

CLAIMS

1. A polypeptide having binding specificity for pyoverdine type I, II, or III, or pyochelin, wherein the polypeptide comprises a human neutrophil gelatinase-associated lipocalin (hNGAL) mutein that is capable of binding pyoverdine type I, II, or III, or pyochelin, wherein:
 - (i) the hNGAL mutein binding to pyoverdine type I comprises ten or more amino acid substitutions at positions 28, 36, 39-41, 46, 49, 52, 54, 55, 59, 65, 68, 70, 72-75, 77, 79-81, 87, 96, 100, 103, 106, 125, 127, 132, 134, and 136 of SEQ ID NO: 1;
 - (ii) the hNGAL mutein binding to pyoverdine type II comprises ten or more amino acid substitutions at positions 28, 36, 40, 41, 49, 52, 54, 65, 68, 70, 72-75, 77, 79, 81, 87, 96, 100, 103, 106, 125, 127, 132, and 134 of SEQ ID NO: 1;
 - (iii) the hNGAL mutein binding to pyoverdine type III comprises ten or more amino acid substitutions at positions 28, 36, 40-42, 45-47, 49, 52, 65, 68, 70, 72, 73, 77, 79, 81, 87, 96, 100, 103, 105, 106, 125, 127, 132, 134, and 145 of SEQ ID NO: 1; or
 - (iv) the hNGAL mutein binding to pyochelin comprises ten or more amino acid substitutions at positions 28, 34, 36, 40, 41, 44-46, 49, 52, 54, 65, 68, 70, 72-74, 77, 79-81, 87, 96, 100, 103, 106, 108, 123, 125, 127, 132, 134, and 141 of SEQ ID NO: 1.
2. The polypeptide of claim 1, wherein the hNGAL mutein binding to pyoverdine type I comprises ten or more of the following amino acid substitutions in SEQ ID NO: 1: L36W, A40T, I41A, Q49P, Y52P, T54V, N65D, S68D, L70Q, R72P, K73E, K75E, D77I, W79S, I80T, R81I, C87S, N96G, Y100S, L103Q, Y106H, K125Y, S127T, Y132G, K134N, and T136A.
3. The polypeptide of claim 1 or 2, wherein the hNGAL mutein binding to pyoverdine type I comprises the amino acid sequence of SEQ ID NO: 16.
4. The polypeptide of claim 1, wherein the hNGAL mutein binding to pyoverdine type II comprises ten or more of the following amino acid substitutions in SEQ

ID NO: 1 L36V, A40T, Q49G, Y52N, T54A, N65D, S68D, L70R, R72I, K73R, D77H, W79Y, R81D, C87S, L103T, Y106Q, K125I, S127R, Y132I, and K134W.

5. The polypeptide of claim 1 or 4, wherein the hNGAL mutein binding to pyoverdine type II comprises the amino acid sequence of SEQ ID NO: 36.
6. The polypeptide of claim 1, wherein the hNGAL mutein binding to pyoverdine type III comprises ten or more of the following amino acid substitutions in SEQ ID NO: 1: L36E, A40L, I41A, L42R, Q49M, Y52M, N65D, S68E, L70R, K73H, D77K, W79D, R81V, C87S, N96L, Y100D, L103Q, S105P, Y106E, S127V, and K134W.
7. The polypeptide of claim 1 or 6, wherein the hNGAL mutein binding to pyoverdine type III comprises the amino acid sequence of SEQ ID NO: 53.
8. The polypeptide of claim 1, wherein the hNGAL mutein binding to pyochelin comprises ten or more of the following amino acid substitutions in SEQ ID NO: 1: L36H, A40Q, I41W, D45G, K46R, Q49R, Y52W, N65D, S68D, L70D, R72A, K73I, D77L, W79R, R81T, C87S, Y100H, L103G, Y106G, K125F, S127I, Y132A, and K134F.
9. The polypeptide of claim 1 or 8, wherein the hNGAL mutein binding to pyochelin comprises the amino acid sequence of SEQ ID NO: 62.
10. The polypeptide of claim 1, wherein
 - (i) the hNGAL mutein binding to pyoverdine type I has at least 80% identity to a sequence selected from the group consisting of SEQ ID NOs: 2-18;
 - (ii) the hNGAL mutein binding to pyoverdine type II has at least 80% identity to a sequence selected from the group consisting of SEQ ID NOs: 19-37;
 - (iii) the hNGAL mutein binding to pyoverdine type III has at least 80% identity to a sequence selected from the group consisting of SEQ ID NOs: 38-53;
 - or
 - (iv) the hNGAL mutein binding to pyochelin has at least 80% identity to a sequence selected from the group consisting of SEQ ID NOs: 54-63.

