

CLAIMS

1. A mutein of human tear lipocalin that is capable of binding lymphocyte-activation gene 3 (LAG-3),

wherein the amino acid sequence of the mutein comprises at least 14 of the following mutated amino acid residues in comparison with the linear polypeptide sequence of mature human tear lipocalin (SEQ ID NO: 1): Ser 14 → Pro; Asp 25 → Ser; Arg 26 → Ser, Phe, Gly, Ala, Asp or Glu; Glu 27 → Asp, Val or Thr; Phe 28 → Cys or Asp; Pro 29 → Phe, Leu or Trp; Glu 30 → Trp, Asn or Tyr; Met 31 → Ile, Val, Asp, Leu or Tyr; Asn 32 → Asp, Glu, Tyr, Trp, Val, Thr or Met; Leu 33 → Asp, Glu or Pro; Glu 34 → Val, Trp or His; Val 36 → Ala; Asn 48 → Asp; Lys 52 → Glu, Ser, Arg or Asn; Val 53 → Ala; Met 55 → Ala or Val; Leu 56 → Asp, Gln or Asn; Ile 57 → Leu; Ser 58 → Phe, Trp or Asp; Arg 60 → Phe or Glu; Cys 61 → Pro, Leu or Trp; Ala 66 → Asn; Ala 79 → Glu; Val 85 → Ala; Ala 86 → Asp; Cys 101 → Ser or Phe; Glu 104 → Tyr; Leu 105 → Cys or Gly; His 106 → Ala, Glu, Thr, Tyr, Gln or Val; Lys 108 → Tyr, Phe, Thr or Trp; Val 110 → Gly or Ala; Arg 111 → Pro; Gly 112 → Met or Thr; Lys 114 → Trp or Ala; Lys 121 → Thr; Ser 140 → Gly; and Cys 153 → Ser, and

wherein the mutein has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 7–22.

2. The mutein of claim 1, wherein the mutein is capable of binding LAG-3 with an affinity measured by K_d of about 500 nM or lower.

3. The mutein of claim 1, wherein the mutein is capable of binding LAG-3 with an affinity measured by K_d of about 160 nM or lower.

4. The mutein of claim 1, wherein the mutein is capable of binding LAG-3 with an affinity measured by K_d of about 30 nM or lower.

5. The mutein of claim 1, wherein the mutein is capable of binding LAG-3 with an affinity measured by EC_{50} of about 109 nM or lower.

6. The mutein of claim 1, wherein the mutein is cross-reactive with both human LAG-3 and cynomolgus LAG-3.

7. The mutein of claim 1, wherein the mutein is capable of binding cynomolgus LAG-3 with an affinity measured by K_d of about 160 nM or lower.

8. The mutein of claim 1, wherein the mutein is capable of interfering or competing with the binding of LAG-3 to major histocompatibility complex (MHC) class II.

9. The mutein of claim 1, wherein the amino acid sequence of the mutein comprises one of the following sets of mutated amino acid residues in comparison with the linear polypeptide sequence of mature human tear lipocalin (SEQ ID NO: 1):

(a) Arg 26 → Ser; Glu 27 → Asp; Phe 28 → Cys; Pro 29 → Phe; Glu 30 → Trp; Met 31 → Ile; Asn 32 → Glu; Leu 33 → Glu; Glu 34 → Trp; Leu 56 → Asp; Ser 58 → Phe; Arg 60 → Phe; Cys 61 → Trp; Cys 101 → Ser; Leu 105 → Cys; His 106 → Ala; Lys 108 → Phe; Arg 111 → Pro; Lys 114 → Trp; and Cys 153 → Ser;

(b) Ser 14 → Pro; Asp 25 → Ser; Arg 26 → Gly; Phe 28 → Asp; Asn 32 → Thr; Lys 52 → Asn; Met 55 → Ala; Ser 58 → Asp; Ala 66 → Asn; Ala 79 → Glu; Ala 86 → Asp; Cys 101 → Phe; Leu 105 → Gly; Lys 108 → Thr; Val 110 → Ala; Gly 112 → Thr; Lys 114 → Ala; and Lys 121 → Thr;

(c) Arg 26 → Phe; Glu 27 → Val; Phe 28 → Cys; Pro 29 → Leu; Glu 30 → Tyr; Met 31 → Asp; Asn 32 → Val; Leu 33 → Pro; Leu 56 → Gln; Ser 58 → Trp; Arg 60 → Glu; Cys 61 → Leu; Cys 101 → Ser; Glu 104 → Tyr; Leu 105 → Cys; His 106 → Val; Lys 108 → Tyr; Arg 111 → Pro; Lys 114 → Trp; and Cys 153 → Ser;

