

CLAIMS

1. A fusion protein comprising a human serum albumin (HSA) binding recombinantly engineered variant of stefin polypeptide and a PD-L1 binding polypeptide, wherein the HSA binding polypeptide binds to HSA with a K_d of $1 \times 10^{-6}M$ or less at pH 6.0.
2. A fusion protein comprising an HSA binding recombinantly engineered variant of stefin polypeptide that binds to HSA and a PD-L1 binding recombinantly engineered variant of stefin polypeptide that binds to PD-L1, wherein the protein has a circulating half-life in human subjects of at least 7 days.
3. The fusion protein of claim 1, wherein the HSA binding polypeptide comprises a loop 2 sequence selected from any one of SEQ ID NOs: 86-138 and/or a loop 4 sequence selected from any one of SEQ ID NOs: 139-191.
4. The fusion protein of claim 1, wherein the PD-L1 binding polypeptide comprises a loop 2 sequence selected from any one of SEQ ID NOs: 16-50 and/or a loop 4 sequence selected from any one of SEQ ID NOs: 51-85.
5. The fusion protein of any one of the preceding claims, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 277, or an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 277, SEQ ID NO: 278, SEQ ID NO: 279, SEQ ID NO: 280, SEQ ID NO: 281, or SEQ ID NO: 282.
6. The fusion protein of claim 1 further comprising a second a PD-L1 binding polypeptide, wherein the second PD-L1 binding polypeptide binds to PD-L1 with a K_d of $1 \times 10^{-6}M$.
7. An in-line fusion protein comprising a human serum albumin (HSA) binding recombinantly engineered variant of stefin, first a PD-L1 binding polypeptide, and a second a PD-L1 binding polypeptide, wherein the HSA binding polypeptide binds to HSA with a K_d of $1 \times 10^{-6}M$ or less at pH 6.0.
8. The fusion protein of claim 1, further comprising a linker.

9. The fusion protein of claim 8, wherein the linker is a rigid linker or a flexible linker.
10. The fusion protein of claim 9, wherein the rigid linker comprises the sequence of SEQ ID NO: 294, or the flexible linker comprises the sequence of SEQ ID NO: 293.
11. The fusion protein of claim 8, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 283, or an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 283, SEQ ID NO: 284, SEQ ID NO: 285, SEQ ID NO: 286, SEQ ID NO: 287, SEQ ID NO: 290, SEQ ID NO: 291, or SEQ ID NO: 293.
12. A polynucleotide comprising a sequence encoding the fusion protein of claim 1.
13. A viral vector, a plasmid, or a minicircle comprising the polynucleotide of claim 12.
14. A cell comprising the fusion protein of claim 1.
15. The fusion protein of claim 1, for use in a method for treating an infectious disease or cancer.
16. The fusion protein of claim 1, further comprising one or more additional polypeptide sequences which may additional therapeutic activity and/or additional PK/ADME properties to the fusion protein.
17. The fusion protein of claim 16, wherein the additional polypeptide sequence is a receptor ligand, and wherein the receptor ligand is an immunostimulatory cytokine, such as IFN- α , IL-2, IL-15, IL-21, and IL-12, or a variant sequence thereof; or wherein the additional polypeptide sequence is a CD3 binding polypeptide sequence that directs the fusion protein to bind to CD3 on the surface of T-cells.
18. The fusion protein of claim 17, wherein the additional polypeptide sequence is a mutant IL-2 polypeptide comprising the amino acid sequence of SEQ ID NO: 1282, or a sequence at least 90% identical thereto, having one or more amino acid substitutions relative to SEQ ID NO: 1282 that abolish or reduce affinity of the mutant IL-2 polypeptide to a high-affinity IL-2 receptor and

preserve affinity of the mutant IL-2 polypeptide to an intermediate-affinity IL-2 receptor, as compared to a wild-type IL-2 polypeptide.

19. The fusion protein of claim 17, wherein the receptor ligand is for a co-stimulatory receptor and the fusion protein agonizes the co-stimulatory receptor upon binding.

20. The fusion protein of claim 17, further comprising a polypeptide sequences that induces dimerization or higher order multimerization of the fusion protein.

