

## SUMMARY

**[0004]** The present disclosure provides improved modified recombinant HA polypeptides with broadened immunogenic profile that extends coverage (i.e., the capability of eliciting a immunogenic response against the HA polypeptide) to antigenically distinct influenza strains, for example to new or additional pandemic and/or seasonal influenza strains. The present disclosure is, in part, based on modifications deduced from *in silico* analysis of sequence variation among circulating influenza strains, mapping of the antigenic region of HA, and/or epitope patterns and structural analyses of the HA peptide. Targeted modifications can be subsequently introduced at various amino acid residue locations and/or specific regions of an HA polypeptide with a known immune profile to yield novel HA polypeptides with improved and more balanced immune profiles. As described in detail below including the Examples section, the present inventors have developed -among other things- various distinct strategies for engineering HA polypeptides to extend a seasonal response profile to cover pandemic strains, or vice versa. These strategies extend the immune profile across clusters of sequences (or clades) of antigenically distinct strains; they can be applied to an engineered recombinant HA molecule over time so that it continues to elicit an immune response against antigenically drifted circulating seasonal strains. In all cases, the strategy is designed to generally preserve specific residues of the receptor binding site (RBS) of a host HA polypeptide with modifications engineered in the region near the RBS. Similar strategies may be used to extend a pandemic response profile to cover seasonal strains. The compositions and methods of the present disclosure are applicable to a wide variety of recombinant HA polypeptides, including those engineered by a variety of methods and those comprising substantially wild-type sequences.

**[0004a]** In one aspect, the present disclosure provides a use of a recombinant influenza hemagglutinin (HA) polypeptide in the manufacture of a pharmaceutical composition for immunizing a subject against seasonal and pandemic influenza virus, wherein the recombinant influenza HA polypeptide comprises: an engineered head region derived from an engineered influenza HA polypeptide with a known immune profile, wherein the engineered head region is a segment encompassed by amino acid residues 59-292 of the engineered influenza HA polypeptide according to California 09 Numbering (CA09 Numbering); and a stem region derived from a second influenza HA polypeptide with a distinct immune profile, wherein the stem region comprises amino acid residues 18-58 and 293-519 of the second influenza HA polypeptide according to CA09 Numbering, wherein the engineered head region comprises:

one or more amino acid substitutions, deletions or insertions at one or more putative N-linked glycosylation sites such that the one or more putative N-linked glycosylation sites are disrupted or one or more additional N-linked glycosylation sites are inserted; and one or more amino acid substitutions in the region of or adjacent to the Receptor Binding Site (RBS) region and/or an insertion of a Lysine (K) or Arginine (R) residue in a loop or loops bounding the RBS region.

**[0005]** Thus, in one example, the present disclosure provides a recombinant hemagglutinin (HA) polypeptide comprising an engineered head region or segment thereof derived from an engineered HA polypeptide with a predominantly seasonal immune profile and a stem region derived from a pandemic strain. In some examples, the engineered head region or segment thereof comprises a sequence at least 95%, 96%, 97%, 98%, 99% or more identical to amino acids corresponding to positions 135 – 269, 125 – 277, or 63 – 278 of SEQ ID NO:1. In some examples, the engineered head region or segment thereof comprises a sequence identical to amino acids corresponding to positions 135 – 269, 125 – 277, or 63 – 278 of SEQ ID NO:1 (SMART\_DO2a sequence).