(d) Arg 26 → Glu; Glu 27 → Thr; Phe 28 → Cys; Pro 29 → Trp; Glu 30 → Trp; Met 31 → Tyr; Asn 32 → Val; Leu 33 → Asp; Glu 34 → His; Leu 56 → Asn; Ile 57 → Leu; Ser 58 → Trp; Arg 60 → Phe; Cys 61 → Trp; Cys 101 → Ser; Leu 105 → Cys; His 106 → Gln; Lys 108 → Trp; Arg 111 → Pro; Lys 114 → Trp; and Cys 153 → Ser;

- (e) Arg 26 → Ser; Glu 27 → Asp; Phe 28 → Cys; Pro 29 → Phe; Glu 30 → Trp; Met 31 → Ile; Asn 32 → Asp; Leu 33 → Asp; Glu 34 → Val; Leu 56 → Asp; Ser 58 → Phe; Arg 60 → Phe; Cys 61 → Trp; Cys 101 → Ser; Leu 105 → Cys; His 106 → Ala; Lys 108 → Tyr; Arg 111 → Pro; Lys 114 → Trp; and Cys 153 → Ser;
- (f) Arg 26 → Ser; Glu 27 → Asp; Phe 28 → Cys; Pro 29 → Phe; Glu 30 → Trp; Met 31 → Ile; Asn 32 → Asp; Leu 33 → Glu; Glu 34 → Val; Leu 56 → Asp; Ser 58 → Phe; Arg 60 → Phe; Cys 61 → Trp; Cys 101 → Ser; Leu 105 → Cys; His 106 → Ala; Lys 108 → Tyr; Arg 111 → Pro; Lys 114 → Trp; and Cys 153 → Ser;
- (g) Arg 26 → Ser; Glu 27 → Asp; Phe 28 → Cys; Pro 29 → Phe; Glu 30 → Trp; Met 31 → Ile; Asn 32 → Glu; Leu 33 → Glu; Glu 34 → Trp; Val 36 → Ala; Asn 48 → Asp; Leu 56 → Asp; Ser 58 → Phe; Arg 60 → Phe; Cys 61 → Trp; Val 85 → Ala; Cys 101 → Ser; Leu 105 → Cys; His 106 → Ala; Lys 108 → Phe; Arg 111 → Pro; Lys 114 → Trp; Ser 140 → Gly; and Cys 153 → Ser;
- (h) Arg 26 → Ser; Glu 27 → Asp; Phe 28 → Cys; Pro 29 → Phe; Glu 30 → Trp; Met 31 → Ile; Asn 32 → Asp; Leu 33 → Glu; Glu 34 → Val; Leu 56 → Asp; Ser 58 → Phe; Arg 60 → Phe; Cys 61 → Trp; Cys 101 → Ser; Leu 105 → Cys; His 106 → Glu; Lys 108 → Phe; Arg 111 → Pro; Lys 114 → Trp; and Cys 153 → Ser;
- (i) Arg 26 → Ser; Glu 27 → Asp; Phe 28 → Cys; Pro 29 → Phe; Glu 30 → Trp; Met 31 → Ile; Asn 32 → Glu; Leu 33 → Glu; Glu 34 → Trp; Val 36 → Ala; Lys 52 → Glu; Val 53 → Ala; Leu 56 → Asp; Ser 58 → Phe; Arg 60 → Phe; Cys 61 → Trp; Cys 101 → Ser; Leu 105 → Cys; His 106 → Ala; Lys 108 → Phe; Arg 111 → Pro; Lys 114 → Trp; and Cys 153 → Ser;
- (j) Arg 26 → Ser; Glu 27 → Asp; Phe 28 → Cys; Pro 29 → Phe; Glu 30 → Trp; Met 31 → Val; Asn 32 → Asp; Leu 33 → Glu; Glu 34 → Val; Leu 56 → Asp; Ser 58 → Phe; Arg 60 → Phe; Cys 61 → Trp; Cys 101 → Ser; Leu 105 → Cys; His 106 → Ala; Lys 108 → Phe; Arg 111 → Pro; Lys 114 → Trp; and Cys 153 → Ser;
- (k) Ser 14 → Pro; Asp 25 → Ser; Arg 26 → Gly; Phe 28 → Asp; Met 31 → Leu; Asn 32 → Trp; Lys 52 → Ser; Met 55 → Ala; Ser 58 → Asp; Ala 66 → Asn; Ala 79 → Glu;

