

CLAIMS

What is claimed:

1. A transthyretin (TTR) protein complex, wherein said TTR protein complex comprises TTR subunits A, B, C, and D;
wherein TTR subunits A and B dimerize to form TTR dimer AB;
wherein TTR subunits C and D dimerize to form TTR dimer CD;
wherein TTR dimers AB and CD further dimerize to form TTR tetramer ABCD; and
wherein each of A, B, C, and D comprise the amino acid sequence of SEQ ID NO: 1, except that at least one amino acid in the interface between TTR dimer AB and TTR dimer CD is mutated such that the formation of an ABCD tetramer is favored over the formation of an ABAB tetramer or a CDCD tetramer.
2. The TTR protein complex according to claim 1, wherein each of A, B, C, and D comprise the amino acid sequence of SEQ ID NO: 1 with the following mutations: C10A, K15A, or both a C10A and a K15A mutation.
3. The TTR protein complex according to claim 1 or 2, wherein both A and B, both C and D, or all four of A, B, C, and D comprise a mutation at one or more amino acids positions selected from the list comprising: 6, 7, 8, 9, 10, 13, 15, 17, 19, 20, 21, 22, 23, 24, 26, 50, 51, 52, 53, 54, 56, 57, 60, 61, 62, 63, 78, 82, 83, 84, 85, 100, 101, 102, 103, 104, 106, 108, 110, 112, 113, 114, 115, 117, 119, 121, 123, 124, 125, 126, and 127 of SEQ ID NO: 1.
4. The TTR protein complex according to claim 3, wherein both A and B, both C and D, or all four of A, B, C, and D comprise a mutation at one or more amino acids positions selected from the list comprising: 15, 17, 20, 21, 22, 23, 24, 51, 52, 84, 106, 108, 112, 114, 115, 119, 121, and 123 of SEQ ID NO: 1.
5. The TTR protein complex according to any one of claims 1-4, wherein both A and B, both C and D, or all four of A, B, C, and D comprise a mutation at one or more amino acids positions selected from the list comprising: 15, 17, 20, 21, 22, 23, 24, 51, 52, 84, 106, 108, 112, 114, 115, 119, 121, and 123 of SEQ ID NO: 1,
wherein said amino acid is mutated to an aspartate or glutamate.
6. The TTR protein complex according to any one of claims 1-4, wherein both A and B, both C and D, or all four of A, B, C, and D comprise a mutation at one or more amino acids positions selected from the list comprising: 15, 17, 20, 21, 22, 23, 24, 51, 52, 84, 106, 108, 112, 114, 115, 119, 121, and 123 of SEQ ID NO: 1,

wherein said amino acid is mutated to an arginine, lysine, or histidine.

7. The TTR protein complex according to any one of claims 1-6, wherein A and B comprise a mutation at one or more amino acids positions selected from the list comprising: 15, 17, 20, 21, 22, 23, 24, 51, 52, 84, 106, 108, 112, 114, 115, 119, 121, and 123 of SEQ ID NO: 1, wherein said amino acid is mutated to an aspartate or glutamate.

8. The TTR protein complex according to any one of claims 1-7, wherein C and D comprise a mutation at one or more amino acids positions selected from the list comprising: 15, 17, 20, 21, 22, 23, 24, 51, 52, 84, 106, 108, 112, 114, 115, 119, 121, and 123 of SEQ ID NO: 1, wherein said amino acid is mutated to an arginine, lysine, or histidine.

9. The TTR protein complex according to any one of claims 1-4 wherein:

A and B comprise a mutation at one or more amino acids positions selected from the list comprising: 15, 17, 20, 21, 22, 23, 24, 51, 52, 84, 106, 108, 112, 114, 115, 119, 121, and 123 of SEQ ID NO: 1, wherein said amino acid is mutated to an aspartate or glutamate; and

C and D comprises a mutation at one or more amino acids positions selected from the list comprising: 15, 17, 20, 21, 22, 23, 24, 51, 52, 84, 106, 108, 112, 114, 115, 119, 121, and 123 of SEQ ID NO: 1, wherein said amino acid is mutated to an arginine, lysine, or histidine.

10. The TTR protein complex according to any one of claims 1-7 and 9, wherein A and B comprise at least one mutation in SEQ ID NO: 1, wherein said mutation is selected from the list comprising: K15D, L17D, V20D, R21D, G22D, S23D, P24D, S52D, I84D, T106D, A108D, S112D, Y114D, S115D, T119D, V121D, S123D, K15E, L17E, V20E, R21E, G22E, S23E, P24E, D51E, S52E, I84E, T106E, A108E, S112E, Y114E, S115E, T119E, V121E, and S123E.