11. The polypeptide of claim 1, wherein
 - (i) the hNGAL mutein binding to pyoverdine type I has at least 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 2-18;
 - (ii) the hNGAL mutein binding to pyoverdine type II has at least 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 19-37;
 - (iii) the hNGAL mutein binding to pyoverdine type III has at least 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 38-53;
or
 - (iv) the hNGAL mutein binding to pyochelin has at least 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 54-63.
12. The polypeptide of claim 1, wherein
 - (i) the hNGAL mutein binding to pyoverdine type I has at least 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 2-18;
 - (ii) the hNGAL mutein binding to pyoverdine type II has at least 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 19-37;
 - (iii) the hNGAL mutein binding to pyoverdine type III has at least 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 38-53;
or
 - (iv) the hNGAL mutein binding to pyochelin has at least 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 54-63.
13. The polypeptide of claim 1, wherein
 - (i) the hNGAL mutein binding to pyoverdine type I has at least 95% identity to a sequence selected from the group consisting of SEQ ID NOs: 2-18;
 - (ii) the hNGAL mutein binding to pyoverdine type II has at least 95% identity to a sequence selected from the group consisting of SEQ ID NOs: 19-37;
 - (iii) the hNGAL mutein binding to pyoverdine type III has at least 95% identity to a sequence selected from the group consisting of SEQ ID NOs: 38-53;
or
 - (iv) the hNGAL mutein binding to pyochelin has at least 95% identity to a sequence selected from the group consisting of SEQ ID NOs: 54-63.
14. The polypeptide of claim 1, wherein

- (i) the hNGAL mutein binding to pyoverdine type I is selected from the group consisting of SEQ ID NOs: 2-18;
 - (ii) the hNGAL mutein binding to pyoverdine type II is selected from the group consisting of SEQ ID NOs: 19-37;
 - (iii) the hNGAL mutein binding to pyoverdine type III is selected from the group consisting of SEQ ID NOs: 38-53; or
 - (iv) the hNGAL mutein binding to pyochelin is selected from the group consisting of SEQ ID NOs: 54-63.
15. The polypeptide of claim 1, wherein the hNGAL mutein is capable of binding pyoverdine type I, II, or III, or pyochelin, when complexed with iron, with a K_D of 200 nM or lower when measured by surface plasmon resonance.
16. A nucleic acid molecule comprising a nucleotide sequence encoding the polypeptide of any one of claims 1 to 15.
17. A host cell containing a nucleic acid molecule of claim 16.
18. A method of producing the polypeptide of any one of claims 1 to 15, wherein the polypeptide is produced starting from the nucleic acid coding for the polypeptide by means of genetic engineering methods.
19. A composition comprising one or more polypeptides selected from the group consisting of (i) a polypeptide of any one of claims 1 to 3 and 10 to 15 specific for pyoverdine type I, (ii) a polypeptide of any one of claims 1, 4, 5, and 10 to 15 specific for pyoverdine type II, (iii) a polypeptide of any one of claims 1, 6, 7, and 10 to 15 specific for pyoverdine type III, and (iv) a polypeptide of any one of claims 1 and 8 to 15 specific for pyochelin.
20. A kit comprising, in one or more containers, separately or in a mixture, a polypeptide of any one of claims 1 to 3 and 10 to 15 specific for pyoverdine type I or composition thereof, a polypeptide of any one of claims 1, 4, 5, and 10 to 15 specific for pyoverdine type II or composition thereof, a polypeptide of any one of claims 1, 6, 7, and 10 to 15 specific for pyoverdine type III or composition

thereof, and/or a polypeptide of any one of claims 1 and 8 to 15 specific for pyochelin or composition thereof.

21. One or more polypeptides selected from the group consisting of (i) a polypeptide of claim 3 binding to pyoverdine type I, (ii) a polypeptide of claim 5 binding to pyoverdine type II, (iii) a polypeptide of claim 7 binding to pyoverdine type III, and (iv) a polypeptide of a claim 9 binding to pyochelin, for use in the treatment or alleviation of *P. aeruginosa* biofilm infection in a subject.
22. Use of one or more polypeptides selected from the group consisting of (i) a polypeptide of claim 3 binding to pyoverdine type I, (ii) a polypeptide of claim 5 binding to pyoverdine type II, (iii) a polypeptide of claim 7 binding to pyoverdine type III, and (iv) a polypeptide of a claim 9 binding to pyochelin, for the manufacture of a medicament for the treatment or alleviation of *P. aeruginosa* biofilm infection in a subject.
23. A combination of two or more polypeptides of any one of claims 1 to 15.