

Compositions and Methods for Modulating Tau Expression

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/618,798, filed January 8, 2024, which is incorporated herein by reference.

SEQUENCE LISTING

[0002] The Sequence Listing written in file DNL-038-03-WO_SeqListing.xml is 237 kilobytes in size, was created January 6, 2025, and is hereby incorporated by reference.

BACKGROUND

[0003] Alzheimer's disease is a progressive neurodegenerative disorder that is currently the seventh leading cause of death in the United States and is the most common cause of dementia among older adults. A prominent pathological feature in Alzheimer's disease and other neurodegenerative diseases is the aberrant aggregation and inclusion formation of microtubule-associated protein tau. Mutations in the microtubule associated protein tau (*MAPT*) gene that encodes tau have been linked to tauopathies that are linked to neurodegeneration.

[0004] *In vivo* delivery of nucleic acid-based molecules, such as antisense oligonucleotides, often requires specific targeting to reach certain tissues or cell types. In particular, delivery to non-hepatic tissues remains an obstacle and has limited the use of such therapies. Delivery of oligonucleotides to the central nervous system (CNS) poses a distinct problem due to the blood brain barrier (BBB). One means to deliver oligonucleotides into the CNS is by intrathecal delivery. However, intrathecal delivery is invasive, has a higher risk of side-effects, and often leads to uneven distribution.

[0005] Thus, there is a continuing need for new and improved methods for delivering nucleic acid-based therapeutics *in vivo*, particularly to CNS tissues.

SUMMARY

[0006] Described herein are Microtubule-associated protein tau (*MAPT*) antisense oligonucleotide (ASO) conjugates comprising a at least one *MAPT* antisense oligonucleotide (ASO) and a transferrin receptor (TfR)-targeting Fc polypeptide dimer, wherein an Fc polypeptide of the Fc polypeptide dimer is modified to bind to TfR. TfR is highly expressed on the blood-brain barrier (BBB), and TfR naturally moves transferrin from the blood into the brain. The described *MAPT* ASO conjugates bind TfR, are transported across the BBB, and transport the attached *MAPT* ASO across the BBB. In some embodiments, the TfR-targeting

Fc polypeptide dimer further comprises at least one cysteine substitution to facilitate attachment of the MAPT ASO. In some embodiments, the Fc dimer comprises a first Fc polypeptide and a second Fc polypeptide, wherein the second Fc polypeptide comprises a modified constant domain that specifically binds to human transferrin receptor 1 (TfR) and comprises a sequence having at least 70% sequence identity to SEQ ID NO:12, wherein second Fc polypeptide forms an Fc dimer with the first Fc polypeptide.

[0007] Described are MAPT antisense oligonucleotides (ASOs) comprising a nucleic acid sequence: 5'-A_Lx_d^mC_Lx_e^mC_Lx_fTTAp_aAp_bGp_cTATTACTx_gT_Lx_hG_Lx_i^mC_L-3' (SEQ ID NO:111) or 5'-^mC_Lx_dT_Lx_eG_Lx_fTp_jTp_kAp_iGp_mACATTCp_nAp_oTTx_g^mC_Lx_hT_Lx_i^mC_L-3' (SEQ ID NO:112) wherein A_L, ^mC_L, G_L, and T_L are adenine, 5-methylcytosine, guanine, and thymine locked nucleosides, respectively; A, C, G, and T are deoxyadenosine, deoxycytidine, deoxyguanine, and deoxythymidine nucleosides, respectively; each **p** is independently a phosphorothioate (PS) internucleoside linkage or a stabilizing internucleoside linkage, and at least 2 of (P)_a, (P)_b, and (P)_c, are not PS internucleoside linkages; each **x** is independently a PS internucleoside linkage, a phosphodiester (PO) internucleoside linkage, or a stabilizing internucleoside linkage; and any internucleoside linkage that is not a stabilizing internucleoside linkage is a PS internucleoside linkage or a phosphodiester internucleoside linkage. The stabilizing internucleoside linkages are independently selected from the group consisting of: phosphorodithioate (PS2) internucleoside linkage, a phosphorylguanidine (PN) internucleoside linkage, a mesylphosphoramidate (MsPA) internucleoside linkage, and an O-isopropyl phosphorothioate (OiPS) internucleoside linkage.

[0008] In some embodiments, each **p** and **x** is a PS internucleoside linkage.

[0009] In some embodiments, x_g, x_h, and x_i are each PS internucleoside linkages. In some embodiments, x_g is a PS internucleoside linkage, and x_h and x_i are each PN internucleoside linkages. In some embodiments, x_g, x_h, and x_i are each PN internucleoside linkages. In some embodiments, x_g and x_h are each PN internucleoside linkages, and x_i is a PO internucleoside linkage. In some embodiments, x_g and x_i are each PN internucleoside linkages, and x_h is a PO internucleoside linkage. In some embodiments, x_h and x_i are each PN internucleoside linkages, and x_g is a PO internucleoside linkage. In some embodiments, x_h and x_i are PN internucleoside linkages. In some embodiments, at least 2 of x_g, x_h, and x_i are PN internucleoside linkages.

[0010] In some embodiments, x_d, x_e, and x_f are each PS internucleoside linkages. In some embodiments, x_d and x_e are PN internucleoside linkages. In some embodiments, x_d and x_e are PN internucleoside linkages, and x_f is a PS internucleoside linkage. In some embodiments, x_d is a PS internucleoside linkage, and x_e and x_f are each PN internucleoside linkages. In some

embodiments, x_d is a PN internucleoside linkage, and x_e and x_f are each PS internucleoside linkages. In some embodiments, x_d , x_e , and x_f are each PN internucleoside linkages. In some embodiments, x_d and x_e are each PN internucleoside linkages, and x_f is a PO internucleoside linkage. In some embodiments, x_d and x_f are each PN internucleoside linkages, and x_e is a PO internucleoside linkage. In some embodiments, x_e and x_f are each PN internucleoside linkages, and x_d is a PO internucleoside linkage.

[0011] In some embodiments, x_d , x_e , x_f , x_g , x_h , and x_i are each PS internucleoside linkages. In some embodiments, x_d , x_e , and x_f are each PS internucleoside linkages, x_g is a PS internucleoside linkage, and x_h and x_i are each PN internucleoside linkages. In some embodiments, x_d , x_e , and x_f are each PS internucleoside linkages, and x_g , x_h , and x_i are each PN internucleoside linkages. In some embodiments, x_d and x_e are PN internucleoside linkages, x_f is a PS internucleoside linkage, and x_g , x_h , and x_i are each PS internucleoside linkages. In some embodiments, x_d and x_e are PN internucleoside linkages, x_f is a PS internucleoside linkage, x_g is a PS internucleoside linkage, and x_h and x_i are each PN internucleoside linkages. In some embodiments, x_d is a PS internucleoside linkage, x_e and x_f are each PN internucleoside linkages, x_g is a PS internucleoside linkage, and x_h and x_i are each PN internucleoside linkages. In some embodiments, x_d , x_e , x_f , x_g , x_h , and x_i are each PN internucleoside linkages. In some embodiments, x_d , x_e , and x_f are each PN internucleoside linkages, x_g and x_h are each PN internucleoside linkages, and x_i is a PO internucleoside linkage. In some embodiments, x_d , x_e , and x_f are each PN internucleoside linkages, x_g and x_i are each PN internucleoside linkages, and x_h is a PO internucleoside linkage. In some embodiments, x_d , x_e , and x_f are each PN internucleoside linkages, x_h and x_i are each PN internucleoside linkages, and x_g is a PO internucleoside linkage. In some embodiments, x_d and x_e are each PN internucleoside linkages, and x_f is a PO internucleoside linkage, and x_g , x_h , and x_i are each PN internucleoside linkages. In some embodiments, x_d and x_f are each PN internucleoside linkages, and x_e is a PO internucleoside linkage, and x_g , x_h , and x_i are each PN internucleoside linkages. In some embodiments, x_e and x_f are each PN internucleoside linkages, and x_d is a PO internucleoside linkage, and x_g , x_h , and x_i are each PN internucleoside linkages. In some embodiments, x_h and x_i or x_g , x_h , and x_i are PN internucleoside linkages and x_d and x_e or x_d , x_e , and x_f are PN internucleoside linkages.

[0012] In some embodiments, the MAPT ASO comprises SEQ ID NO:111 and: (a) p_a , p_b , and p_c are PS internucleoside linkages; (b) p_a is a stabilizing internucleoside linkage, and p_b and p_c

are PS internucleoside linkages; (c) p_b is a stabilizing internucleoside linkage, and p_a and p_c are PS internucleoside linkages; (d) p_a and p_b are stabilizing internucleoside linkages and p_c is a PS internucleoside linkage; (e) p_b and p_c are stabilizing internucleoside linkages and p_a is a PS internucleoside linkage; (f) p_a , p_b , and p_c are stabilizing internucleoside linkages; or (g) p_a and p_b are PS2 internucleoside linkages.

[0013] In some embodiments, the MAPT ASO comprises SEQ ID NO:111 and: (a) p_a is a PS2 linkage, and p_b and p_c are PS internucleoside linkages; (b) p_b is a PS2 linkage, and p_a and p_c are PS internucleoside linkages; (c) p_b is a PN linkage, and p_a and p_c are PS internucleoside linkages; (d) p_a and p_b are PN internucleoside linkages and p_c is a PS internucleoside linkage; (e) p_a and p_b are PS2 internucleoside linkages and p_c is a PS internucleoside linkage; (f) p_a and p_b are MsPA internucleoside linkages and p_c is a PS internucleoside linkage; (g) p_a and p_b are OiPS internucleoside linkages and p_c is a PS internucleoside linkage; (h) p_b and p_c are PN internucleoside linkages and p_a is a PS internucleoside linkage; (i) p_a , p_b , and p_c are PN internucleoside linkages; or (j) p_a , p_b , and p_c are MsPA internucleoside linkages.

[0014] In some embodiments, the MAPT ASO comprises SEQ ID NO:111 and p_a and p_b are PS2 internucleoside linkages. In some embodiments, MAPT ASO comprises SEQ ID NO:111 and: (a) x_h and x_i or x_g , x_h , and x_i are PN internucleoside linkages, x_d and x_e or x_d , x_e , and x_f are PN internucleoside linkages, and p_a and p_b are PS2 internucleoside linkages; (b) x_d , x_e , x_h , and x_i are PN internucleoside linkages, and p_a and p_b are PS2 internucleoside linkages; (c) x_d , x_e , x_f , x_g , x_h , and x_i are PN internucleoside linkages, and p_a and p_b are PS2 internucleoside linkages; (d) x_d , x_e , x_h , and x_i are PN internucleoside linkages, p_a and p_b are PS2 internucleoside linkages, and all other internucleoside linkages are PS linkages; or (e) x_d , x_e , x_f , x_g , x_h , and x_i are PN internucleoside linkages, p_a and p_b are PS2 internucleoside linkages, and all other internucleoside linkages are PS linkages.

[0015] In some embodiments, the MAPT ASO comprises SEQ ID NO:111 and p_b and p_c are PN internucleoside linkages. In some embodiments, MAPT ASO comprises SEQ ID NO:111 and: (a) x_h and x_i or x_g , x_h , and x_i are PN internucleoside linkages, x_d and x_e or x_d , x_e , and x_f are PN internucleoside linkages, and p_b and p_c are PN internucleoside linkages; (b) x_h and x_i are PN internucleoside linkages, and p_b and p_c are PN internucleoside linkages; or (c) x_h and x_i are PN internucleoside linkages, p_b and p_c are PN internucleoside linkages, and all other internucleoside linkages are PS linkages.

[0016] In some embodiments, the MAPT ASO comprises SEQ ID NO:111 and p_a and p_b are MsPA internucleoside linkages or p_a , p_b , and p_c are MsPA internucleoside linkages. In some embodiments, MAPT ASO comprises SEQ ID NO:111 and: (a) x_h and x_i or x_g , x_h , and x_i are

PN internucleoside linkages, x_d and x_e or x_d , x_e , and x_f are PN internucleoside linkages, and p_a and p_b or p_a , p_b , and p_c are MsPA internucleoside linkages; (b) x_g , x_h , and x_i are PN internucleoside linkages, and p_a , p_b , and p_c are MsPA internucleoside linkages; (c) x_d , x_e , x_h , and x_i are PN internucleoside linkages, and p_a and p_b are MsPA internucleoside linkages; (d) x_g , x_h , and x_i are PN internucleoside linkages, p_a , p_b , and p_c are MsPA internucleoside linkages, and all other internucleoside linkages are PS linkages; or (e) x_d , x_e , x_h , and x_i are PN internucleoside linkages, p_a and p_b are MsPA internucleoside linkages, and all other internucleoside linkages are PS linkages.

[0017] In some embodiments, the MAPT ASO comprises SEQ ID NO:111 and p_a and p_b are OiPS internucleoside linkages or p_a , p_b , and p_c are OiPS internucleoside linkages. In some embodiments, MAPT ASO comprises SEQ ID NO:111 and: (a) x_h and x_i or x_g , x_h , and x_i are PN internucleoside linkages, x_d and x_e or x_d , x_e , and x_f are PN internucleoside linkages, and p_a and p_b or p_a , p_b , and p_c are OiPS internucleoside linkages; (b) x_d , x_e , x_f , x_g , x_h , and x_i are PN internucleoside linkages, and p_a and p_b are OiPS internucleoside linkages; or (c) x_d , x_e , x_f , x_g , x_h , and x_i are PN internucleoside linkages, p_a and p_b are OiPS internucleoside linkages, and all other internucleoside linkages are PS linkages.

