

Claims

CLAIMS

We claim:

1. ~~An~~ A conjugate or fusion construct comprising an anti-TfR antibody or antigen-binding fragment thereof for delivering an agent ~~respectively coupled to the brain or~~ covalently linked to a subject in need second antibody or an antigen binding fragment thereof that binds a brain target, wherein the anti-TfR antibody or antigen-binding fragment thereof binds to a transferrin receptor (TfR), preferably human TfR1, with a dissociation constant K_D of at least 1 nM, preferably 1-500 nM, at neutral pH and an off-rate constant k_d of at least 10^{-4} sec^{-1} , preferably 10^{-4} to 10^{-1} sec^{-1} , at an acidic pH, preferably pH 5-
2. ~~The~~, and the anti-TfR antibody or antigen-binding fragment thereof ~~of claim 1, having~~ has an off-rate constant k_d of 2×10^{-2} to $2 \times 10^{-4} \text{ sec}^{-1}$, preferably $2.0 \times 10^{-3} \text{ sec}^{-1}$ at the neutral pH, wherein the second antibody or an antigen binding fragment thereof binds a brain target selected from Tau and a cancer of the central nervous system (CNS) or brain, more particularly, the brain target is selected from the group consisting of epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), Tau, CD20, leucine rich repeat kinase 2 (LRRK2), parkin, death receptor 6 (DR6), p75 neurotrophin receptor (p75NTR), and caspase 6.
3. ~~The~~ 2. The conjugate or fusion construct of claim 1, wherein the anti-TfR antibody or antigen-binding fragment thereof ~~of claim 1 or 2, comprising~~ comprises:
 - (1) a heavy chain variable region comprising heavy chain complementarity determining regions (HCDRs) HCDR1, HCDR2 and HCDR3, and a light chain variable region comprising light chain complementarity determining regions (LCDRs) LCDR1, LCDR2 and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 have the amino acid sequences of:
 - (i) SEQ ID NOs: 292, 293, 294, 295, 296, and 297, respectively;
 - (ii) SEQ ID NOs: 279, 280, 281, 282, 283 and 284, respectively;
 - (iii) SEQ ID NOs: 29, 30, 31, 32, 33 and 34, respectively;
 - (iv) SEQ ID NOs: 57, 58, 59, 60, 61 and 62, respectively;

- (v) SEQ ID NOs: 85, 86, 87, 88, 89 and 90, respectively;
 - (vi) SEQ ID NOs: 110, 111, 112, 113, 114 and 115, respectively;
 - (vii) SEQ ID NOs: 135, 136, 137, 138, 139 and 140, respectively;
 - (viii) SEQ ID NOs: 191, 192, 193, 194, 195 and 196, respectively;
 - (ix) SEQ ID NOs: 244, 245, 246, 247, 248 and 249, respectively;
 - (x) SEQ ID NOs: 263, 264, 265, 266, 267 and 268, respectively;
 - (xi) SEQ ID NOs: 345, 346, 347, 348, 349 and 350, respectively;
 - (xii) SEQ ID NOs: 355, 356, 357, 358, 359 and 360, respectively;
 - (xiii) SEQ ID NOs: 365, 366, 367, 368, 369 and 370, respectively;
 - (xiv) SEQ ID NOs: 375, 376, 377, 378, 379 and 380, respectively;
 - (xv) SEQ ID NOs: 385, 386, 387, 388, 389 and 390, respectively;
 - (xvi) SEQ ID NOs: 395, 396, 377, 398, 399 and 400, respectively;
 - (xvii) SEQ ID NOs: 405, 406, 407, 408, 409 and 410, respectively;
 - (xviii) SEQ ID NOs: 415, 416, 417, 418, 419 and 420, respectively;
 - (xix) SEQ ID NOs: 425, 426, 427, 428, 429 and 430, respectively;
 - (xx) SEQ ID NOs: 435, 436, 437, 438, 439 and 440, respectively;
 - (xxi) SEQ ID NOs: 445, 446, 447, 448, 449 and 450, respectively;
 - (xxii) SEQ ID NOs: 455, 456, 457, 458, 459 and 460, respectively;
 - (xxiii) SEQ ID NOs: 465, 466, 467, 468, 469 and 470, respectively;
 - (xxiv) SEQ ID NOs: 475, 476, 477, 478, 479 and 480, respectively;
 - (xxv) SEQ ID NOs: 485, 486, 487, 488, 489 and 490, respectively;
 - (xxvi) SEQ ID NOs: 495, 496, 497, 498, 499 and 500, respectively;
 - (xxvii) SEQ ID NOs: 505, 506, 507, 508, 509 and 510, respectively;
 - (xxviii) SEQ ID NOs: 515, 516, 517, 518, 519 and 520, respectively;
 - (xxix) SEQ ID NOs: 525, 526, 527, 528, 529 and 530, respectively;
 - (xxx) SEQ ID NOs: 535, 536, 537, 538, 539 and 540, respectively; or
 - (xxxi) SEQ ID NOs: 545, 546, 547, 548, 549 and 550, respectively; or
- (2) a single variable domain on a heavy chain (VHH) comprising heavy chain complementarity determining regions (HCDRs) HCDR1, HCDR2 and HCDR3 having the amino acid sequences of:
- (i) SEQ ID NOs: 7, 8 and 9, respectively;

