglobulin heavy chain variable region domain fused to the N-terminus of (a) a polypeptide segment consisting of amino acids 117 to 421 of SEQ ID NO: 11, which is the CH1 domain of human IgG1 fused to the stalk region, the transmembrane domain, and the intracellular domain of the vaccinia virus A56R protein, or (b) a polypeptide segment consisting of amino acids 117 to 653 of SEQ ID NO: 30, which is the full constant region of human IgG1 fused to the stalk region, the transmembrane domain, and the intracellular domain of a vaccinia virus A56R protein; wherein the fusion

protein can be expressed on the surface of a vaccinia extracellular enveloped virus (EEV) virus particle, and wherein the fusion protein when combined with a light chain variable domain can form a functional antibody or antigen binding fragment thereof.

14. The fusion protein of claim 13, further comprising a signal peptide for facilitating expression of the fusion polypeptides on the surface of EEV.

15. A polynucleotide encoding the fusion protein of claim 13 or claim 14.

16. A vector comprising the polynucleotide of claim 15.

17. A recombinant vaccinia virus comprising the polynucleotide of claim 15 or the vector of claim 16.

18. A host cell infected with the recombinant vaccinia virus of claim 17.

19. A recombinant vaccinia library comprising a library of polynucleotides encoding a plurality of immunoglobulin fusion polypeptides, each immunoglobulin fusion polypeptide comprising a heavy chain variable domain fused to a CH1 constant domain fused to a polypeptide segment comprising the transmembrane domain of an EEV-specific membrane protein, wherein the library of polynucleotides is constructed in a vaccinia virus vector, and wherein each polynucleotide in the library comprises the following elements:
(a) a first polynucleotide encoding a first polypeptide segment comprising a heavy chain IgG CH1 domain;
(b) a second polynucleotide encoding a second polypeptide segment comprising the stalk region, the transmembrane domain, and the intracellular domain of vaccinia virus A56R EEV-specific membrane protein, situated downstream of the first polynucleotide encoding the first polypeptide segment; and
(c) a third polynucleotide encoding an immunoglobulin heavy chain variable region or fragment thereof situated upstream of the first polynucleotide encoding the first polypeptide segment;
wherein the immunoglobulin fusion protein can be expressed on the surface of a vaccinia EEV particle, and wherein the fusion protein can combine with an immunoglobulin light chain to form an antigen-binding domain of an immunoglobulin molecule.

20. The recombinant vaccinia library of claim 19, wherein each immunoglobulin fusion polypeptide further comprises a signal peptide for facilitating expression of the plurality of immunoglobulin fusion polypeptides on the surface of EEV.

21. The recombinant vaccinia library of claim 19 or claim 20, wherein the immunoglobulin fusion polypeptides each comprise a VH or fragment thereof fused to the N-terminus of amino acids 117 to 421 of SEQ ID NO: 11.

22. The recombinant vaccinia library of claim 19 or claim 20, wherein the first polynucleotide of (a) encodes a first polypeptide segment comprising a full-length human IgG constant region.

23. The recombinant vaccinia library of claim 22, wherein the immunoglobulin fusion polypeptides each comprise a VH or fragment thereof fused to the N-terminus of amino acids 117 to 653 of SEQ ID NO: 30.