[0018] In some embodiments, the MAPT ASO comprises SEQ ID NO:111 and the modified MAPT ASO comprises:

- (a) 5' $AL^{*m}CL^{*m}CL^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^{*m}C^*T^*TL^{*GL^{*m}CL}3'$;
- (b) 5' $AL^{*m}CL^{*m}CL^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*TL^{*GL^{*m}CL}3'$;
- (c) 5' $AL^{*m}CL^{*m}CL^{*T}T^*AnAnGnT^*A^*T^*T^*A^*C^*T^*TL^{*GL^{*m}CL}3'$;
- (d) 5' $AL^{*m}CL^{*m}CL^{*T}T^*A^*AnGnT^*A^*T^*T^*A^*C^*T^*TLnGln^{*m}CL}3'$;
- (e) 5' $AL^{*m}CL^{*m}CL^{*T}T^*AnAnGnT^*A^*T^*T^*A^*C^*T^*TLnGln^{*m}CL}3'$;
- (f) 5' $AL^{*m}CL^{*m}CL^{*T}T^*AuAuGuT^*A^*T^*T^*A^*C^*T^*TnTlnGln^{*m}CL}3'$;
- (g) 5' $ALn^{*m}CL^{*m}CL^{*T}T^*AnAnG^*T^*A^*T^*T^*A^*C^*T^*TLnGln^{*m}CL}3'$;
- (h) 5' $AL^{*m}CLn^{*m}CLn^{*T}T^*AnAnG^*T^*A^*T^*T^*A^*C^*T^*TLnGln^{*m}CL}3'$;
- (i) 5' $ALn^{*m}CLn^{*m}CL^{*T}T^*AnAnG^*T^*A^*T^*T^*A^*C^*T^*TL^{*GL^{*m}CL}3'$;
- (j) 5' $ALn^{*m}CLn^{*m}CL^{*T}T^*A^*AnGnT^*A^*T^*T^*A^*C^*T^*TL^{*GL^{*m}CL}3'$;
- (k) 5' $ALn^{*m}CLn^{*m}CL^{*T}T^*A^*AnG^*T^*A^*T^*T^*A^*C^*T^*TLnGln^{*m}CL}3'$;
- (l) 5' $ALn^{*m}CLn^{*m}CL^{*T}T^*AuAuG^*T^*A^*T^*T^*A^*C^*T^*TLnGln^{*m}CL}3'$;
- (m) 5' $ALn^{*m}CLn^{*m}CL^{*T}T^*AtAtG^*T^*A^*T^*T^*A^*C^*T^*TLnGln^{*m}CL}3'$;
- (n) 5' $ALn^{*m}CLn^{*m}CL^{*T}T^*A^*AnGnT^*A^*T^*T^*A^*C^*T^*TLnGln^{*m}CL}3'$;
- (o) 5' $ALn^{*m}CLn^{*m}CLn^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*TnTlnGln^{*m}CL}3'$;
- (p) 5' $ALn^{*m}CLn^{*m}CLn^{*T}T^*AtA^*G^*T^*A^*T^*T^*A^*C^*T^*TnTlnGln^{*m}CL}3'$;

- (q) 5' AL^mCLⁿ^mCLⁿT*^tA*AtG*^tA*^tT*^tA*^c*TnTⁿLⁿGⁿ^mCL 3';
- (r) 5' ALⁿ^mCLⁿ^mCLⁿT*^tAtAtG*^tA*^tT*^tA*^c*TnTⁿLⁿGⁿ^mCL 3';
- (s) 5' ALⁿ^mCLⁿ^mCLⁿT*^tAoAoG*^tA*^tT*^tA*^c*TnTⁿLⁿGⁿ^mCL 3';
- (t) 5' ALⁿ^mCLⁿ^mCLⁿT*^tAtAtG*^tA*^tT*^tA*^c*TnTⁿLⁿGⁿ^mCL 3';
- (u) 5' ALⁿ^mCLⁿ^mCLⁿT*^tAtAtG*^tA*^tT*^tA*^c*TnTⁿLⁿPⁿGⁿ^mCL 3';
- (v) 5' ALⁿ^mCLⁿ^mCLⁿT*^tAtAtG*^tA*^tT*^tA*^c*TPTⁿLⁿGⁿ^mCL 3';
- (w) 5' ALⁿ^mCLⁿ^mCPT*^tAtAtG*^tA*^tT*^tA*^c*TnTⁿLⁿGⁿ^mCL 3';
- (x) 5' ALⁿ^mCLⁿ^mCnT*^tAtAtG*^tA*^tT*^tA*^c*TnTⁿLⁿGⁿ^mCL 3'; or
- (y) 5' ALⁿ^mCLⁿ^mCnT*^tAtAtG*^tA*^tT*^tA*^c*TnTⁿLⁿGⁿ^mCL 3';

wherein AL, ^mCL, TL, and GL are adenine locked nucleic acid, 5-methylcytosine locked nucleic acid, thymine locked nucleic acid; and guanine locked nucleic acid, respectively; A, C, T, and G are deoxyadenosine, deoxycytidine, deoxythymidine, deoxyguanosine, respectively; s is a PS internucleoside linkage; n is a PN internucleoside linkage; t is a PS2 internucleoside linkage; u is a MsPA internucleoside linkage, o is an OiPS internucleoside linkage. Chemical structures representing the MAPT ASOs are shown in FIGs. 10, 11, 12, 13, and 14. The MAPT ASO can also be a salt of any of the structures shown in any of FIGs. 10, 11, 12, 13, and 14.

[0019] In some embodiments, the MAPT ASO comprises SEQ ID NO:112 and: (a) p_k, p_l, p_n, and p_o are stabilizing internucleoside linkages; (b) p_k, p_l, p_n, and p_o are stabilizing internucleoside linkages, and p_j and p_m are PS internucleoside linkages; (c) p_j, p_l, and p_o are stabilizing internucleoside linkages; (d) p_j, p_l, and p_o are stabilizing internucleoside linkages, and p_k, p_m, and p_n are PS internucleoside linkages; (e) p_l, p_m, p_n, and p_o are stabilizing internucleoside linkages; or (d) p_l, p_m, p_n, and p_o are stabilizing internucleoside linkages, and p_j and p_k are PS internucleoside linkages.

[0020] In some embodiments, the MAPT ASO comprises SEQ ID NO:112 and (a) p_k, p_l, p_n, and p_o are PN internucleoside linkages; (b) p_k, p_l, p_n, and p_o are PN internucleoside linkages, and p_j and p_m are PS internucleoside linkages; (c) p_j is a PN linkage, and p_l and p_o are PS2 internucleoside linkages; (d) p_j is a PN linkage, p_l and p_o are PS2 internucleoside linkages, and p_k, p_m, and p_n are PS internucleoside linkages; (e) p_l and p_m are PS2 internucleoside linkages, and p_n, and p_o are PN internucleoside linkages; or (d) p_l and p_m are PS2 internucleoside linkages, p_n, and p_o are PN internucleoside linkages, and p_j and p_k are PS internucleoside linkages.

[0021] In some embodiments, the MAPT ASO comprises SEQ ID NO:112 and the modified MAPT ASO comprises:

- (a) ^mCLⁿTⁿLⁿGⁿ*^tT*^tTnAnG*^tA*^c*A*^tT*^tCnAnT*^t^mCL*^tTL*^mCL;

- (b) ${}^m\text{C}_L * \text{T}_L * \text{G}_L * \text{T} * \text{TnAnG} * \text{A} * \text{C} * \text{A} * \text{T} * \text{T} * \text{CnAnT} * \text{T} * {}^m\text{C}_L \text{nT}_L \text{n} {}^m\text{C}_L$;
- (c) ${}^m\text{C}_L \text{nT}_L \text{nG}_L \text{nTnT} * \text{AtG} * \text{A} * \text{C} * \text{A} * \text{T} * \text{T} * \text{C} * \text{AtT} * \text{Tn} {}^m\text{C}_L \text{nT}_L \text{n} {}^m\text{C}_L$; or
- (d) ${}^m\text{C}_L \text{nT}_L \text{nG}_L * \text{T} * \text{T} * \text{AtGtA} * \text{C} * \text{A} * \text{T} * \text{T} * \text{CnAnT} * \text{T} * {}^m\text{C}_L \text{nT}_L \text{n} {}^m\text{C}_L$

wherein ${}^m\text{C}_L$, T_L , and G_L are 5-methylcytosine locked nucleoside, thymine locked nucleoside, and guanosine locked nucleoside, respectively; A, C, T, and G are deoxyadenosine, deoxycytidine, deoxythymidine, and deoxyguanosine, respectively; * is a PS internucleoside linkage; n is a PN internucleoside linkage; and t is a PS2 internucleoside linkage.

[0022] In some embodiments, the oligonucleotide contains a 5' terminal group, wherein the 5' terminal group can be, but is not limited to, a phosphodiester (PO) group, a phosphorothioate (PS) group, a phosphorodithioate (PS2) group, a mesyl-phosphoramidate (MsPA) group, a cyclic phosphoryl guanidine (PN) group, or an *O*-isopropyl phosphorothioate (OiPS) group. In some embodiments, the oligonucleotide (or stabilized oligonucleotide) contains a 5' terminal group selected from the group consisting of: PO, PS, and PN. In some embodiments, the oligonucleotide (or stabilized oligonucleotide) contains a PO 5' terminal group. In some embodiments, the oligonucleotide (or stabilized oligonucleotide) contains a PS 5' terminal group. In some embodiments, the oligonucleotide (or stabilized oligonucleotide) contains a PN 5' terminal group. In some embodiments, the oligonucleotide (or stabilized oligonucleotide) contains a PS2 5' terminal group. In some embodiments, the oligonucleotide (or stabilized oligonucleotide) contains a MsPA 5' terminal group. In some embodiments, the oligonucleotide (or stabilized oligonucleotide) contains a OiPS 5' terminal group.

[0023] Described are MAPT ASOs conjugated to transferrin receptor (TfR)-targeting Fc polypeptide dimer. Any of the described MAPT ASOs can be conjugated to the TfR-targeting Fc polypeptide dimer. In some embodiments, the TfR-targeting Fc polypeptide dimer comprises a first Fc polypeptide and a second Fc polypeptide, wherein the second Fc polypeptide comprises a modified constant domain that specifically binds to human transferrin receptor 1 (TfR) and wherein second Fc polypeptide forms an Fc dimer with the first Fc polypeptide. In some embodiments, the TfR-targeting Fc polypeptide dimer comprises a first Fc polypeptide that does not contain a modified constant domain that specifically binds to human transferrin receptor 1 (TfR) and a second Fc polypeptide comprising a modified constant domain that specifically binds to TfR, wherein first and second Fc polypeptide form an Fc dimer. In some embodiments, the first Fc polypeptide, the second Fc polypeptide, or both the first and second Fc polypeptides are modified to reduce effector function. In some embodiments, the first Fc polypeptide and the second Fc polypeptide are modified to reduce effector function. In some embodiments, the first Fc polypeptide comprises a cysteine at

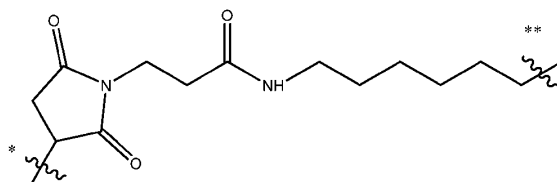
position 239, an A at position 234, an A at position 235, and a serine position 329, each according to EU numbering. In some embodiments, the second Fc polypeptide comprising an A at position 234, an A at position 235, and serine at position 329, each according to EU numbering and comprises a sequence having at least 90% sequence identity to SEQ ID NO:12.

[0024] In some embodiments, the TfR-targeting Fc polypeptide dimer further comprises a non-targeting Fab (NTF) fused to the first Fc polypeptide via a hinge region to form a first Fab-Fc fusion polypeptide. In some embodiments, the TfR-targeting Fc polypeptide dimer further comprises a NTF fused to the second Fc polypeptide via a hinge region to form a second Fab-Fc fusion polypeptide. In some embodiments, the TfR-targeting Fc polypeptide dimer comprises a non-targeting Fab (NTF) fused to the first Fc polypeptide via a hinge region to form a first Fab-Fc fusion polypeptide, and a non-targeting Fab (NTF) fused to the second Fc polypeptide via a hinge region to form a second Fab-Fc fusion polypeptide wherein the first and second Fab-Fc fusion polypeptides for a Fab-Fc dimer. In some embodiments, the first and second NTFs each comprise a heavy chain segment comprising SEQ ID NO:109 or 110 and a light chain comprising SEQ ID NO:108. In some embodiments, the first and second NTFs each comprise a heavy chain segment comprising SEQ ID NO:130 or 131 and a light chain comprising SEQ ID NO:129. In some embodiments, the hinge regions each comprise SEQ ID NO:121.

[0025] In some embodiments, the TfR-targeting Fc polypeptide dimer further comprises a non-binding variable region (NBVR) fused to the first Fc polypeptide via a hinge region to form a NBVR-Fc fusion polypeptide. In some embodiments, the TfR-targeting Fc polypeptide dimer further comprises a NBVR fused to the second Fc polypeptide via a hinge region to form a NBVR-Fc fusion polypeptide. In some embodiments, the NBVRs each comprise the variable region of SEQ ID NO:109 or 110. In some embodiments, the hinge regions each comprise SEQ ID NO:121.

[0026] The MAPT ASO is linked to the TfR-targeting Fc polypeptide dimer via a linking group. The linking group can be any linking group available in the art suitable for linking an oligonucleotide to a polypeptide. The MAPT ASO can be linked to the first Fc polypeptide, the second Fc polypeptide, the first NTF (if present), and/or the second NTF (if present). In some embodiments, the MAPT ASO is covalently linked to the first Fc polypeptide, the second Fc polypeptide, the first NTF (if present), and/or the second NTF (if present). In some embodiments, the MAPT ASO is linked the first Fc polypeptide at a cysteine at position 239. In some embodiments, the first Fc polypeptide comprises a CH1 domain and the MAPT ASO is linked the first Fc polypeptide at a cysteine at position 114 (according to Kabat numbering)

or position 124 (according to EU numbering (*i.e.*, the CH1 domain comprises a A114C substitution or an S124C substitution). In some embodiments, the MAPT ASO is linked to first Fc polypeptide via the 5' end of the MAPT ASO. In some embodiments, the first Fc polypeptide is linked to the MAPT ASO via a linking group attached to the cysteine at position 239 and the 5' end of the MAPT ASO (or a 5' terminal group of the MAPT ASO), wherein the linking group comprises:



wherein “~” that intersects a bond in a chemical structure indicates the point of attachment of the bond that the wavy bond intersects in the chemical structure to the remainder of a molecule.