11. The TTR protein complex according to claim 10, wherein A and B comprise at least one mutation in SEQ ID NO: 1, wherein said mutation is selected from the list comprising: L17D, L17E, V20D, V20E, G22D, G22E, S112D, S112E, T119D, T119E, V121D, and V121E.

12. The TTR protein complex according to any one of claims 1-6, 8, and 9, wherein C and D comprise at least one mutation in SEQ ID NO: 1, wherein said mutation is selected from the list comprising: K15R, L17R, V20R, G22R, S23R, P24R, D51R, S52R, I84R, T106R, A108R, S112R, Y114R, S115R, T119R, V121R, S123R, L17K, V20K, R21K, G22K, S23K, P24K, D51K, S52K, I84K, T106K, A108K, S112K, Y114K, S115K, T119K, V121K, S123K, K15H, L17H, V20H, R21H, G22H, S23H, P24H, D51H, S52H, I84H, T106H, A108H, S112H, Y114H, S115H, T119H, V121H, and S123H.

13. The TTR protein complex according to claim 12, wherein C and D comprise at least one mutation in SEQ ID NO: 1, wherein said mutation is selected from the list comprising: L17R, L17K, L17H, V20R, V20K, V20H, G22R, G22K, G22H, S112R, S112K, S112H, T119R, T119K, T119H, V121R, V121K, and V121H.

14. The TTR protein complex according to any one of claims 1-13, wherein both A and B, both C and D, or all four of A, B, C, and D independently comprise one said mutation.

15. The TTR protein complex according to any one of claims 1-13, wherein both A and B, both C and D, or all four of A, B, C, and D independently comprise two said mutations.

16. The TTR protein complex according to any one of claims 1-15, wherein each of A, B, C, and D comprise the amino acid sequence of SEQ ID NO: 1 with the following mutations:

both A and B comprise C10A/K15A/L17D, and both C and D comprise C10A/K15A/L17R;
both A and B comprise C10A/K15A/L17E, and both C and D comprise C10A/K15A/L17R;
both A and B comprise C10A/K15A/V20D, and both C and D comprise C10A/K15A/L17R;
both A and B comprise C10A/K15A/V20E, and both C and D comprise C10A/K15A/L17R;
both A and B comprise C10A/K15A/G22D, and both C and D comprise C10A/K15A/L17R;
both A and B comprise C10A/K15A/G22E, and both C and D comprise C10A/K15A/L17R;
both A and B comprise C10A/K15A/S112D, and both C and D comprise C10A/K15A/L17R;
both A and B comprise C10A/K15A/S112E, and both C and D comprise C10A/K15A/L17R;
both A and B comprise C10A/K15A/T119D, and both C and D comprise C10A/K15A/L17R;
both A and B comprise C10A/K15A/T119E, and both C and D comprise C10A/K15A/L17R;
both A and B comprise C10A/K15A/V121D, and both C and D comprise C10A/K15A/L17R;
both A and B comprise C10A/K15A/V121E, and both C and D comprise C10A/K15A/L17R;
both A and B comprise C10A/K15A/L17D, and both C and D comprise C10A/K15A/L17K;
both A and B comprise C10A/K15A/L17E, and both C and D comprise C10A/K15A/L17K;
both A and B comprise C10A/K15A/V20D, and both C and D comprise C10A/K15A/L17K;
both A and B comprise C10A/K15A/V20E, and both C and D comprise C10A/K15A/L17K;
both A and B comprise C10A/K15A/G22D, and both C and D comprise C10A/K15A/L17K;
both A and B comprise C10A/K15A/G22E, and both C and D comprise C10A/K15A/L17K;
both A and B comprise C10A/K15A/S112D, and both C and D comprise C10A/K15A/L17K;
both A and B comprise C10A/K15A/S112E, and both C and D comprise C10A/K15A/L17K;
both A and B comprise C10A/K15A/T119D, and both C and D comprise C10A/K15A/L17K;
both A and B comprise C10A/K15A/T119E, and both C and D comprise C10A/K15A/L17K;
both A and B comprise C10A/K15A/V121D, and both C and D comprise C10A/K15A/L17K;
both A and B comprise C10A/K15A/V121E, and both C and D comprise C10A/K15A/L17K;
both A and B comprise C10A/K15A/L17D, and both C and D comprise C10A/K15A/V20R;