- (ii) SEQ ID NOs: 317, 318 and 319, respectively;
- (iii) SEQ ID NOs: 324, 325 and 326, respectively;
- (iv) SEQ ID NOs: 331, 332 and 333, respectively; or
- (v) SEQ ID NOs: 338, 339 and 340, respectively.

~~4. The~~3. The conjugate or fusion construct of claim 2, wherein the anti-TfR antibody or antigen-binding fragment thereof ~~of any one of claims 1-3, being~~is a VHH fragment comprising an amino acid sequence having at least 80%, such as at least 85%, 90%, 95% or 100%, sequence identity to SEQ ID NO: 6, 316, 323, 330, or 337.

~~5. The~~4. The conjugate or fusion construct of claim 2, wherein the anti-TfR antibody or antigen-binding fragment thereof ~~of any one of claims 1-3, being~~is a single-chain variable fragment (scFv) comprising the heavy chain variable region covalently linked to the light chain variable region via a linker, preferably, the linker has the amino acid sequence of SEQ ID NO: 314, more preferably, the scFv comprises an amino acid sequence having at least 80%, such as at least 85%, 90%, 95% or 100%, sequence identity to the amino acid sequences of SEQ ID NO: 278, 291, 28, 56, 84, 109, 134, 162, 190, 218, 243, 262, 344, 354, 364, 374, 384, 394, 404, 414, 424, 434, 444, 454, 464, 474, 484, 494, 504, 514, 524, 534 or 544.

~~6. A conjugate comprising the anti-TfR antibody or antigen-binding fragment thereof of any one of claims 1-5 coupled to a therapeutic or diagnostic agent, preferably, the conjugate is a multi-specific antibody comprising a first antigen binding region which binds the TfR and comprises the antigen-binding fragment of any one of claims 1 to 5, and a second antigen binding region which binds a brain target, such as a brain target selected from the group consisting of beta-secretase 1 (BACE1), amyloid beta (Abeta), epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), Tau, apolipoprotein E4 (ApoE4), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma-secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), and caspase 6.~~

~~7. A fusion construct comprising the anti-TfR antibody or antigen-binding fragment thereof of any one of claims 1 to 5 covalently linked to a second antibody or an antigen-binding fragment~~

thereof that binds to a brain target, such as a brain target selected from the group consisting of beta-secretase 1 (BACE1), amyloid beta (Abeta), epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), Tau, apolipoprotein E4 (ApoE4), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma-secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), and caspase 6.

~~8. The fusion construct of claim 75.~~ The fusion construct of any one of claims 1-4, wherein the anti-TfR antibody or antigen-binding fragment thereof is covalently linked to the carboxy terminus of only one of the two heavy chains of the second antibody or antigen binding fragment thereof via a linker, preferably the linker has the amino acid sequence of SEQ ID NO: 312 or SEQ ID NO: 313.

~~96.~~ The fusion construct of claim ~~85~~, wherein each of the two heavy chains of the second antibody or antigen binding fragment thereof comprises one or more heterodimeric mutations, such as a modified heterodimeric CH3 domain, or one or more knob and hole mutations, as compared to a wild-type CH3 domain polypeptide; and/or the second antibody or antigen binding fragment thereof comprises one or more mutations in the Fc domain that enhance binding of the fusion to the neonatal Fc receptor (RcRn), preferably the one or more mutations enhance the binding at an acidic pH; and/or the second antibody or antigen binding fragment thereof comprises one or more mutations in the Fc domain that reduce or eliminate the effector function.