[0027] In some embodiments, the modified constant domain of the second Fc polypeptide comprises: a glutamate (E), leucine (L), serine (S), valine (V), tryptophan (W), or tyrosine (Y) at position 153; a Y, phenylalanine (F), W, methionine (M), proline (P), or V at position 157; a threonine (T), an asparagine (N), or V at position 159; an E, Isoleucine (I), P, or V at position 160; a W at position 161; an alanine (A), I, V, serine (S), or T at position 162; a N, S, arginine (R), or T at position 163; a T, histidine (H), or S at position 186; an E, S, aspartate (D), glycine (G), T, P, glutamine (Q), or R at position 188; an E or R at position 189; a Q at position 191; a Q at position 192; a F, H, lysine (K), Y, or W at position 194; a S, T, or W at position 197; and a S, C, P, M, or W at position 199, and as numbered with reference to SEQ ID NO:1.

[0028] In some embodiments, the modified constant domain of the second Fc polypeptide comprises: an E, L, S, V, W, or Y at position 380; a Y, F, W, M, P, or V at position 384; a T, N, or V at position 386; an E, I, P, or V at position 387; a W at position 388; an A, I, V, S, or T at position 389; a N, S, R, or T at position 390; a T, H, or S at position 413; an E, S, D, G, T, P, Q, or R at position 415; an E or R at position 416; a Q at position 418; a Q at position 419; a F, H, K, Y, or W at position 421; a S, T, or W at position 424; and a S, C, P, M, or W at position 426, according to EU numbering.

[0029] In some embodiments, the modified constant domain of the second Fc polypeptide comprises: an E at position 153; a Y at position 157; a T at position 159; an E at position 160; a W at position 161; a A at position 162; a N at position 163; a T at position 186; an E at

position 188; an E at position 189; a Q at position 191; a Q at position 192; a F at position 194; a S at position 197; and a S at position 199, and as numbered with reference to SEQ ID NO:1.

[0030] In some embodiments, the modified constant domain of the second Fc polypeptide further comprises a E at position 380, a Y at position 384, a T at position 386, a E at position 387, a W at position 388, an A at position 389, an N at position 390, a T at position 413, a E at position 415, a E at position 416, and a F at position 421, according to EU numbering.

[0031] In some embodiments, the modified constant domain of the second Fc polypeptide comprises: an E at position 380; a Y at position 384; a T at position 386; an E at position 387; a W at position 388; an A at position 389; a N at position 390; a T at position 413; an E at position 415; an E at position 416; a Q at position 418; a Q at position 419; a F at position 421; a S at position 424; and a S at position 426, according to EU numbering.

[0032] In some embodiments, the second Fc polypeptide further comprises a W at position 366, according to EU numbering and the first Fc polypeptide further comprises a serine a position 366, an A at position 368, and a valine at position 407, according to EU numbering. In some embodiments, the second Fc polypeptide further comprises a serine a position 366, an A at position 368, and a valine at position 407, according to EU numbering, and the first Fc polypeptide further comprises a W at position 366, according to EU numbering.

[0033] In some embodiments, the second Fc polypeptide comprises the amino acid sequence of SEQ ID NO:12. In some embodiments, the first Fc polypeptide comprises the amino acid sequence of SEQ ID NO:63 or 80. In some embodiments, the second Fc polypeptide comprises the amino acid sequence of SEQ ID NO:12, and the first Fc polypeptide comprises the amino acid sequence of SEQ ID NO:63 or 80.

[0034] In some embodiments, the first and second NTFs each comprise a heavy chain variable region comprising SEQ ID NO:109 or 110 and a light chain variable region comprising SEQ ID NO:108.

[0035] In some embodiments, the first Fab-Fc fusion polypeptide comprises SEQ ID NO:100 or 101 and the second Fab-Fc fusion polypeptide comprises SEQ ID NO:98 or 99.

[0036] Certain embodiments provide a pharmaceutical composition comprising a MAPT ASO conjugate as described herein and a pharmaceutically acceptable carrier or diluent.

[0037] Certain embodiments provide a method of generating a neuronal cell with decreased Tau expression, the method comprising delivering to the neuron cell a MAPT ASO conjugate as described herein, wherein the MAPT ASO decreases the expression level of an endogenous MAPT gene. The neuronal cell can be, but is not limited to, a brain cell, a deep brain cell, or a spinal cord cell.

[0038] Certain embodiments provide a method of modifying a neuronal cell to decrease Tau expression, the method comprising delivering to the neuron cell a MAPT ASO conjugate as described herein, wherein the MAPT ASO decreases the expression level of an endogenous MAPT gene. The neuronal cell can be, but is not limited to, a brain cell, a deep brain cell, or a spinal cord cell.

[0039] Certain embodiments provide a method of delivering a MAPT ASO to the CNS or cell of the CNS of a human subject in need thereof, comprising administering to the subject a MAPT ASO conjugate as described herein.

[0040] Delivery of a MAPT ASO to a neuronal cell using the described MAPT ASO conjugates can be used to treat a neurodegenerative disorder. Delivery of a described MAPT ASO to a neuronal cell or the can be used to treat a tau-associated neurodegenerative disorder. The neurodegenerative disorder can be, but is not limited to, Alzheimer's disease.

[0041] Certain embodiments provide a method of reducing MAPT messenger ribonucleic acid (mRNA) expression or levels in a human subject in need thereof, the method comprising administering to the human subject the MAPT ASO conjugates as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] FIG. 1 illustrates plasma pharmacokinetic profile of modified MAPT ASO OTVs.

[0043] FIG. 2 illustrates single dose brain ASO concentration of modified MAPT ASO OTVs.

[0044] FIG. 3 illustrates multidose brain ASO concentration of modified MAPT ASO OTVs.

[0045] FIG. 4 illustrates target MAPT knockdown after 4 doses modified MAPT ASO OTVs.

[0046] FIG. 5 illustrates target MAPT knockdown after 8 doses of modified MAPT ASO OTVs.

[0047] FIG. 6 illustrates brain ASO concentration of unmodified MAPT ASO OTV.

[0048] FIG. 7 illustrates target MAPT knockdown of unmodified MAPT ASO OTV.

[0049] FIG. 8 illustrates MAPT ASO potency.

[0050] FIG. 9 illustrates ASO stability in brain tissue.

[0051] FIG. 10. Chemical structure drawing representing one embodiment of a MAPT ASO.

[0052] FIG. 11. Chemical structure drawing representing one embodiment of a MAPT ASO.

[0053] FIG. 12. Chemical structure drawing representing one embodiment of a MAPT ASO.

[0054] FIG. 13. Chemical structure drawing representing one embodiment of a MAPT ASO.

[0055] FIG. 14. Chemical structure drawing representing one embodiment of a MAPT ASO.

DETAILED DESCRIPTION

I. Definitions

[0056] Before describing the present teachings in detail, it is to be understood that the disclosure is not limited to specific compositions or process steps, as such may vary. It should be noted that, as used in this specification and the appended claims, the singular form “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. Thus, for example, reference to “an oligonucleotide” includes a plurality of oligonucleotides and the like. The conjunction “or” is to be interpreted in the inclusive sense, *i.e.*, as equivalent to “and/or,” unless the inclusive sense would be unreasonable in the context.

[0057] In general, the term “about” indicates insubstantial variation in a quantity of a component of a composition not having any significant effect on the activity or stability of the composition. When the specification discloses a specific value for a parameter, the specification should be understood as alternatively disclosing the parameter at “about” that value. The terms “about” and “approximately,” when used to modify an amount specified in a numeric value or range, indicate that the numeric value as well as reasonable deviations (*e.g.*, standard margin of error of measurement (SEM)) from the value known to the skilled person in the art, for example $\pm 20\%$, $\pm 10\%$, or $\pm 5\%$, are within the intended meaning of the recited value.

[0058] Compositions or methods “comprising” or “including” one or more recited elements may include other elements not specifically recited. For example, a composition that “comprises” or “includes” an Fc polypeptide dimer may contain the Fc polypeptide dimer alone or in combination with other ingredients.

[0059] Designation of a range of values includes all integers within or defining the range, and all subranges defined by integers within the range.

[0060] The use of “comprise,” “comprises,” “comprising,” “contain,” “contains,” “containing,” “include,” “includes,” and “including” are not intended to be limiting and may include other elements not specifically recited. For example, a composition that “comprises” or “includes” an Fc polypeptide dimer may contain the Fc polypeptide dimer alone or in combination with other ingredients. It is to be understood that both the foregoing general description and detailed description are exemplary and explanatory only and are not restrictive of the teachings. To the extent that any material incorporated by reference is inconsistent with the express content of this disclosure, the express content controls.

[0061] Designation of a range of values includes all integers within or defining the range, and all subranges defined by integers within the range.

[0062] As used herein, the term “antibody” refers to a protein with an immunoglobulin fold that specifically binds to an antigen via its variable regions. The term encompasses intact polyclonal antibodies, intact monoclonal antibodies, single chain antibodies, multispecific antibodies such as bispecific antibodies, monospecific antibodies, monovalent antibodies, chimeric antibodies, humanized antibodies, and human antibodies. The term “antibody,” as used herein, also includes antibody fragments that retain antigen-binding specificity, including but not limited to Fab, F(ab')₂, Fv, scFv, and bivalent scFv. Antibodies can contain light chains that are classified as either kappa or lambda. Antibodies can contain heavy chains that are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE, respectively.

[0063] The “EU numbering scheme” is generally used in the art when referring to a residue in an antibody heavy chain constant region. The EU numbering scheme is shown below with respect to SEQ ID NO:5 (Clone CH3C.35.23.2 knob):

5				3	13	23
EU				230	240	250
				PCPAPELLGGPSVLFPPKPKDT		
5	33	43		53	63	73
EU	260	270		280	290	300
	LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY					
5	83	93		103	113	123
EU	310	320		330	340	350
	RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT					
5	133	143		153	163	173
EU	360	370		380	390	400
	LPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWANYKTTPPVLD					
5	183	193		203	213	
EU	410	420		430	440	
	DGSFFLYSKLTVTKEEWQOGFVFSCSVMEALHNHYTQKSLSLSPGK					

[0064] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two pairs of polypeptide chains, each pair having one “light” (about 25 kD) and one “heavy” chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen

recognition. The terms “variable light chain” (V_L) and “variable heavy chain” (V_H) refer to these light and heavy chains, respectively.

[0065] The term “variable region” or “variable domain” refers to a domain in an antibody heavy chain or light chain that is derived from a germline Variable (V) gene, Diversity (D) gene, or Joining (J) gene (and not derived from a Constant (C_μ and C_δ) gene segment), and that gives an antibody its specificity for binding to an antigen. Typically, an antibody variable region comprises four conserved “framework” regions interspersed with three hypervariable “complementarity determining regions.”

[0066] The term “complementarity determining region” or “CDR” refers to the three hypervariable regions in each chain that interrupt the four framework regions established by the light and heavy chain variable regions. The CDRs are primarily responsible for antibody binding to an epitope of an antigen. The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus, and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 or CDR-H3 is located in the variable region of the heavy chain of the antibody in which it is found, whereas a V_L CDR1 or CDR-L1 is the CDR1 from the variable region of the light chain of the antibody in which it is found.

[0067] The “framework regions” or “FRs” of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three-dimensional space. Framework sequences can be obtained from public DNA databases or published references that include germline antibody gene sequences. For example, germline DNA sequences for human heavy and light chain variable region genes can be found in the “VBASE2” germline variable gene sequence database for human and mouse sequences.

[0068] The amino acid sequences of the CDRs and framework regions can be determined using various well-known definitions in the art, *e.g.*, Kabat, Chothia, international ImMunoGeneTics database (IMGT), AbM, and observed antigen contacts (“Contact”). In some embodiments, CDRs are determined according to the Contact definition. *See*, MacCallum *et al.*, *J. Mol. Biol.* 262:732-745, 1996. In some embodiments, CDRs are determined by a combination of Kabat, Chothia, and/or Contact CDR definitions.

[0069] The term “Fd portion” refers to an N-terminal portion of an immunoglobulin heavy chain. Typically, an Fd portion includes the heavy chain variable (V_H) region and a heavy chain constant (CH1) region.

[0070] The term “Fab” refers to an antigen-binding fragment consisting of a light chain variable region, a light chain constant region, a heavy chain variable region, and a heavy chain CH1 constant region.

[0071] The term “single-chain variable fragment” or “scFv” refers to an antigen-binding fragment consisting of a heavy chain variable region and a light chain variable region linked together via a peptide linker. The linker can either connect the N-terminus of the V_H with the C-terminus of the V_L or the N-terminus of the V_L with the C-terminus of the V_H. An scFv lacks constant regions.

[0072] The term “epitope” refers to the area or region of an antigen to which a molecule, *e.g.*, the CDRs of an antibody, specifically binds and can include a few amino acids or portions of a few amino acids, *e.g.*, 5 or 6, or more, *e.g.*, 20 or more amino acids, or portions of those amino acids. In some cases, the epitope includes non-protein components, *e.g.*, from a carbohydrate, nucleic acid, or lipid. In some cases, the epitope is a three-dimensional moiety. Thus, for example, where the target is a protein, the epitope can be comprised of consecutive amino acids (*e.g.*, a linear epitope), or amino acids from different parts of the protein that are brought into proximity by protein folding (*e.g.*, a discontinuous or conformational epitope).

[0073] As used herein, the phrase “recognizes an epitope,” as used with reference to an antibody, means that the antibody CDRs interact with or specifically bind to the antigen at that epitope or a portion of the antigen containing that epitope.

[0074] The term “specifically binds” refers to a molecule (*e.g.*, a Fab, or an scFv) that binds to an epitope or target with greater affinity, greater avidity, and/or greater duration to that epitope or target in a sample than it binds to another epitope or non-target compound (*e.g.*, a structurally different antigen). In some embodiments, a Fab or an scFv that specifically binds to an epitope or target, is a Fab or an scFv that binds to the epitope or target with at least 5-fold greater affinity than other epitopes or non-target compounds, *e.g.*, at least 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 25-fold, 50-fold, 100-fold, 1000-fold, 10,000-fold, or greater affinity. The term “specific binding,” “specifically binds to,” or “is specific for” a particular epitope or target, as used herein, can be exhibited, for example, by a molecule having an equilibrium dissociation constant K_D for the epitope or target to which it binds of, *e.g.*, 10⁻⁴ M or smaller, *e.g.*, 10⁻⁵ M, 10⁻⁶ M, 10⁻⁷ M, 10⁻⁸ M, 10⁻⁹ M, 10⁻¹⁰ M, 10⁻¹¹ M, or 10⁻¹² M. It will be recognized by one of skill that a Fab or scFv that specifically binds to a target from one species may also specifically bind to orthologs of that target.