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[illegible]

both A and B comprise C10A/K15A/G22D, and both C and D comprise C10A/K15A/V121K;
both A and B comprise C10A/K15A/G22E, and both C and D comprise C10A/K15A/V121K;
both A and B comprise C10A/K15A/S112D, and both C and D comprise C10A/K15A/V121K;
both A and B comprise C10A/K15A/S112E, and both C and D comprise C10A/K15A/V121K;
both A and B comprise C10A/K15A/T119D, and both C and D comprise C10A/K15A/V121K;
both A and B comprise C10A/K15A/T119E, and both C and D comprise C10A/K15A/V121K;
both A and B comprise C10A/K15A/V121D, and both C and D comprise C10A/K15A/V121K; or
both A and B comprise C10A/K15A/V121E, and both C and D comprise C10A/K15A/V121K.

17. The TTR protein complex according to any one of claims 1-15, wherein:

both A and B comprise C10A/K15A/L17D, and both C and D comprise C10A/K15A/V121R;
both A and B comprise C10A/K15A/L17D, and both C and D comprise C10A/K15A/V121K;
both A and B comprise C10A/K15A/L17E, and both C and D comprise C10A/K15A/V121R;
both A and B comprise C10A/K15A/V20D, and both C and D comprise C10A/K15A/V20R;
both A and B comprise C10A/K15A/V20D, and both C and D comprise C10A/K15A/V20K;
both A and B comprise C10A/K15A/V20E, and both C and D comprise C10A/K15A/V20R;
both A and B comprise C10A/K15A/V20E, and both C and D comprise C10A/K15A/V20K;
both A and B comprise C10A/K15A/T119D, and both C and D comprise C10A/K15A/L17R;
both A and B comprise C10A/K15A/T119D, and both C and D comprise C10A/K15A/L17K; or
both A and B comprise C10A/K15A/V121E, and both C and D comprise C10A/K15A/L17K.

18. The TTR protein complex according to any one of claims 1-15, wherein each of A, B, C, and D comprise the amino acid sequence of SEQ ID NO: 1 with the following mutations:

both A and B comprise C10A/K15A/L17D/V20D, and both C and D comprise C10A/K15A/L17K/V20K;
both A and B comprise C10A/K15A/L17D/V20E, and both C and D comprise C10A/K15A/L17K/V20R;
both A and B comprise C10A/K15A/L17E/V20D, and both C and D comprise C10A/K15A/L17R/V20K;
both A and B comprise C10A/K15A/L17E/V20E, and both C and D comprise C10A/K15A/L17R/V20R;
both A and B comprise C10A/K15A/L17D/T119D, and both C and D comprise C10A/K15A/L17K/V121K;
both A and B comprise C10A/K15A/L17D/V121E, and both C and D comprise C10A/K15A/L17K/V121R;
both A and B comprise C10A/K15A/L17E/T119D, and both C and D comprise C10A/K15A/L17R/V121K;

both A and B comprise C10A/K15A/L17E/V121E, and both C and D comprise C10A/K15A/L17R/V121R;

both A and B comprise C10A/K15A/V20D/T119D, and both C and D comprise C10A/K15A/V20K/V121K;

both A and B comprise C10A/K15A/V20D/V121E, and both C and D comprise C10A/K15A/V20K/V121R;

both A and B comprise C10A/K15A/V20E/T119D, and both C and D comprise C10A/K15A/V20R/V121K; or

both A and B comprise C10A/K15A/V20E/V121E, and both C and D comprise C10A/K15A/V20R/V121R.

19. The TTR protein complex according to any one of claims 1-15, wherein each of A, B, C, and D comprise the amino acid sequence of SEQ ID NO: 1 with the following mutations:

both A and B comprise C10A/K15A/V20E/T119D, and both C and D comprise C10A/K15A/V20R/V121K;

both A and B comprise C10A/K15A/L17D/T119D, and both C and D comprise C10A/K15A/L17K/V121K;

both A and B comprise C10A/K15A/L17E/T119D, and both C and D comprise C10A/K15A/L17R/V121K;

both A and B comprise C10A/K15A/L17E/V20D, and both C and D comprise C10A/K15A/L17R/V20K;

both A and B comprise C10A/K15A/L17D/V20D, and both C and D comprise C10A/K15A/L17K/V20K; or

both A and B comprise C10A/K15A/L17E/V121E, and both C and D comprise C10A/K15A/L17R/V121R.