~~10. The fusion construct of claim 9, wherein~~preferably, the heterodimeric mutations comprise the modified heterodimeric CH3 domain of the first heavy chain comprises amino acid modifications at positions T350, L351, F405, and Y407, and the modified heterodimeric CH3 domain of the second heavy chain comprises amino acid modifications at positions T350, T366, K392 and T394, wherein the amino acid modification at position T350 is T350V, T350I, T350L or T350M; the amino acid modification at position L351 is L351Y; the amino acid modification at position F405 is F405A, F405V, F405T or F405S; the amino acid modification at position Y407 is Y407V, Y407A or Y407I; the amino acid modification at position T366 is T366L, T366I,

T366V or T366M, the amino acid modification at position K392 is K392F, K392L or K392M, and the amino acid modification at position T394 is T394W, ~~and wherein the numbering of amino acid residues is according to the EU index as set forth in Kabat.; more preferably,~~

~~11. The fusion construct of claim 10, wherein the modified heterodimeric CH3 domain of the first heavy chain comprises mutations T350V, L351Y, F405A and Y407V, and the modified heterodimeric CH3 domain of the second heavy chain comprises mutations T350V, T366L, K392L and T394W-;~~ and/or

~~12. The fusion of any one of claims 7-11, wherein the second antibody or antigen binding fragment thereof comprises one or more mutations in the Fc domain that enhance binding of the fusion to the neonatal Fc receptor (FcRn), preferably the one or more mutations enhance the binding at an acidic pH, more preferably the Fc has the M252Y/S254T/T256E (YTE) mutations, wherein the numbering of amino acid residues is according to the EU index as set forth in Kabat.~~

~~13. The fusion of any one of claims 7-12, wherein the second antibody or antigen binding fragment thereof comprises one or more mutations in the Fc domain that reduce or eliminate the effector function, preferably the Fc has the M252Y/S254T/T256E (YTE) mutations; and/or the Fc has one or more amino acid modifications at positions L234, L235, D270, N297, E318, K320, K322, P331, and P329, such as one, two or three mutations of L234A, L235A and P331S, wherein the numbering of amino acid residues is according to the EU index as set forth in Kabat.~~

~~14~~wherein the numbering of amino acid residues is according to the EU index as set forth in Kabat.

7. The fusion construct of any one of claims ~~7-13~~1-6, wherein the second antibody or antigen binding fragment thereof binds to Tau and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 having the amino acid sequences of SEQ ID NOs: 554 to 559, respectively, preferably, the second antibody is a monoclonal antibody comprising a heavy chain having the amino acid sequence of SEQ ID NO: 310 and a light chain having the amino acid sequence of SEQ ID NO: 311.

~~15~~8. The fusion construct of ~~any one of claims 7-14~~claim 2, comprising:

(1) a first heavy chain having an amino acid sequence that is at least 80%, such as at least 85%, 90%, 95% or 100%, identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 301, 304, 307, 285, 288, 298, 10, 13, 16, 19, 22, 25, 35, 38, 41, 44, 47, 50, 53, 63, 66, 69, 72, 75, 78, 81, 91, 94, 97, 100, 103, 106, 116, 119, 122, 125, 128, 131, 141, 144, 147, 150, 153, 156, 159, 169, 172, 175, 178, 181, 184, 187, 197, 200, 203, 206, 209, 212, 215, 225, 228, 231, 234, 237, 240, 250, 252, 256, 259, 269, 272, 275, 320, 327, 334, 341, 351, 361, 371, 381, 391, 401, 411, 421, 431, 441, 451, 461 and 471;

(2) two light chains each independently having an amino acid sequence that is at least 80%, such as at least 85%, 90%, 95% or 100%, identical to an amino acid sequence selected from the group consisting of 302, 305, 308, 286, 289, 299, 11, 14, 17, 20, 23, 26, 36, 39, 42, 45, 48, 51, 54, 64, 67, 70, 73, 76, 79, 82, 92, 95, 98, 101, 104, 107, 117, 120, 123, 126, 129, 132, 142, 145, 148, 151, 154, 157, 160, 170, 173, 176, 179, 182, 185, 188, 198, 201, 204, 207, 210, 213, 216, 226, 229, 232, 235, 238, 241, 251, 253, 257, 260, 270, 273, 276, 321, 328, 335, 342, 352, 362, 372, 382, 392, 402, 412, 422, 432, 442, 452, 462 and 472, respectively; and

(3) a second heavy chain having an amino acid sequence that is at least 80%, such as at least 85%, 90%, 95% or 100%, identical to an amino acid sequence selected from the group consisting of 303, 306, 309, 287, 290, 300, 12, 15, 18, 21, 24, 27, 37, 40, 43, 46, 49, 52, 55, 65, 68, 71, 74, 77, 80, 83, 93, 96, 99, 102, 105, 108, 118, 121, 124, 127, 130, 133, 143, 146, 149, 152, 155, 158, 161, 171, 174, 177, 180, 183, 186, 189, 199, 202, 205, 208, 211, 214, 217, 227, 230, 233, 236, 239, 242, 252, 254, 258, 261, 271, 274, 277, 322, 329, 336, 343, 353, 363, 373, 383, 393, 403, 413, 423, 433, 443, 453, 463 and 473, respectively.