[0075] The term “binding affinity” is used herein to refer to the strength of a non-covalent interaction between two molecules, *e.g.*, between a Fab or scFv and an antigen. Thus, for

example, the term may refer to 1:1 interaction between a Fab or scFv and an antigen, unless otherwise indicated or clear from context. Binding affinity may be quantified by measuring an equilibrium dissociation constant (K_D), which refers to the dissociation rate constant (k_d , time^{-1}) divided by the association rate constant (k_a , $\text{time}^{-1} \text{ M}^{-1}$). K_D can be determined by measurement of the kinetics of complex formation and dissociation, *e.g.*, using Surface Plasmon Resonance (SPR) methods, *e.g.*, a Biacore™ system; kinetic exclusion assays such as KinExA®; and BioLayer interferometry (*e.g.*, using the ForteBio® Octet platform). As used herein, “binding affinity” includes not only formal binding affinities, such as those reflecting 1:1 interaction between a Fab or scFv and an antigen, but also apparent affinities for which K_D 's are calculated that may reflect avid binding.

[0076] Monoclonal antibodies and fragments thereof (including Fabs and conjugates), or other biological entities are typically provided in isolated form. An antibody (or fragment or conjugate thereof) is typically at least 50% w/w pure of interfering proteins and other contaminants arising from its production or purification but does not exclude the possibility that the antibody or Fab is combined with an excess of pharmaceutically acceptable carrier(s) or other vehicle intended to facilitate its use. Antibodies (or fragment or conjugate thereof) can be at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or at least 99% w/w pure of interfering proteins and contaminants from production or purification. The isolated antibody (or fragment or conjugate thereof) can be the predominant macromolecular species remaining after its purification.

[0077] As used herein, the term “Fc region” refers to the C-terminal region of a naturally occurring immunoglobulin heavy chain polypeptide that is characterized by an Ig fold as a structural domain. An Fc polypeptide typically contains constant region sequences including at least the CH2 domain and/or the CH3 domain and may contain at least part of the hinge region. Two Fc peptides dimerize to form an Fc region or Fc fragment.

[0078] The terms “polypeptide” and “peptide” refer to a polymer of amino acid residues in a single chain. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. A “protein” can refer to either a polypeptide, a polypeptide dimer, or a polypeptide multimer. The single chain polypeptides of a protein dimer or multimer may be joined by a covalent bond (*e.g.*, a disulfide bond) or by non-covalent interactions.

[0079] For purposes of classifying amino acid substitutions as conservative or nonconservative, amino acids are grouped as follows: Group I (hydrophobic side chains): met,

ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

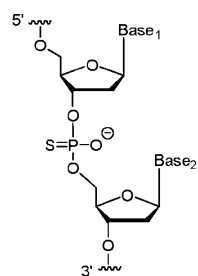
[0080] The terms “identical” or percent “identity,” in the context of two or more polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues, *e.g.*, at least 60% identity, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% or greater, that are identical over a specified region when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one a sequence comparison algorithm or by manual alignment and visual inspection. For sequence comparison of polypeptides, typically one amino acid sequence acts as a reference sequence, to which a candidate sequence is compared. Alignment can be performed using various methods available to one of skill in the art, *e.g.*, visual alignment or using publicly available software using known algorithms to achieve maximal alignment. Such programs include the BLAST programs, ALIGN, ALIGN-2 (Genentech, South San Francisco, Calif.) or Megalign (DNASTAR). The parameters employed for an alignment to achieve maximal alignment can be determined by one of skill in the art. For sequence comparison of polypeptide sequences for purposes of this application, the BLASTP algorithm standard protein BLAST for aligning two proteins sequence with the default parameters is used.

[0081] Percentage sequence identities are determined with antibody or Fc polypeptide sequences maximally aligned by the Kabat numbering convention. After alignment, if a subject antibody region (*e.g.*, the entire mature variable region of a heavy or light chain or Fc polypeptide) is being compared with the same region of a reference antibody, the percentage sequence identity between the subject and reference antibody regions is the number of positions occupied by the same amino acid in both the subject and reference antibody region divided by the total number of aligned positions of the two regions, with gaps not counted, multiplied by 100 to convert to percentage.

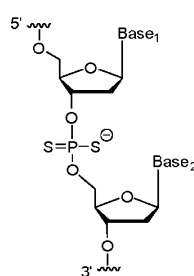
[0082] Nucleic acid sequence identity can be determined by aligning sequences using algorithms, such as BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, Wis.), using default gap parameters, or by inspection, and the best alignment (*i.e.*, resulting in the highest

percentage of sequence similarity over a comparison window). Percentage of sequence identity is calculated by comparing two optimally aligned sequences over a window of comparison, determining the number of positions at which the identical residues occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of matched and mismatched positions not counting gaps in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. Unless otherwise indicated the window of comparison between two sequences is defined by the entire length of the shorter of the two sequences.

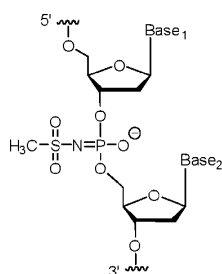
[0083] A modified internucleoside linkage is an internucleoside linkage other than a naturally occurring phosphate linkage. Shown below are modified internucleoside linkages linking the sugar groups (included in the structure) of nucleosides 5' and 3' of the modified internucleoside linkages.



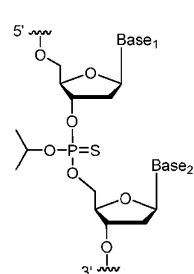
Phosphorothioate (PS)



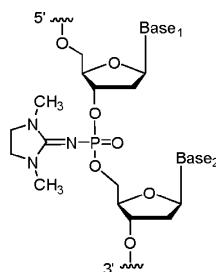
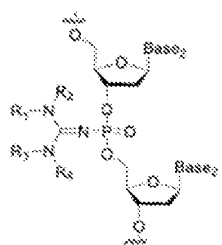
phosphorodithioate (PS2)



mesylphosphoramidate (MsPA)



O-isopropyl phosphorothioate (OiPS)



(a) phosphorylguanidine

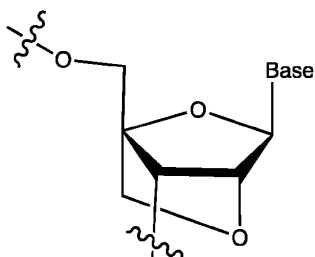
(b) Phosphorylguanidine (PN)

As used herein, PN refers to structure (b).

[0084] As used herein “stabilizing internucleoside linkage” refers to a modified internucleoside linkage that is specifically introduced into the backbone of an oligonucleotide of an oligonucleotide polypeptide conjugate to remove a soft spot (*e.g.*, nuclease sensitive site), increase resistance of the oligonucleotide to cleavage, or otherwise modulate one or more pharmacokinetic or pharmacodynamic properties of the oligonucleotide and/or oligonucleotide polypeptide conjugate into which it is introduced. Stabilizing internucleoside linkages do not include phosphorothioates or phosphodiester. Oligonucleotides and/or oligonucleotide polypeptide conjugates containing one or more stabilizing internucleoside linkages (stabilized oligonucleotides) have improved properties compared to an oligonucleotide or oligonucleotide polypeptide conjugate that has the same oligonucleotide sequence but does not contain the one or more stabilizing internucleoside linkages. Stabilized oligonucleotides and oligonucleotide polypeptide conjugates have improved properties over those having only phosphorothioate internucleoside linkages or a combination of phosphodiester and phosphorothioate internucleoside linkages. A stabilized oligonucleotide may contain phosphorothioate internucleoside linkage(s) and/or phosphodiester internucleoside linkage(s) in addition to the stabilizing internucleoside linkages. In some embodiments, one or more phosphorothioate and/or phosphodiester internucleoside linkages in an oligonucleotide (*e.g.*, an ASO; *e.g.*, a gapmer) are replaced by stabilizing internucleoside linkages. In some embodiments, the one or more phosphorothioate and/or phosphodiester internucleoside linkages can be replaced by one or more stabilizing internucleoside linkages at or near a position identified in the oligonucleotide as being susceptible to cleavage (*e.g.*, a soft spot). In some embodiments, one or more phosphorothioate and/or phosphodiester internucleoside linkages in a wing segment of a gapmer are replaced by stabilizing internucleoside linkages. In some embodiments, one or more phosphorothioate and/or phosphodiester internucleoside linkages in a gap segment of a gapmer are replaced by stabilizing internucleoside linkages. Examples of modified internucleoside linkages that can be used as a stabilizing internucleoside linkage include, but are not limited to, phosphorodithioate, phosphoramidates, mesylphosphoramidates, phosphonates, phosphotriesters, and phosphoryl guanidines (*see*: Nucleic Acids Res., 47, 5465–5479 (2019); Proc. Natl. Acad. Sci. U.S.A., 116, 1229–1234 (2019); Nucleic Acids Research, 50(10), 5401–5423 (2022); Vasquez G., Nucleic Acid Therapeutics, 32(1), 40-50 (2022); and Molecular Therapy: Nucleic Acids; 29: 176-188 (2022)). Additional examples of

modified internucleoside linkages that can be used as a stabilizing internucleoside linkage include, but are not limited to, modified internucleoside linkage s1, s2, s3, s4, s5, s6, s7, s8, s8, s10, s11, s12, s13, s14, s15, s16, s17, or s18 as described in WO 2017210647, the disclosure of which is incorporated by reference in its entirety. Further examples of modified internucleoside linkages that can be used as a stabilizing internucleoside linkage include, but are not limited to, modified internucleoside linkages n001, n002, n003, n004, n005, n006, n007, n008, n009, n010, n020, n025, or n026 as described in WO 2022099159, the disclosure of which is incorporated by reference in its entirety. In some embodiments, the stabilizing internucleoside linkage are selected from the group consisting of PS2, MsPA, OiPS, and PN internucleoside linkages.

[0085] “LNA” refers to a bicyclic nucleoside analogue which comprises a bridge between the 2' and 4' position in the ribose ring (2' to 4' bicyclic nucleotide analogue), and is known as “Locked Nucleic Acid” or “Locked Nucleoside.” As used herein, “LNA oligonucleotide,” refers to an oligonucleotide containing one or more such bicyclic nucleoside analogues. *Biochemistry*, 43(42):13233-13240 (2004). In some embodiments, a LNA provided herein has the following structure, wherein “Base” is a nucleobase: %



[0086] “Expression” refers to the transcription and/or translation of an endogenous gene, heterologous gene or nucleic acid segment, or a transgene in cells. For example, expression may refer to the transcription and stable accumulation of sense (mRNA) or functional RNA. Expression may also refer to the production of protein.

[0087] The term “pharmaceutically acceptable” means that the carrier, diluent, excipient, or auxiliary is compatible with the other ingredients of the formulation and not substantially deleterious to the recipient thereof.

[0088] The terms “subject,” “individual,” and “patient,” refer to a mammal, including, but not limited to, a human, a non-human primate, a rodent (e.g., rat, mouse, and guinea pig), a rabbit, a cow, a pig, a horse, and other mammalian species. In some embodiments, the subject is a

human. The term “subject” includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

[0089] The term “disease” or “condition” refers to any abnormal condition that impairs physiological function. The term is used broadly to encompass any disorder, illness, abnormality, pathology, sickness, condition, or syndrome in which physiological function is impaired, irrespective of the nature of the etiology.

[0090] The terms “treat,” “treatment,” and the like, mean the methods or steps taken to provide relief from or alleviation of the number, severity, and/or frequency of one or more symptoms of a disease or condition in a subject. Treating generally refers to obtaining a desired pharmacological and/or physiological effect. The effect can be, but does not necessarily have to be, prophylactic in terms of preventing or partially preventing a disease, symptom, or condition thereof. The effect can be therapeutic in terms of a partial or complete cure of a disease, condition, symptom, or adverse effect attributed to the disease, disorder, or condition. The term treatment can include: (a) preventing the disease from occurring in a subject who may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *i.e.*, arresting its development; and (c) relieving the disease, *i.e.*, mitigating or ameliorating the disease and/or its symptoms or conditions. Treating can refer to both therapeutic treatment alone, prophylactic treatment alone, or both therapeutic and prophylactic treatment. Those in need of treatment (subjects in need thereof) can include those already with disease or condition or those in which disease or condition is to be prevented. Treating can include inhibiting the disease, disorder, or condition, *e.g.*, impeding its progress; and relieving the disease, disorder, or condition, *e.g.*, causing regression of the disease, disorder, and/or condition. Treating the disease, disorder, or condition can include ameliorating at least one symptom of the particular disease, disorder, or condition, even if the underlying pathophysiology is not affected, *e.g.*, such as treating the symptom without affecting or removing an underlying cause of the symptom. The treatment or amelioration of symptoms can be based on objective or subjective parameters. The effect of treatment can be compared to an individual or pool of individuals not receiving the treatment, or to the same patient prior to treatment or at a different time during treatment.

[0091] A “pharmacologically effective amount,” “therapeutically effective amount,” or simply “effective amount” refers to that amount (dose) of a described active pharmaceutical ingredient or pharmaceutical composition to produce the intended pharmacological, therapeutic, or preventive result. An “effective amount” can also refer to the amount of, for example an excipient, in a pharmaceutical composition that is sufficient to achieve the desired property of

the composition. An effective amount can be administered in one or more administrations, applications, or dosages.

II. Overview

[0092] Oligonucleotide therapies for CNS disorders caused by genetic abnormalities or increased protein accumulation are becoming an increasingly popular approach to modulate gene expression of such neurological disorders. The blood brain barrier (BBB) represents a challenge to the delivery of systemically administered oligonucleotides to the relevant sites of action within the CNS. Intrathecal (IT) delivery, in which drugs are administered directly into the cerebrospinal fluid (CSF) space, enables the bypass of the BBB. However, one limitation of this approach is that delivery of these oligonucleotide therapies directly to the CSF via the IT approach does not achieve uniform distribution throughout the CNS.