Ala 86 → Asp; Cys 101 → Phe; Leu 105 → Gly; His 106 → Tyr; Lys 108 → Thr; Val 110 → Gly; Gly 112 → Met; Lys 114 → Ala; and Lys 121 → Thr;

(l) Ser 14 → Pro; Asp 25 → Ser; Arg 26 → Ala; Phe 28 → Asp; Met 31 → Leu; Asn 32 → Val; Lys 52 → Ser; Met 55 → Ala; Ser 58 → Asp; Ala 66 → Asn; Ala 79 → Glu; Ala 86 → Asp; Cys 101 → Phe; Leu 105 → Gly; Lys 108 → Thr; Val 110 → Ala; Gly 112 → Thr; Lys 114 → Ala; and Lys 121 → Thr;

(m) Ser 14 → Pro; Asp 25 → Ser; Arg 26 → Asp; Phe 28 → Asp; Asn 32 → Thr; Lys 52 → Ser; Met 55 → Ala; Ser 58 → Asp; Ala 66 → Asn; Ala 79 → Glu; Ala 86 → Asp; Cys 101 → Phe; Leu 105 → Gly; His 106 → Gln; Lys 108 → Thr; Val 110 → Gly; Gly 112 → Met; Lys 114 → Ala; and Lys 121 → Thr;

(n) Ser 14 → Pro; Asp 25 → Ser; Arg 26 → Glu; Phe 28 → Asp; Asn 32 → Thr; Lys 52 → Ser; Met 55 → Ala; Ser 58 → Asp; Ala 66 → Asn; Ala 79 → Glu; Ala 86 → Asp; Cys 101 → Phe; Leu 105 → Gly; Lys 108 → Thr; Val 110 → Gly; Gly 112 → Met; Lys 114 → Ala; and Lys 121 → Thr;

(o) Ser 14 → Pro; Asp 25 → Ser; Arg 26 → Gly; Phe 28 → Asp; Asn 32 → Met; Lys 52 → Arg; Met 55 → Val; Ser 58 → Asp; Ala 66 → Asn; Ala 79 → Glu; Ala 86 → Asp; Cys 101 → Phe; Leu 105 → Gly; His 106 → Gln; Lys 108 → Thr; Val 110 → Gly; Gly 112 → Met; Lys 114 → Ala; and Lys 121 → Thr; or

(p) Arg 26 → Phe; Glu 27 → Val; Phe 28 → Cys; Pro 29 → Leu; Glu 30 → Asn; Met 31 → Asp; Asn 32 → Tyr; Leu 33 → Pro; Leu 56 → Gln; Ser 58 → Trp; Arg 60 → Glu; Cys 61 → Pro; Cys 101 → Ser; Glu 104 → Tyr; Leu 105 → Cys; His 106 → Thr; Lys 108 → Tyr; Arg 111 → Pro; Lys 114 → Trp; and Cys 153 → Ser.

10. The mutein of claim 1, wherein the mutein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7–22 or a variant thereof.

11. The mutein according of claim 1, wherein the mutein has at least 85% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 7–22.

12. The mutein of claim 1, wherein the mutein is conjugated to a compound selected from the group consisting of an organic molecule, an enzyme label, a radioactive label, a colored label, a fluorescent label, a chromogenic label, a luminescent label, a hapten, digoxigenin, biotin, a cytostatic agent, a toxin, a metal complex, a metal, and colloidal gold.
13. The mutein of claim 1, wherein the mutein is fused at its N-terminus and/or its C-terminus, with or without an intervening linker, to a fusion partner selected from the group consisting of an antibody, an antibody fragment, a protein, a protein domain, a peptide, or a lipocalin mutein.
14. The mutein of claim 1, wherein the mutein is conjugated to a compound that extends the serum half-life of the mutein.
15. The mutein of claim 1, wherein the mutein is capable of binding LAG-3 with an affinity measured by K_d of about 1 nM or lower.
16. A diagnostic or analytical kit comprising a mutein of claim 1.
17. An immunoconjugate or fusion protein comprising the mutein of claim 1 linked to a therapeutic agent.
18. A pharmaceutical composition comprising a mutein of claim 1 and a pharmaceutically acceptable excipient.
19. A method of detecting the presence of LAG-3, comprising the steps of
 - (a) contacting a sample suspected of containing LAG-3 with a mutein of claim 1 under conditions that allow the formation of a complex between the mutein and LAG-3, and
 - (b) detecting the complex of the mutein and LAG-3 for the presence of LAG-3.