~~469.~~ An isolated nucleic acid encoding the ~~antibody or antigen-binding fragment of~~ conjugate of any one of claims 1-5, ~~the conjugate of claim 64~~ or the fusion construct of any one of claims 7-~~451-8.~~

~~4710.~~ A vector comprising the isolated nucleic acid of claim ~~469.~~

~~4811.~~ A host cell comprising the nucleic acid of claim ~~469~~ or the vector of claim ~~4710.~~

~~4912.~~ A method of producing the ~~antibody or antigen-binding fragment of~~ conjugate of any one of claims 1-5, ~~the conjugate of claim 64~~, or the fusion construct of any one of claims 7-~~451-8,~~

comprising culturing a cell comprising a nucleic acid encoding the ~~antibody or antigen-binding fragment, the conjugate or the fusion construct~~ under conditions to produce the ~~antibody or antigen-binding fragment, the conjugate or the fusion construct~~, and recovering the ~~antibody or antigen-binding fragment, the conjugate or the fusion construct~~ from the cell or cell culture.

2013. A pharmaceutical composition comprising the ~~antibody or antigen-binding fragment of conjugate of any one of claims 1-5, the conjugate of claim 6~~ 4, or the fusion construct of any one of claims 7-151-8, and a pharmaceutically acceptable carrier.

21. A method of treating or detecting a disorder, preferably a neurological disorder, in a subject in need thereof, comprising administering to the subject the ~~antibody or antigen-binding fragment of~~ 14. The conjugate of any one of claims 1-5, the conjugate of claim 64, or the fusion construct of any one of claims 7-151-8, or the pharmaceutical composition of claim 2013 for use in therapy.

15. Use of the conjugate of any one of claims 1-4, or the fusion construct of any one of claims 1-8, or the pharmaceutical composition of claim 13 in the manufacture of a medicament for the treatment or detection of a disorder, preferably a neurological disorder, preferably, the neurological disorder is selected from the group consisting of neurodegenerative diseases (such as Lewy body disease, ~~postpoliomyelitis syndrome, Shy Drager syndrome, olivopontocerebellar atrophy, Parkinson's disease, multiple system atrophy, striatonigral degeneration, spinocerebellar ataxia, spinal muscular atrophy~~), tauopathies (such as Alzheimer disease and supranuclear palsy), prion diseases (such as bovine spongiform encephalopathy, ~~scrapie, Creutz-feldt-Jakob syndrome, kuru, Gerstmann-Straussler-Scheinker disease, chronic wasting disease, and fatal familial insomnia~~), bulbar palsy, motor neuron disease, and nervous system heterodegenerative disorders (such as Canavan disease, Huntington's disease, neuronal ceroid lipofuscinosis, Alexander's disease, Tourette's syndrome, Menkes kinky hair syndrome, Cockayne syndrome, Halervorden-Spatz syndrome, ~~lafora disease, Rett syndrome, hepatolenticular degeneration, Lesch-Nyhan syndrome, and Unverricht-Lundborg syndrome~~), dementia (such as Pick's disease, and spinocerebellar ataxia), and cancer of the CNS and/or brain (such as brain metastases resulting from cancer elsewhere in the body)-), preferably, the conjugate or the pharmaceutical

composition is to be administered intravenously, more preferably the medicament is for reducing Fc-mediated effector function and/or does not induce rapid reticulocyte depletion.

~~22~~16. The ~~method~~use of claim ~~21~~15, wherein the medicament is for delivering the second antibody or antigen-binding fragment thereof, ~~the conjugate, or the pharmaceutical composition is administered intravenously.~~

~~23. A method of delivering a therapeutic or diagnostic agent across the blood-brain barrier (BBB) of a subject in need thereof, comprising administering to the subject a complex comprising the therapeutic or diagnostic agent coupled to, preferably covalently conjugated to, the antibody or antigen-binding fragment thereof of any one of claims 1 to 5.~~

~~24. The method of any one of claims 21 to 24, wherein the administration reduces Fc-mediated effector function and/or does not induce rapid reticulocyte depletion.~~

~~25. A method of~~17. The use of claim 15, wherein the medicament is for inducing antibody dependent phagocytosis (ADP) without stimulating secretion of a pro-inflammatory cytokine in a subject in need thereof, comprising administering to the subject a complex comprising a therapeutic antibody or antigen-binding fragment thereof coupled to, preferably covalently conjugated to, the antigen-binding fragment thereof of any one of claims 1 to 5, wherein the therapeuticwherein the second antibody or antigen binding fragment thereof comprises one or more amino acid modifications at positions L234, L235, D270, N297, E318, K320, K322, P331, and P329, such as one, two or three mutations of L234A, L235A and P331S, and wherein the numbering of amino acid residues is according to the EU index as set forth in Kabat,

~~26. The method of claim 25, wherein preferably, the therapeutic antibody or antigen binding fragment thereof binds specifically to tau aggregates.~~