[0093] Described are MAPT ASO conjugates that can be administered to a subject intravenously and facilitate transport of the MAPT ASO across the BBB and delivery of the MAPT ASO to CNS cells, where the MAPT ASO provides knockdown of MAPT expression in the brain and spinal cord. The MAPT ASO conjugates comprise a transferrin-targeting Fc polypeptide dimer linked to a MAPT ASO. Conjugation of a MAPT ASO to an Fc polypeptide dimer can be used to increase targeting efficacy of the molecule following administration into a subject. The Fc polypeptide dimer-cargo molecule conjugate has increased targeting efficacy compared to unconjugated molecule. The MAPT ASO comprises modifications that decrease *in vivo* plasma clearance and/or degradation/cleavage when the MAPT ASO is linked to the transferrin-targeting Fc polypeptide dimer.

[0094] In some embodiments, the described MAPT ASO conjugates provide for knockdown of MAPT expression across CNS regions including across brain regions that include deep brain regions, as well as the frontal lobe, parietal lobe, temporal lobe, occipital lobe, and cerebellum. In some embodiments, the described MAPT ASO conjugates provide knockdown of MAPT expression across multiple CNS cell types, including endothelial cells, neurons, astrocytes, oligodendrocytes, and microglia. In some embodiments, the described MAPT ASO conjugates provide for knockdown of MAPT expression in the spinal cord.

[0095] In some embodiments, the described MAPT ASO conjugates provide for knockdown of MAPT expression in skeletal and cardiac muscle. Knockdown of MAPT expression in skeletal, cardiac, and diaphragm muscle can be used to treat neuromuscular disorders.

[0096] The described MAPT ASO conjugates provide for targeted delivery of therapeutic levels of MAPT ASOs to various tissues, including the CNS, following systemic

administration. This targeted delivery allows for the use of a lower dose of the MAPT ASO compared with administration of the MAPT ASO alone, *i.e.*, non-targeted delivery.

[0097] Described are MAPT ASO conjugates comprising a transferrin receptor (TfR)-targeting Fc polypeptide dimer and a MAPT antisense oligonucleotide (ASO), wherein:

(a) the TfR-targeting Fc polypeptide dimer comprises:

(i) a first Fc polypeptide comprising a cysteine at position 239, an A at position 234, an A at position 235, and a serine position 329, each according to EU numbering;

(ii) a second Fc polypeptide comprising an A at position 234, an A at position 235, and serine at position 329, each according to EU numbering, and a modified constant domain that specifically binds to human transferrin receptor 1 (TfR) wherein the second Fc polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:11 or 12, wherein second Fc polypeptide forms an Fc dimer with the first Fc polypeptide; and

(iii) a first non-targeting Fab (NTF) fused to the first Fc polypeptide via a first hinge region to form a first Fab-Fc fusion polypeptide and a second NTF fused to the second Fc polypeptide via a second hinge region to form a second Fab-Fc fusion polypeptide, wherein first and second NTFs each comprise SEQ ID NO:109 or 110, wherein the hinge regions each comprise SEQ ID NO:121; and

(b) the MAPT ASO comprises

5'-ALx_d^mCLx_e^mCLx_fTTAp_aAp_bGp_cTATTACTx_gTLx_hGLx_i^mCL-3' (SEQ ID NO:111)

wherein

AL, ^mCL, GL, and TL are adenine, 5-methylcytosine, guanosine, and thymine locked nucleosides, respectively,

A, C, G, and T are deoxyadenosine, deoxycytidine, deoxyguanine, and deoxythymidine nucleosides, respectively,

each **p** is independently a phosphorothioate (PS) internucleoside linkage, a phosphorodithioate (PS2) internucleoside linkage, a mesylphosphoramidate (MsPA) internucleoside linkage, an *O*-isopropyl phosphorothioate (OiPS) internucleoside linkage, or a phosphorylguanidine (PN) internucleoside linkage,

each **x** is independently a PN internucleoside linkage, a PS internucleoside linkage, or a phosphodiester (PO) internucleoside linkage, and

any internucleoside linkage that is not a PO, PS2, MsPA, OiPS, or PN

internucleoside linkage is a PS internucleoside linkage;

wherein the first Fc polypeptide is linked to the MAPT ASO via a linking group.

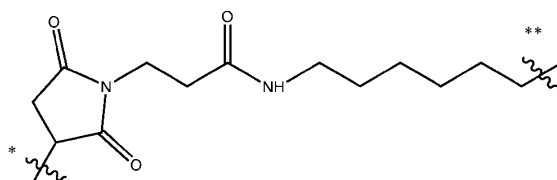
[0098] In some embodiments, at least one of **p_a**, **p_b**, and **p_c**, is not a PS internucleoside linkage.

In some embodiments, at least two of **p_a**, **p_b**, and **p_c**, are not a PS internucleoside linkages.

[0099] In some embodiments, at least two of **x_g**, **x_h**, and **x_i** are PN internucleoside linkages.

[0100] In some embodiments, the modified constant domain of the second Fc polypeptide comprises: an E at position 380; a Y at position 384; a T at position 386; an E at position 387; a W at position 388; an A at position 389; a N at position 390; a T at position 413; an E at position 415; an E at position 416; a Q at position 418; a Q at position 419; a F at position 421; a S at position 424; and a S at position 426, according to EU numbering.

[0101] In some embodiments, the MAPT ASO is linked to the cysteine at position 239 (EU numbering) of the first Fc polypeptide. In some embodiments, the 5' end of the MAPT ASO is linked to the cysteine at position 239 of the first Fc polypeptide, wherein the linking group is



wherein “*wavy*” that intersects a bond in a chemical structure indicates the point of attachment of the bond that the wavy bond intersects in the chemical structure to the remainder of a molecule.

[0102] In some embodiments, the NTF comprises SEQ ID NO:109 or 110 and SEQ ID NO:108.

[0103] Described are MAPT ASO conjugates comprising a transferrin receptor (TfR)-targeting Fc polypeptide dimer and a MAPT antisense oligonucleotide (ASO), wherein:

(a) the TfR-targeting Fc polypeptide dimer comprises:

(i) a first Fc polypeptide comprising a cysteine at position 239, an A at position 234, an A at position 235, and a serine position 329, each according to EU numbering;

(ii) a second Fc polypeptide comprising an A at position 234, an A at position 235, and serine at position 329, each according to EU numbering, and a modified constant domain that specifically binds to human transferrin receptor 1 (TfR) wherein the second Fc polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:11 or 12, wherein second Fc polypeptide forms an Fc dimer with the first Fc polypeptide; and

(iii) a first non-targeting Fab (NTF) fused to the first Fc polypeptide via a first hinge region to form a first Fab-Fc fusion polypeptide and a second NTF fused to the

second Fc polypeptide via a second hinge region to form a second Fab-Fc fusion polypeptide, wherein first and second NTFs each comprise SEQ ID NO:109 or 110, wherein the hinge regions each comprise SEQ ID NO:121; and

(b) the MAPT ASO comprises

5'-CL_xdTL_xeGL_xfTp_jTp_kAp_iGp_mACATTCP_nAp_oTT_xgCL_xhTL_xiCL-3' (SEQ ID NO:112)

wherein

^mCL, GL, and TL are 5-methylcytosine, guanosine, and thymine locked nucleosides, respectively,

A, C, G, and T are deoxyadenosine, deoxycytidine, deoxyguanine, and deoxythymidine nucleosides, respectively,

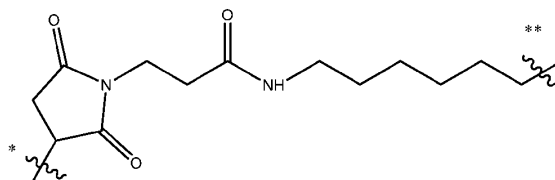
each **p** is independently a phosphorothioate (PS) internucleoside linkage, a phosphorodithioate (PS2) internucleoside linkage, a mesylphosphoramidate (MsPA) internucleoside linkage, an *O*-isopropyl phosphorothioate (OiPS) internucleoside linkage, or a phosphorylguanidine (PN) internucleoside linkage, and at least 2 of p_a, p_b, and p_c, are not PS internucleoside linkages,

each **x** is independently a PN internucleoside linkage or a PS internucleoside linkage, and at least 2 of x_g, x_h, and x_i are PN internucleoside linkages, and

any internucleoside linkage that is not a PS2, MsPA, OiPS, or PN internucleoside linkage is a PS internucleoside linkage;

wherein the first Fc polypeptide is linked to the MAPT ASO via a linking group.

[0104] In some embodiments, the modified constant domain of the second Fc polypeptide comprises: an E at position 380; a Y at position 384; a T at position 386; an E at position 387; a W at position 388; an A at position 389; a N at position 390; a T at position 413; an E at position 415; an E at position 416; a Q at position 418; a Q at position 419; a F at position 421; a S at position 424; and a S at position 426, according to EU numbering. In some embodiments, the MAPT ASO is linked to the cysteine at position 239 (EU numbering) of the first Fc polypeptide. In some embodiments, the 5' end of the MAPT ASO is linked to the cysteine at position 239 of the first Fc polypeptide, wherein the linking group is:



wherein “” that intersects a bond in a chemical structure indicates the point of attachment of the bond that the wavy bond intersects in the chemical structure to the remainder of a molecule.

[0105] In some embodiments, the NTF comprises SEQ ID NO:109 or 110 and SEQ ID NO:108.

A. Transferrin Receptor (TfR)-targeting Fc polypeptide dimer

[0106] In some embodiments, the first Fc polypeptide or first Fab-Fc fusion polypeptide comprises a sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to a human Fc peptide. In some embodiments, the first Fc polypeptide comprises a cysteine at position 239, 442, 330, and 289, according to EU numbering (*i.e.*, the Fc domain comprises a S239C substitution, a S442C substitution, a A330C substitution, a K149C substitution, or a T289C substitution). In some embodiments, the first Fc polypeptide comprises a cysteine at position 239, according to EU numbering. In some embodiments, the first Fc polypeptide further comprises a CH1 domain (*i.e.*, a Fab-Fc fusion polypeptide), wherein the CH1 domain comprises a cysteine at position 114 (according to Kabat numbering) or position 124 (according to EU numbering) (*i.e.*, the first Fc polypeptide comprises an A114C substitution or an S124C substitution). In some embodiments, the first Fc polypeptide comprises LALA-PS mutations (an A at position 234, an A at position 235, and a serine position 329, each according to EU numbering). In some embodiments, the first FC polypeptide comprises a cysteine at position 239 according to EU numbering, LALA-PS mutations, and either knob or hole mutations. In some embodiments, the first Fc polypeptide comprises, LALA-PS mutations, either knob or hole mutations, and further comprises a CH1 domain, wherein the CH1 domain comprises a cysteine at position 114 (according to Kabat numbering) or position 124 (according to EU numbering). In some embodiments, the first Fc polypeptide comprises a sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% to any one of SEQ ID NOs:47-48, 51-54, 61-63, 66, 73-77, and 80. In some embodiments, the first Fc polypeptide comprises the amino acid sequence of SEQ ID NO:63 or 80. In some embodiments, the first Fc polypeptide comprises a cysteine at position 239, an A at position 234, an A at position 235, and a serine position 329 (each according to EU numbering) and has at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to a SEQ ID NO:63 or 80. In some embodiments, the first Fc polypeptide comprises an amino acid sequence consisting of the amino acid sequence of SEQ ID NO:63 or 80.

[0107] In some embodiments, the modified constant domain of the second Fc polypeptide comprises: an E at position 380; a Y at position 384; a T at position 386; an E at position 387; a W at position 388; an A at position 389; a N at position 390; a T at position 413; an E at position 415; an E at position 416; a Q at position 418; a Q at position 419; a F at position 421; a S at position 424; and a S at position 426, according to EU numbering. In some embodiments, the second Fc polypeptide or Fab-Fc fusion polypeptide comprises a E at position 153, Y at position 157, T at position 159, E at position 160, W at position 161, A at position 162, N at position 163, T at position 186, E at position 188, E at position 189, and F at position 194, as numbered with reference to SEQ ID NO:5. In some embodiments, the modified constant domain of the second Fc polypeptide comprises: an E at position 380; a Y at position 384; a T at position 386; an E at position 387; a W at position 388; an A at position 389; a N at position 390; a T at position 413; an E at position 415; an E at position 416; a Q at position 418; a Q at position 419; a F at position 421; a S at position 424; and a S at position 426 (according to EU numbering) and has at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identify to SEQ ID NO:4, 5, 11, or 12. In some embodiments, the second Fc polypeptide comprises a E at position 153, Y at position 157, T at position 159, E at position 160, W at position 161, A at position 162, N at position 163, T at position 186, E at position 188, E at position 189, and F at position 194 as numbered with reference to SEQ ID NO:5 and has at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identify to SEQ ID NO:4, 5, 11, or 12. In some embodiments, the second Fc polypeptide comprises LALA-PS mutations (an A at position 234, an A at position 235, and a serine position 329, each according to EU numbering). In some embodiments, the second Fc polypeptide comprises LALA-PS mutations, and either knob or hole mutations. In some embodiments, the modified constant domain of the second Fc polypeptide comprises: an E at position 380; a Y at position 384; a T at position 386; an E at position 387; a W at position 388; an A at position 389; a N at position 390; a T at position 413; an E at position 415; an E at position 416; a Q at position 418; a Q at position 419; a F at position 421; a S at position 424; and a S at position 426 (according to EU numbering) and has at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to any of SEQ ID NOs:4-29, 36, and 43-44. In some embodiments, the second Fc polypeptide comprises a E at position 153, Y at position 157, T at position 159, E at position 160, W at position 161, A at position 162, N at position 163, T at position 186, E at position 188, E at position 189, and F at position 194 as numbered with reference to SEQ ID NO:5 and has at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identify to any of SEQ ID NOs:4-29, 36, and 43-44.