20. The TTR protein complex according to any one of claims 1-19, wherein said TTR protein complex is attached to 1, 2, 3, 4, 5, 6, 7, or 8 antigen binding proteins or peptides.

21. The TTR protein complex according to claim 20, wherein said TTR protein complex is attached to 1, 2, 3, or 4 antigen binding proteins or peptides.

22. The TTR protein complex according to claim 20 or 21, wherein said antigen binding protein or peptide is attached to the TTR protein complex at the C-terminus of a TTR subunit.

23. The TTR protein complex according to claim 20 or 21, wherein said antigen binding protein or peptide is attached to the TTR protein complex at the N-terminus of a TTR subunit.

24. The TTR protein complex according to any one of claims 20-23, wherein said TTR protein complex is:

directly attached to 1, 2, 3, 4, 5, 6, 7, or 8 antigen binding proteins; or
attached to 1, 2, 3, 4, 5, 6, 7, or 8 antigen binding proteins via a linker.

25. The TTR protein complex according to any one of claims 20-23, wherein said TTR protein complex is:

directly attached to 1, 2, 3, or 4 antigen binding proteins; or
attached to 1, 2, 3, or 4 antigen binding proteins via a linker.

26. The TTR protein complex according to claim 25, wherein said linker is an amino acid-based linker comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 amino acids.

27. The TTR protein complex according to claim 26, wherein said linker is an amino acid-based linker comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 amino acids.

28. The TTR protein complex according to claim 27, wherein said linker is an amino acid-based linker comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids.

29. The TTR protein complex according to claim 28, wherein said linker is an amino acid-based linker comprising 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids.

30. The TTR protein complex according to claim 26, wherein said linker is G, GG, GGG, GGGG, GGGGG, GGGGGG, GGGGGGG, GGGGGGGG, GGGGGGGGG, or GGGGGGGGGG.

31. The TTR protein complex according to claim 26, wherein said linker is $G(G_xB_y)_rG_z$ and wherein:

G = glycine;
B = any amino acid;
x = 1-15;
y = 1-5;
z = 1-15; and
r = 1-20.

32. The TTR protein complex according to claim 30, wherein:

B = Q, S, A, E, P, T, K, R, D or N;
x = 4;
y = 1;
z = 4; and

$r=1$.

33. The TTR protein complex according to claim 26, wherein said linker is selected from the list comprising: GG, GGGG, GGGSGG, GGGSGGGG, and GGAGGGAGGG.

34. The TTR protein complex according to any one of claims 20-33, wherein said TTR protein complex is attached to two antigen binding proteins, wherein said antigen binding proteins bind different antigens.

35. The TTR protein complex according to any one of claims 20-33, wherein said TTR protein complex is attached to four antigen binding proteins, wherein said antigen binding proteins bind to at least two different antigens.

36. The TTR protein complex according to any one of claims 20-35, wherein the antigen binding proteins are antibodies.

37. The TTR protein complex according to any one of claims 20-35, wherein the antigen binding proteins are Fabs or scFvs.

38. The TTR protein complex according to claim 37, wherein the antigen binding proteins are Fabs.

39. The TTR protein complex according to any one of claims 20-35, wherein the antigen binding proteins are a mixture of antibodies and Fabs.

40. A pharmaceutical composition comprising a TTR protein complex according to any one of claims 1-39.

41. A method of treating cancer using a TTR protein complex according to any one of claims 1-39.

42. Use of a TTR protein complex according to any one of claims 1-39 in the treatment of cancer.

43. A TTR protein complex according to any one of claims 1-39 for use in the treatment of cancer.

44. One or more isolated nucleic acid(s) encoding a TTR subunit, a TTR subunit fused or linked to an antigen binding protein or peptide (such as an antibody or Fab), or a TTR protein complex according to any one of claims 1-39.

45. An expression vector comprising a nucleic acid of claim 44.

46. A recombinant host cell comprising a nucleic acid of claim 44 or a vector of claim 45.

47. The recombinant host cell of claim 45, wherein said host cell is a Chinese hamster ovary (CHO) cell, E5 cell, baby hamster kidney (BHK) cell, monkey kidney (COS) cell, human hepatocellular carcinoma cell, or human embryonic kidney 293 (HEK 293) cell.

48. A method of making a TTR protein complex according to any one of claims 1-39, said method comprising:

- a) culturing the recombinant host cell of claim 46 or 47; and
- b) isolating the TTR protein complex from said culture.