## CLAIMS

### We claim:

1. Use of a recombinant influenza hemagglutinin (HA) polypeptide in the manufacture of a pharmaceutical composition for immunizing a subject against seasonal and pandemic influenza virus,

wherein the recombinant influenza HA polypeptide comprises:

an engineered head region derived from an engineered influenza HA polypeptide with a known immune profile, wherein the engineered head region is a segment encompassed by amino acid residues 59-292 of the engineered influenza HA polypeptide according to California 09 Numbering (CA09 Numbering); and

a stem region derived from a second influenza HA polypeptide with a distinct immune profile, wherein the stem region comprises amino acid residues 18-58 and 293-519 of the second influenza HA polypeptide according to CA09 Numbering,

wherein the engineered head region comprises:

one or more amino acid substitutions, deletions or insertions at one or more putative N-linked glycosylation sites such that the one or more putative N-linked glycosylation sites are disrupted or one or more additional N-linked glycosylation sites are inserted; and

one or more amino acid substitutions in the region of or adjacent to the Receptor Binding Site (RBS) region and/or an insertion of a Lysine (K) or Arginine (R) residue in a loop or loops bounding the RBS region.

2. The use of claim 1, wherein the engineered head region comprises a sequence identical to amino acids corresponding to residues 63-278, 125-277, or 135-269 of SEQ ID NO: 1.

3. The use of claim 1 or 2, wherein the engineered influenza HA polypeptide has a pandemic immune profile, and wherein the stem region is derived from a strain with a seasonal immune profile.

4. The use of claim 1 or 2, wherein the engineered influenza HA polypeptide has a seasonal immune profile, and wherein the stem region is derived from a strain with a pandemic immune profile.

5. The use of claim 4, wherein the strain with a pandemic immune profile is a naturally-occurring pandemic strain.
6. The use of claim 5, wherein the naturally-occurring pandemic strain is selected from A/California/07/2009, A/New Jersey/10/1976, or A/South Carolina/1/1918.
7. The use of claim 1 or 2, wherein the stem region is derived from an engineered influenza HA polypeptide that has a pandemic immune profile.
8. The use of claim 7, wherein the engineered influenza HA polypeptide that has a pandemic immune profile is engineered by a computationally optimized broadly reactive antigens (COBRA) technology, a mosaic technology, influenza consensus sequences based combinations of influenza strains, deletion and/or rearrangement of structural domains, domain swapping, or combinations of neutralizing or cross-reactive epitopes among multiple influenza strains.
9. The use of any one of claims 1-8, wherein the one or more putative N-linked glycosylation sites or the one or more additional N-linked glycosylation sites are defined by a consensus sequence of NxS/Ty, wherein x and y are not proline (P).
10. The use of claim 9, wherein the recombinant influenza HA polypeptide comprises a Lysine (K) or Arginine (R) residue inserted within 1-5 amino acids of the NxS/Ty consensus sequence.
11. The use of claim 9 or 10, wherein the one or more amino acid substitutions, deletions or insertions to disrupt the one or more putative N-linked glycosylation sites comprise modifying the consensus sequence NxS/Ty to  $z^1z^2z^3z^4$  wherein
  - $z^1$  is N, D, K or S;
  - $z^2$  is Y or is unchanged;
  - $z^3$  is E, D or N; and
  - $z^4$  is I, L, P, S or T, or is unchanged.

12. The use of any one of claims 1-11, wherein the one or more putative N-linked glycosylation sites are at positions corresponding to positions 142-145 and/or 177-179 of SEQ ID NO: 2.
13. The use of any one of claims 1-12, wherein the RBS region comprises all amino acids residues within 15 angstroms of a position corresponding to residue W167 of SEQ ID NO: 2 in a three-dimensional (3-D) structure.
14. The use of any one of claims 1-13, wherein the RBS region comprises the epitope bound by the paratope of monoclonal antibody CH65.
15. The use of any one of claims 1-14, wherein the recombinant influenza HA polypeptide comprises one or more amino acid substitutions, deletions or insertions at positions corresponding to residues 137, 144, 145, 154, 155, 156, 157, 158, 159, 177, 210, 211, 212, 213, 214, 244, 245 and/or 262 of SEQ ID NO: 2.
16. The use of any one of claims 1-15, wherein the recombinant influenza HA polypeptide comprises one or more amino acid substitutions, deletions or insertions at positions corresponding to residue 137, 144, 145, 154, 155, 156, 157, 158, 159, 177, 210, 211, 212, 213 and/or 214 of SEQ ID NO: 2.