[0108] In some embodiments, the first Fc polypeptide and/or the second Fc polypeptide comprises one or more mutations or sets of mutations selected from the group consisting of: a knob mutation (*e.g.*, T139W as numbered with reference to SEQ ID NO:5), hole mutations (*e.g.*, T139S, L141A, and Y180V as numbered with reference to SEQ ID NO:5), one or more mutations that modulate effector function (*e.g.*, L7A, L8A, and/or P102G or P102S (*e.g.*, L7A and L8A; L7A, L8A, and P102G; or L7A, L8A, and P102S)) as numbered with reference to SEQ ID NO:5), and/or one or more mutations that increase serum stability (*e.g.*, (i) M25Y, S27T, and T29E as numbered with reference to SEQ ID NO:5, or (ii) N207S with or without M201L as numbered with reference to SEQ ID NO:5). If the first Fc polypeptide contains a knob mutation, then the second Fc polypeptide contains hole mutations. If the second Fc polypeptide contains a knob mutation, then the first Fc polypeptide contains hole mutations.

[0109] In some embodiments, both the first and second Fc polypeptides contain one or more mutations that reduce effector function. In some embodiments, the first and second Fc polypeptides each contain LALA mutations. In some embodiments, the first and second Fc polypeptides each contain LALA-PG mutations. In some embodiments, the first and second Fc polypeptides each contain LALA-PD mutations.

[0110] In some embodiments, the first Fc polypeptide or the second Fc polypeptide contains a knob mutation (*e.g.*, T139W as numbered with reference to SEQ ID NO:5). In some embodiments, the second Fc polypeptide contains a knob mutation and has at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity or at least 99% identity to the sequence of SEQ ID NO:5 or 6. In some embodiments, the second Fc polypeptide comprises the sequence of SEQ ID NO:5 or 6.

[0111] In some embodiments, the first Fc polypeptide or the second Fc polypeptide contains a knob mutation (*e.g.*, T139W as numbered with reference to SEQ ID NO:5), and one or more mutations that modulate effector function (*e.g.*, L7A, L8A, and/or P102G or P102S (*e.g.*, L7A and L8A; L7A, L8A, and P102G; or L7A, L8A, and P102S)) as numbered with reference to SEQ ID NO:5). In some embodiments, the second Fc polypeptide contains a knob mutation and one or more mutations that modulate effector function and has at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity or at least 99% identity to the sequence of SEQ ID NO:7, 9, 11, 8, 10, or 12. In some embodiments, the second Fc polypeptide comprises the sequence of SEQ ID NO:7, 9 or 11. In some embodiments, the second Fc polypeptide comprises the sequence of SEQ ID NO:8, 10, or 12.

[0112] In some embodiments, the first Fc polypeptide or the second Fc polypeptide contains a knob mutation (*e.g.*, T139W as numbered with reference to SEQ ID NO:5), and one or more mutations that increase serum stability (*e.g.*, M25Y, S27T, and T29E as numbered with reference to SEQ ID NO:5). In some embodiments, the second Fc polypeptide contains a knob mutation and one or more mutations that increase serum stability has at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity or at least 99% identity to the sequence of SEQ ID NO:13. In some embodiments, the second Fc polypeptide comprises the sequence of SEQ ID NO:13.

[0113] In some embodiments, the first Fc polypeptide or the second Fc polypeptide contains a knob mutation (*e.g.*, T139W as numbered with reference to SEQ ID NO:5) and one or more mutations that increase serum stability (*e.g.*, N207S with or without M201L as numbered with reference to SEQ ID NO:5). In some embodiments, the second Fc polypeptide contains a knob mutation and one or more mutations that increase serum stability and has at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity or at least 99% identity to the sequence of SEQ ID NO:14. In some embodiments, the second Fc polypeptide comprises the sequence of SEQ ID NO:14.

[0114] In some embodiments, the first Fc polypeptide or the second Fc polypeptide contains a knob mutation (*e.g.*, T139W as numbered with reference to SEQ ID NO:5), one or more mutations that modulate effector function (*e.g.*, L7A, L8A, and/or P102G or P102S (*e.g.*, L7A and L8A; L7A, L8A, and P102G; or L7A, L8A, and P102S)) as numbered with reference to SEQ ID NO:5), and one or more mutations that increase serum stability (*e.g.*, M25Y, S27T, and T29E as numbered with reference to SEQ ID NO:5). In some embodiments, the second Fc polypeptide contains a knob mutation, one or more mutations that modulate effector function, one or more mutations that increase serum stability, and has at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity or at least 99% identity to the sequence of SEQ ID NO:15 or 16. In some embodiments, the second Fc polypeptide comprises the sequence of SEQ ID NO:15 or 16.

[0115] In some embodiments, the first Fc polypeptide or the second Fc polypeptide contains a knob mutation (*e.g.*, T139W as numbered with reference to SEQ ID NO:5), one or more mutations that modulate effector function (*e.g.*, L7A, L8A, and/or P102G or P102S (*e.g.*, L7A and L8A; L7A, L8A, and P102G; or L7A, L8A, and P102S)) as numbered with reference to SEQ ID NO:5), one or more mutations that increase serum stability (*e.g.*, N207S with or without M201L as numbered with reference to SEQ ID NO:5). In some embodiments, the second Fc polypeptide contain a knob mutation, one or more mutations that modulate effector

function, one or more mutations that increase serum stability, and has at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity or at least 99% identity to the sequence of SEQ ID NO:17 or 18. In some embodiments, the second Fc polypeptide comprises the sequence of SEQ ID NO:17 or 18.

[0116] In some embodiments, the first Fc polypeptide or the second Fc polypeptide contains hole mutations (*e.g.*, T139S, L141A, and Y180V as numbered with reference to SEQ ID NO:5). In some embodiments, the second Fc polypeptide contains hole mutations and has at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity or at least 99% identity to the sequence of SEQ ID NO:19. In some embodiments, the second Fc polypeptide comprises the sequence of SEQ ID NO:19.

[0117] In some embodiments, the first Fc polypeptide or the second Fc polypeptide contains hole mutations (*e.g.*, T139S, L141A, and Y180V as numbered with reference to SEQ ID NO:5) and one or more mutations that modulate effector function (*e.g.*, L7A, L8A, and/or P102G or P102S (*e.g.*, L7A and L8A; L7A, L8A, and P102G; or L7A, L8A, and P102S)) as numbered with reference to SEQ ID NO:5). In some embodiments, the second Fc polypeptide may contains hole mutations and one or more mutations that modulate effector function, and has at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity or at least 99% identity to the sequence of SEQ ID NO:20 or 21. In some embodiments, the second Fc polypeptide comprises the sequence of SEQ ID NO:20 or 21.

[0118] In some embodiments, the first Fc polypeptide or the second Fc polypeptide contains hole mutations (*e.g.*, T139S, L141A, and Y180V as numbered with reference to SEQ ID NO:5), one or more mutations that increase serum stability (*e.g.*, M25Y, S27T, and T29E as numbered with reference to SEQ ID NO:5). In some embodiments, the second Fc polypeptide contains hole mutations and one or more mutations that increase serum stability and has at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity or at least 99% identity to the sequence of SEQ ID NO:22. In some embodiments, the second Fc polypeptide comprises the sequence of SEQ ID NO:22.

[0119] In some embodiments, the first Fc polypeptide or the second Fc polypeptide contains hole mutations (*e.g.*, T139S, L141A, and Y180V as numbered with reference to SEQ ID NO:5) and one or more mutations that increase serum stability (*e.g.*, N207S with or without M201L as numbered with reference to SEQ ID NO:5). In some embodiments, the second Fc polypeptide contains hole mutations and one or more mutations that increase serum stability and has at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity,

at least 97% identity, at least 98% identity or at least 99% identity to the sequence of SEQ ID NO:23. In some embodiments, the second Fc polypeptide comprises the sequence of SEQ ID NO:23.

[0120] In some embodiments, the first Fc polypeptide or the second Fc polypeptide contains hole mutations (*e.g.*, T139S, L141A, and Y180V as numbered with reference to SEQ ID NO:5), one or more mutations that modulate effector function (*e.g.*, L7A, L8A, and/or P102G or P102S (*e.g.*, L7A and L8A; L7A, L8A, and P102G; or L7A, L8A, and P102S)) as numbered with reference to SEQ ID NO:5), and one or more mutations that increase serum stability (*e.g.*, M25Y, S27T, and T29E as numbered with reference to SEQ ID NO:5). In some embodiments, the second Fc polypeptide contains hole mutations, one or more mutations that modulate effector function, and one or more mutations that increase serum stability, and has at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity or at least 99% identity to the sequence of SEQ ID NO:24 or 25. In some embodiments, the second Fc polypeptide comprises the sequence of SEQ ID NO:24 or 25.

[0121] In some embodiments, the first Fc polypeptide or the second Fc polypeptide contains hole mutations (*e.g.*, T139S, L141A, and Y180V as numbered with reference to SEQ ID NO:5), one or more mutations that modulate effector function (*e.g.*, L7A, L8A, and/or P102G or P102S (*e.g.*, L7A and L8A; L7A, L8A, and P102G; or L7A, L8A, and P102S)) as numbered with reference to SEQ ID NO:5), and one or more mutations that increase serum stability (*e.g.*, N207S with or without M201L as numbered with reference to SEQ ID NO:5). In some embodiments, the second Fc polypeptide contains hole mutations, one or more mutations that modulate effector function, and one or more mutations that increase serum stability, and has at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity or at least 99% identity to the sequence of SEQ ID NO:26 or 27. In some embodiments, the second Fc polypeptide comprises the sequence of SEQ ID NO:26 or 27.

[0122] In some embodiments, the N-terminus of the second Fc polypeptide includes a hinge sequence or a portion of a hinge sequence (*e.g.*, SEQ ID NO:49 or 50 for the second Fc polypeptide). In some embodiments, the N-terminus of the first Fc polypeptide is further joined to a CH1 region (*e.g.*, SEQ ID NO:86, 87, or 94) or a NTF heavy chain sequence.

[0123] In some embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises a sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to any one of SEQ ID NOs:4, 5, 6, 7, 9, 11, 50, 86, and 87, wherein the polypeptide comprises a E at position 153, Y at position 157, T at position 159, E at position 160, W at

position 161, A at position 162, N at position 163, T at position 186, E at position 188, E at position 189, and F at position 194, as numbered with reference to SEQ ID NO:5; and the first Fc polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of any one of SEQ ID NOs:54, 63, 66, and 90. In some embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises a Q at position 192; a Q at position 193; a S at position 197; and a S at position 199, as numbered with reference to SEQ ID NO:5. In some embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:7, and the first Fc polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:54. In some embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:9, and the first Fc polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:66. In some embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:11, and the first Fc polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:63

[0124] In some embodiments, the N-terminus of the first and/or the second Fc polypeptide includes all or a portion of the hinge region (*e.g.*, TCPPCP (SEQ ID NO:121), DKHTTCP (SEQ ID NO:91), or DKHTCPCP (SEQ ID NO:92)). Thus, in some embodiments, the second Fc polypeptide comprises the sequence of SEQ ID NO:50, and the first Fc polypeptide comprises the sequence of SEQ ID NO:90.

[0125] In certain embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises a sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to any one of SEQ ID NOs:6, 8, 10, 12, 43, 44, 49, and 94, wherein the polypeptide comprises a E at position 153, Y at position 157, T at position 159, E at position 160, W at position 161, A at position 162, N at position 163, T at position 186, E at position 188, E at position 189, and F at position 194, as numbered with reference to SEQ ID NO:5; and the first Fc polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of any one of SEQ ID NOs:48 77, 78, 80, and 101. In some embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises a Q at position 192; a Q at position 193; a S at position 197; and a S at position 199, as numbered with reference to SEQ ID NO:5. In certain embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:8, and the first Fc polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:77. In some embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:10, and the first Fc polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:78.

In some embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:12, and the first Fc polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:80. In some embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:49, and the first Fc polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:48. In some embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:94, and the first Fc polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:101. In certain embodiments, the N-terminus of the first and/or second Fc polypeptide includes a portion of the hinge region (e.g., DKHTTCP (SEQ ID NO:91 or DKHTCPCPP (SEQ ID NO:92)).

[0126] In certain embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises a sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to any one of SEQ ID NOs:29, 36, and 44, wherein the polypeptide comprises a E at position 153, Y at position 157, T at position 159, E at position 160, W at position 161, A at position 162, N at position 163, T at position 186, E at position 188, E at position 189, and F at position 194 (as numbered with reference to SEQ ID NO:5), and a Ser at position 239 (according to EU numbering); and the first Fc polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of any one of SEQ ID NOs:47, 48, 51-54, 61-63, 66, 73-78, 80, and 90. In some embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises a Q at position 192; a Q at position 193; a S at position 197; and a S at position 199, as numbered with reference to SEQ ID NO:5. In certain embodiments, the second polypeptide or second Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:29, and the first polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:48, 53, 54, 74, or 77. In some embodiments, the second polypeptide or second Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:36, and the first polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:61, 66, 75, or 78. In some embodiments, the second polypeptide or second Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:44, and the first polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:74 or 77.

[0127] In certain embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises a sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:44 or 49, wherein the polypeptide comprises a E at position 153, Y at position 157, T at position 159, E at position 160, W at position 161, A at position 162, N at position 163, T at position 186, E at position 188, E at position 189, and F at position

194, as numbered with reference to SEQ ID NO:5; and the first Fc polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of any one of SEQ ID NOs:48, 73-75, and 77. In certain embodiments, the second Fc polypeptide or second Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:44, and the first Fc polypeptide or first Fab-Fc fusion polypeptide comprises the sequence of SEQ ID NO:77.

[0128] In certain embodiments, the second Fab-Fc fusion polypeptide comprises a sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to any one of SEQ ID NOs:4, 5, 7, 9, 50, 86, and 87, wherein the polypeptide comprises a E at position 153, Y at position 157, T at position 159, E at position 160, W at position 161, A at position 162, N at position 163, T at position 186, E at position 188, E at position 189, and F at position 194, as numbered with reference to SEQ ID NO:5; and the first Fab-Fc fusion polypeptide comprises the sequence of any one of SEQ ID NOs:52, 53, and 61.

[0129] In certain embodiments, the second Fab-Fc fusion polypeptide comprises a sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to any one of SEQ ID NOs:6, 8, 10, 43, 49, and 94 wherein the polypeptide comprises a E at position 153, Y at position 157, T at position 159, E at position 160, W at position 161, A at position 162, N at position 163, T at position 186, E at position 188, E at position 189, and F at position 194, as numbered with reference to SEQ ID NO:5; and the first Fab-Fc fusion polypeptide comprises the sequence of any one of SEQ ID NOs:73-75.

[0130] In certain embodiments, the second Fab-Fc fusion polypeptide comprises a sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity any one of SEQ ID NOs:6, 8, 10, 43, 49, and 94 wherein the polypeptide comprises a E at position 153, Y at position 157, T at position 159, E at position 160, W at position 161, A at position 162, N at position 163, T at position 186, E at position 188, E at position 189, and F at position 194, as numbered with reference to SEQ ID NO:5; and the first Fab-Fc fusion polypeptide comprises the sequence of any one of SEQ ID NOs:73-75.

[0131] In some embodiments, the second Fc polypeptide comprises the amino acid sequence of SEQ ID NO:11 or 12. In some embodiments, the first Fc polypeptide comprises an A at position 234, an A at position 235, a serine position 329, a E at position 380, a Y at position 384, a T at position 386, a E at position 387, a W at position 388, an A at position 389, an N at position 390, a T at position 413, a E at position 415, a E at position 416, and a F at position 421, according to EU numbering (each according to EU numbering) and has at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to a

SEQ ID NO:11 or 12. In some embodiments, the first Fc polypeptide comprises an amino acid sequence consisting of the amino acid sequence of SEQ ID NO:11 or 12.

B. Non-targeting Fab

[0132] A non-targeting Fab, (NTFs) comprises a light chain and a heavy chain, wherein the light chain comprises a V_L region and a light chain constant region (CL) and the heavy chain comprises a V_H region and a heavy chain CH1 constant region. In some embodiments, a NTF does not specifically bind to a naturally occurring epitope in a subject. In some embodiments, a NTF does not specifically bind to an antigen expressed in a given mammal, mammalian tissue, or mammalian cell type. The antigen can be a mammalian antigen or an antigen found in the mammal such as from an infectious organism such as a virus, bacteria, fungus, or parasite. The mammal can be, but is not limited to, a non-human primate, a human, or a rodent (*e.g.*, a mouse).

[0133] Specific binding of an antibody to an antigen means an affinity of at least 10⁶ M⁻¹. Specific binding is detectably higher in magnitude and distinguishable from non-specific binding occurring to at least one unrelated target. Nonspecific binding is often the result of van der Waals forces. Non-targeting does not imply the NTF does not bind any antigen with any affinity. Rather, in some embodiments, a NTF does not exhibit specific binding to (a) any protein or epitope in mammalian cell, mammalian tissue, or mammal; (b) any surface accessible protein or epitope on a mammalian cell or mammalian tissue; or (c) any serum accessible protein or epitope in a mammalian tissue, or mammal.

[0134] A NTF comprises three light chain CDRs and three heavy chain CDRs. In some embodiments, the heavy chain CDRs, CDR-H1, CDR-H2, and CDR-H3, comprise: SEQ ID NOs:105, 106 or 128, and 107, respectively, and the light chain CDRs, CDR-L1, CDR-L2, and CDR-L3, comprise SEQ ID NOs:102, 103, and 104, respectively. In some embodiments, the heavy chain CDRs, CDR-H1, CDR-H2, and CDR-H3, comprise: SEQ ID NOs:125, 126 or 128, and 127, respectively, and the light chain CDRs, CDR-L1, CDR-L2, and CDR-L3, comprise SEQ ID NOs:122, 123, and 124, respectively.

[0135] In some embodiments, an NTF comprises a heavy chain comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%, or 100% identity to the amino acid sequence of SEQ ID NO:109 or 110; and a light chain comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%, or 100% identity to the amino acid sequence of SEQ ID NO:108.

[0136] In some embodiments, an NTF comprises a heavy chain comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%, or 100% identity to the amino acid sequence of SEQ ID NO:130 or 131; and a light chain comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%, or 100% identity to the amino acid sequence of SEQ ID NO:129.

[0137] In some embodiment, the NTF comprises a heavy chain sequence comprising SEQ ID NO:109 or 110 and a light chain sequence comprising SEQ ID NO:108. In some embodiment, the NTF comprises a heavy chain sequence comprising SEQ ID NO:109 and a light chain sequence comprising SEQ ID NO:108. In some embodiment, the NTF comprises a heavy chain sequence comprising SEQ ID NO:110 and a light chain sequence comprising SEQ ID NO:108.

[0138] In some embodiments, the NTF comprises a heavy chain sequence comprising SEQ ID NO:130 or 131 and a light chain sequence comprising SEQ ID NO:129. In some embodiments, the NTF comprises a heavy chain sequence comprising SEQ ID NO:130 and a light chain sequence comprising SEQ ID NO:129. In some embodiments, the NTF comprises a heavy chain sequence comprising SEQ ID NO:131 and a light chain sequence comprising SEQ ID NO:129.

[0139] In some embodiments, a NTF comprises a light chain containing an amino acid sequence that is at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:108, and a heavy chain containing an amino acid sequence that is at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:109 or 110, and contains the CDR sequences of SEQ ID NO:102 (CDR-L1), SEQ ID NO:103 (CDR-L2), SEQ ID NO:104 (CDR-L3), SEQ ID NO:105 (CDR-H1), SEQ ID NO:106 (CDR-H2) and SEQ ID NO:107 (CDR-H3) and maintains the non-targeting properties of a NTF comprising a light chain comprising SEQ ID NO:108 and a heavy chain comprising SEQ ID NO:109 or 110.

[0140] In some embodiments, a NTF comprises a light chain containing an amino acid sequence that is at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:129, and a heavy chain containing an amino acid sequence that is at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:130 or 131, and contains the CDR sequences of SEQ ID NO:122 (CDR-L1), SEQ ID NO:123 (CDR-L2), SEQ ID NO:124 (CDR-L3), SEQ ID NO:125

(CDR-H1), SEQ ID NO:126 (CDR-H2) and SEQ ID NO:127 (CDR-H3) and maintains the non-targeting properties of a NTF comprising a light chain comprising SEQ ID NO:129 and a heavy chain comprising SEQ ID NO:130 or 131.

[0141] An NTF light chain and/or heavy chain can contain one or more modifications that facilitate conjugation to one or more cargo molecules. The modifications can be amino acid substitutions or insertions. The amino acid substitutions can be, but are not limited to, a lysine to cysteine substitution at positions 149 (EU numbering) of the light chain, a serine to cysteine substitution at position 156 (EU numbering) of the light chain, an A to cysteine substitution at position 118 (EU numbering (position 114 according to Kabat numbering)) of the heavy chain, or a serine to cysteine substitution at position 124 (UE numbering) of the heavy chain, or a combination thereof.

[0142] In some embodiments, the first Fab-Fc fusion polypeptide comprises a cysteine at position 239, an A at position 234, an A at position 235, and a serine position 329 (each according to EU numbering) and has at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to a SEQ ID NO:100 or 101. In some embodiments, the first Fab-Fc fusion polypeptide comprises a cysteine at position 114 (according to Kabat numbering) or position 124 (according to EU numbering), and an A at position 234, an A at position 235, and a serine position 329 (according to EU numbering). In some embodiments, the second Fab-Fc fusion polypeptide comprises an A at position 234, an A at position 235, a serine position 329, a E at position 380, a Y at position 384, a T at position 386, a E at position 387, a W at position 388, an A at position 389, an N at position 390, a T at position 413, a E at position 415, a E at position 416, and a F at position 421, according to EU numbering (each according to EU numbering) and has at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to a SEQ ID NO:98 or 99. In some embodiments, the first Fab-Fc comprises an amino acid sequence consisting of SEQ ID NO:100 or 101. In some embodiments, the second Fab-Fc comprises an amino acid sequence consisting of SEQ ID NO:98 or 99. In some embodiments, the first Fab-Fc comprises an amino acid sequence of consisting of SEQ ID NO:100 or 101 and the second Fab-Fc comprises an amino acid sequence consisting of SEQ ID NO:98 or 99. In some embodiments, the first Fab-Fc comprises an amino acid sequence of consisting of SEQ ID NO:100 and the second Fab-Fc comprises an amino acid sequence consisting of SEQ ID NO:98. In some embodiments, the first Fab-Fc comprises an amino acid sequence of consisting of SEQ ID NO:100 and the second Fab-Fc comprises an amino acid sequence consisting of SEQ ID NO:99. In some embodiments, the first Fab-Fc comprises an amino acid sequence of consisting of SEQ ID NO:101 and the

second Fab-Fc comprises an amino acid sequence consisting of SEQ ID NO:98. In some embodiments, the first Fab-Fc comprises an amino acid sequence consisting of SEQ ID NO:101 and the second Fab-Fc comprises an amino acid sequence consisting of SEQ ID NO:99.

C. MAPT ASO

[0143] In some embodiments, the nucleobase sequence of the MAPT ASO comprises the nucleobase sequence of SEQ ID NO:111 (5' ACCTTAAGTATTACTTGC 3'). In some embodiments, the nucleobase sequence of the MAPT ASO consists of the nucleobase sequence of SEQ ID NO:111.

[0144] In some embodiments, the nucleobase sequence of the MAPT ASO comprises the nucleobase sequence of SEQ ID NO:112 (5' CTGTTAGACATTCATTCTC 3'). In some embodiments, the nucleobase sequence of the MAPT ASO consists of the nucleobase sequence of SEQ ID NO:112.

[0145] In some embodiments, the MAPT ASO is linked to a delivery vehicle (*e.g.*, a TfR-targeting Fc polypeptide dimer) via the 5' end of the oligonucleotide (*e.g.*, the delivery vehicle is linked to the 5' terminus of the MAPT ASO).

[0146] Described are MAPT ASOs comprising SEQ ID NO:111 or 112 and having modifications that decrease non-specific plasma clearance and/or degradation/cleavage of the MAPT ASO when the MAPT ASO is linked to the peptide delivery vehicle (*e.g.*, a TfR-targeting Fc polypeptide dimer). In some embodiments, the MAPT ASO is a gapmer. In some embodiments, the MAPT ASO sequence consists of the nucleobase sequence of SEQ ID NO:111 or 112.

[0147] In some embodiments, the MAPT gapmer comprises a 5' wing segment having 3, 4, or 5 nucleosides. In some embodiments, the MAPT gapmer comprises a 3' wing segment having 3, 4, or 5 nucleosides. In some embodiments, the MAPT gapmer comprises a 5' wing segment having 3, 4, or 5 nucleosides and a 3' wing segment having 3, 4, or 5 nucleosides.

[0148] In some embodiments, the MAPT gapmer comprises a 5' wing segment having 3 nucleosides. In some embodiments, the MAPT gapmer comprises a 3' wing segment having 3 nucleosides. In some embodiments, the MAPT gapmer comprises a 5' wing segment having 3 nucleosides and a 3' wing segment having 3 nucleosides.

[0149] In some embodiments, the MAPT gapmer comprises a gap segment comprising 8-16 nucleosides. In some embodiments, the MAPT gapmer comprises a gap segment comprising 8, 9, 10, 11, 12, 13, 14, 15, or 16 nucleosides. In some embodiments, the MAPT gapmer

comprises a gap segment comprising 10-14 nucleosides. In some embodiments, the MAPT gapmer comprises a gap segment comprising 12 nucleosides. In some embodiments, the MAPT gapmer comprises a gap segment comprising 13 nucleosides. In some embodiments, the MAPT gapmer comprises a gap segment comprising 14 nucleosides. In some embodiments, each nucleoside in the gap segment comprises a 2'-deoxyribose sugar.

[0150] In some embodiments, a MAPT gapmer comprises: $X_a-Y_b-Z_c$, wherein a is an integer from 2 to 5, b is an integer from 8 to 16, and c is an integer from 2 to 5, each X nucleoside comprises a modified sugar, each Y nucleoside has a 2' deoxyribose, and each Z nucleoside comprises a modified sugar. In some embodiments, a MAPT gapmer comprises a 3-12-3 gapmer. In some embodiments, a MAPT gapmer comprises a 3-13-3 gapmer. In some embodiments, a and c are each 3, b is 12, and each X and Z nucleoside is an LNA.

[0151] In some embodiments, the wing nucleosides of the MAPT gapmer comprise at least one nucleoside having a modified sugar (modified ribose). In some embodiments, every nucleoside in a 5' wing segment, a 3' wing segment, or the 5' and 3' wing segments of a MAPT gapmer comprises a modified sugar. The modified sugar can be, but is not limited to, a 2'-O-methoxyethyl (MOE) sugar moiety or a bicyclic sugar moiety comprising a 2'-4' bridge. The 2'-4' bridge can be, but is not limited to, a $-O-CH_2-$ (locked nucleic acid or locked nucleoside (LNA)) or $-O-CH(CH_3)-$ (constrained ethyl (cEt)). In some embodiments, each wing nucleoside of a MAPT gapmer comprises a bicyclic sugar moiety comprising a $-O-CH_2-$ 2'-4' bridge (LNA).

i. Internucleoside linkages:

[0152] In some embodiments, every internucleoside linkage in a MAPT ASO comprises a modified linkage (*i.e.*, a linkage other than a naturally occurring phosphate linkage).

[0153] In some embodiments, a MAPT ASO comprises at least two stabilizing internucleoside linkages at the end of the MAPT ASO opposite the end linked to the delivery vehicle. If the delivery vehicle is linked to the 5' end of the MAPT ASO, then at least two internucleoside linkages at the 3' end of the MAPT ASO comprise stabilizing internucleoside linkages. In some embodiments, the MAPT ASO further comprises stabilizing internucleoside linkages at the 5' end. In some embodiments, the stabilizing internucleoside linkages having increased stability or nuclease resistance relative to a PS internucleoside linkage. In some embodiments, the stabilizing internucleoside linkages are PN internucleoside linkages.

[0154] In some embodiments, a MAPT ASO comprises at least two PN linkages at the 3' end (*i.e.*, PN linkages between nucleosides at positions $n-2$ (n minus 2) and $n-1$ and between nucleosides at positions $n-1$ and n , where n is 3' terminal nucleoside). In some embodiments,

the MAPT ASO further comprises at least two PN linkages at the 5' end (*i.e.*, PN linkages between nucleosides at positions 1 and 2 and between nucleosides at positions 2 and 3).

[0155] In some embodiments, a MAPT ASO comprises at least three PN linkages at the 3' end (*i.e.*, PN linkages between nucleosides at positions $n-3$ and $n-2$, between nucleosides at positions $n-2$ and $n-1$ and between nucleosides at positions $n-1$ and n , where n is 3' terminal nucleoside). In some embodiments, the MAPT ASO further comprises at least three PN linkages at the 5' end (*i.e.*, PN linkages between nucleosides at positions 1 and 2, between nucleosides at positions 2 and 3, and between nucleosides at positions 3 and 4).

[0156] In some embodiments, the MAPT ASO comprises at least two stabilizing internucleoside linkages between nucleosides at positions 6-9. In some embodiments, the stabilizing internucleoside linkages have increased stability or nuclease resistance relative to a PS internucleoside linkage. In some embodiments, the stabilizing internucleoside linkages are positioned at and/or near a site identified as a soft spot or site of catabolism of the MAPT ASO. A stabilizing internucleoside linkage can be, but is not limited to, a PS2 linkage, a MsPA linkage, an OiPS linkage, or a PN linkage.

[0157] In some embodiments, a MAPT ASO comprises at least two PS2 internucleoside linkages between nucleosides at positions 6-9. In some embodiments, the MAPT ASO comprises PS2 internucleoside linkages between nucleosides at positions 6 and 7 and between nucleosides at positions 7 and 8.

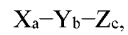
[0158] In some embodiments, a MAPT ASO comprises at least two MsPA internucleoside linkages between nucleosides at positions 6-9. In some embodiments, the MAPT ASO comprises MsPA internucleoside linkages between nucleosides at positions 6 and 7 and between nucleosides at positions 7 and 8. In some embodiments, the MAPT ASO comprises MsPA internucleoside linkages between nucleosides at positions 6 and 7, between nucleosides at positions 7 and 8, and between nucleosides and positions 8 and 9.

[0159] In some embodiments, a MAPT ASO comprises at least two OiPS internucleoside linkages between nucleosides at positions 6-9. In some embodiments, the MAPT ASO comprises OiPS internucleoside linkages between nucleosides at positions 6 and 7 and between nucleosides at positions 7 and 8. In some embodiments, the MAPT ASO comprises OiPS internucleoside linkages between nucleosides at positions 6 and 7, between nucleosides at positions 7 and 8, and between nucleosides and positions 8 and 9.

[0160] In some embodiments, a MAPT ASO comprises at least two PN internucleoside linkages between nucleosides at positions 6-9. In some embodiments, the MAPT ASO comprises PN internucleoside linkages between nucleosides at positions 7 and 8 and between

nucleosides at positions 8 and 9. In some embodiments, the MAPT ASO comprises PN internucleoside linkages between nucleosides at positions 6 and 7, between nucleosides at positions 7 and 8, and between nucleosides and positions 8 and 9.

[0161] In some embodiments, a MAPT ASO comprises:



wherein

(a) a is an integer from 3 to 5, b is an integer from 8 to 16, and c is an integer from 3 to 5;

(b) each X is a nucleoside comprising a modified sugar, each Y is a nucleoside having a 2' deoxyribose, and each Z is nucleoside comprising a modified sugar;

(c) each internucleoside linkage between X nucleosides comprises a PS linkage or a PN linkage and each internucleoside linkage between Z nucleosides comprises a PN linkage;

(d) the internucleoside linkage between X and Y nucleosides and the internucleoside linkage between Y and Z nucleosides independently comprise a PS linkage or a PN linkage;

(e) each internucleoside linkage between Y nucleosides comprises a modified linkage wherein Y_b comprises two to three contiguous PS2 linkages, two to three contiguous MsPA linkages, two to three contiguous OiPS linkages, or two to three contiguous PN linkages, and wherein the other internucleoside linkages between Y nucleosides comprise PS linkages.

[0162] In some embodiments, a and c are each 3. In some embodiments, a and c are each 3, and b is 10-14. In some embodiments, a and c are each 3 and b is 12. In some embodiments, a and c are each 3 and b is 13.

[0163] In some embodiments, a and c are each 3 and b is 12-13, and each internucleoside linkage between X nucleosides comprises a PS linkage.

[0164] In some embodiments, a and c are each 3 and b is 12-13, and each internucleoside linkage between X nucleosides comprises a PN linkage.

[0165] In some embodiments, a and c are each 3 and b is 12-13, each internucleoside linkage between X nucleosides comprises a PS linkage, the internucleoside linkage between X and Y nucleosides comprises a PS linkage, and the internucleoside linkage between Y and Z nucleosides comprises a PS linkage.

[0166] In some embodiments, a and c are each 3 and b is 12-13, each internucleoside linkage between X nucleosides comprises a PS linkage, the internucleoside linkage between X and Y nucleosides comprises a PS linkage, and the internucleoside linkage between Y and Z nucleosides comprises a PN linkage.

[0167] In some embodiments, a and c are each 3 and b is 12-13, each internucleoside linkage between X nucleosides comprises a PN linkage, the internucleoside linkage between X and Y nucleosides comprises a PS linkage and the internucleoside linkage between Y and Z nucleosides comprise a PS linkage.

[0168] In some embodiments, a and c are each 3 and b is 12-13, and each internucleoside linkage between X nucleosides comprises a PN linkage, and the internucleoside linkage between X and Y nucleosides comprises a PN linkage and the internucleoside linkage between Y and Z nucleosides comprise a PN linkage.

Gapmer with PS2, MsPA, OiPS, or PN linkages in gap

[0169] In some embodiments, a and c are each 3 and b is 12-13, and Yb comprises two to three contiguous PS2 linkages. In some embodiments, Yb comprises two contiguous PS2 linkages. In some embodiments, the two contiguous PS2 linkages are between the 3rd and 4th and between the 4th and 5th nucleosides of Yb. In some embodiments, Yb comprises three contiguous PS2 linkages.

[0170] In some embodiments, a and c are each 3 and b is 12-13, and Yb comprises two to three contiguous MsPA linkages. In some embodiments, Yb comprises two contiguous MsPA linkages. In some embodiments, the two contiguous MsPA linkages are between the 3rd and 4th and between the 4th and 5th nucleosides of Yb. In some embodiments, Yb comprises three contiguous MsPA linkages. In some embodiments, the three contiguous MsPA linkages are between the 3rd and 4th, between the 4th and 5th, between the 5th and 6th nucleosides of Yb.

[0171] In some embodiments, a and c are each 3 and b is 12-13, and Yb comprises two to three contiguous OiPS linkages. In some embodiments, Yb comprises two contiguous OiPS linkages. In some embodiments, the two contiguous OiPS linkages are between the 3rd and 4th and between the 4th and 5th nucleosides of Yb. In some embodiments, Yb comprises three contiguous OiPS linkages. In some embodiments, the three contiguous OiPS linkages are between the 3rd and 4th, between the 4th and 5th, between the 5th and 6th nucleosides of Yb.

[0172] In some embodiments, a and c are each 3 and b is 12-13, and Yb comprises two to three contiguous PN linkages. In some embodiments, Yb comprises two contiguous PN linkages. In some embodiments, the two contiguous PN linkages are between the 4th and 5th and between the 5th and 6th nucleosides of Yb. In some embodiments, Yb comprises three contiguous PN linkages.

[0173] In some embodiments, a and c are each 3 and b is 12-13, and Yb comprises two to four stabilizing internucleoside linkages. In some embodiments, Yb comprises PN linkages between the 2nd and 3rd, between the 3rd and 4th, between the 10th and 11th, and between the 11th and 12th

nucleosides of Yb. In some embodiments, Yb comprises two different stabilizing internucleoside linkages. In some embodiments, Yb comprises a PN linkage between the 1st and 2nd nucleosides of Yb and PS2 linkages between the 3rd and 4th and between the 11th and 12th nucleosides of Yb. In some embodiments, Yb comprises two contiguous PS2 linkages and two contiguous PN linkages. In some embodiments, the two contiguous PS2 linkages are between the 3rd and 4th and between the 4th and 5th nucleosides of Yb and the two contiguous PN linkages are between the 10th and 11th and between the 11th and 12th nucleosides of Yb.

[0174] In some embodiments, at least one C nucleoside in X_a or Z_c, if present, comprises a 5-methylcytosine. In some embodiments, any C nucleoside in X_a or Z_c, if present, comprises a 5-methylcytosine.

ii. Specific Combinations

[0175] In some embodiments, a and c are each 3 and b is 12-13, each internucleoside linkage between X nucleosides comprises a PS linkage, the internucleoside linkage between X and Y nucleosides comprises a PS linkage, the internucleoside linkage between Y and Z nucleosides comprises a PS linkage, the internucleoside linkages linking the 4th and 5th and the 5th and 6th nucleosides of Yb comprise PN linkages, and any C nucleoside in X_a or Z_c, if present, comprises a 5-methylcytosine.

[0176] In some embodiments, a MAPT ASO comprises:

5' AL*^mCL*^mCL*T*T*A*AⁿGnT*A*T*T*A*C*T*T_LnGLⁿCL 3' (SEQ ID NO:111)

wherein:

AL is an adenine locked nucleic acid;

^mCL is a 5-methylcytosine locked nucleic acid;

TL is a thymine locked nucleic acid;

GL is a guanine locked nucleic acid;

A is a deoxyadenosine;

C is a deoxycytidine;

T is a deoxythymidine;

G is a deoxyguanosine;

* is a PS internucleoside linkage; and

ⁿ is a PN internucleoside linkage (*see* FIG. 10).

[0177] In some embodiments, a and c are each 3 and b is 12, each internucleoside linkage between X nucleosides comprises a PN linkage, the internucleoside linkage between X and Y nucleosides comprises a PN linkage, the internucleoside linkage between Y and Z nucleosides comprises a PN linkage, the internucleoside linkages linking the 3rd and 4th and the 4th and 5th

nucleosides of Yb comprise PS2 linkages, and any C nucleoside in X_a or Z_c, if present, comprises a 5-methylcytosine.

[0178] In some embodiments, a MAPT ASO comprises:

5' A_L**n**^mC_L**n**^mC_L**n**T*T*As₂As₂G*T*A*T*T*A*C*T**n**T_L**n**G_L**n**^mC_L 3' (SEQ ID NO:111)

wherein:

A_L is an adenine locked nucleic acid;

^mC_L is a 5-methylcytosine locked nucleic acid;

T_L is a thymine locked nucleic acid;

G_L is a guanine locked nucleic acid;

A is a deoxyadenosine;

C is a deoxycytidine;

T is a deoxythymidine;

G is a deoxyguanosine;

* is a PS internucleoside linkage;

s₂ is a PS2 internucleoside linkage; and

n is a PN internucleoside linkage (*see* FIG. 11).

[0179] In some embodiments, a and c are each 3 and b is 12, each internucleoside linkage between X nucleosides comprises a PN linkage, the internucleoside linkage between X and Y nucleosides comprises a PS linkage, the internucleoside linkage between Y and Z nucleosides comprises a PS linkage, the internucleoside linkages linking the 3rd and 4th and the 4th and 5th nucleosides of Yb comprise PS2 linkages, and any C nucleoside in X_a or Z_c, if present, comprises a 5-methylcytosine.

[0180] In some embodiments, a MAPT ASO comprises:

5' A_L**n**^mC_L**n**^mC_L*T*T*As₂As₂G*T*A*T*T*A*C*T*T_L**n**G_L**n**^mC_L 3' (SEQ ID NO:111)

wherein:

A_L is an adenine locked nucleic acid;

^mC_L is a 5-methylcytosine locked nucleic acid;

T_L is a thymine locked nucleic acid;

G_L is a guanine locked nucleic acid;

A is a deoxyadenosine;

C is a deoxycytidine;

T is a deoxythymidine;

G is a deoxyguanosine;

* is a PS internucleoside linkage;

s_2 is a PS2 internucleoside linkage; and

n is a PN internucleoside linkage (*see* FIG. 12).

[0181] In some embodiments, a and c are each 3 and b is 12, each internucleoside linkage between X nucleosides comprises a PS linkage, the internucleoside linkage between X and Y nucleosides comprises a PS linkage, the internucleoside linkage between Y and Z nucleosides comprises a PN linkage, the internucleoside linkages linking the 3rd and 4th, the 4th and 5th, and the 5th and 6th nucleosides of Yb comprise MsPA linkages, and any C nucleoside in X_a or Z_c , if present, comprises a 5-methylcytosine.

[0182] In some embodiments, a MAPT ASO comprises:

5' $ALs^mCLs^mCL^*T^*T^*AuAuGuT^*A^*T^*T^*A^*C^*TnTlnGLn^mCL$ 3' (SEQ ID NO:111)

wherein:

AL is an adenine locked nucleic acid;

mCL is a 5-methylcytosine locked nucleic acid;

TL is a thymine locked nucleic acid;

GL is a guanine locked nucleic acid;

A is a deoxyadenosine;

C is a deoxycytidine;

T is a deoxythymidine;

G is a deoxyguanosine;

$*$ is a PS internucleoside linkage;

u is a MsPA internucleoside linkage; and

n is a PN internucleoside linkage (*see* FIG. 13).

[0183] In some embodiments, a and c are each 3 and b is 12, each internucleoside linkage between X nucleosides comprises a PN linkage, the internucleoside linkage between X and Y nucleosides comprises a PS linkage, the internucleoside linkage between Y and Z nucleosides comprises a PS linkage, the internucleoside linkages linking the 3rd and 4th and the 4th and 5th nucleosides of Yb comprise MsPA linkages, and any C nucleoside in X_a or Z_c , if present, comprises a 5-methylcytosine.

[0184] In some embodiments, a MAPT ASO comprises:

5' $ALn^mCLn^mCL^*T^*T^*AuAuG^*T^*A^*T^*T^*A^*C^*T^*TlnGLn^mCL$ 3' (SEQ ID NO:111)

wherein:

AL is an adenine locked nucleic acid;

mCL is a 5-methylcytosine locked nucleic acid;

TL is a thymine locked nucleic acid;

G_L is a guanine locked nucleic acid;
A is a deoxyadenosine;
C is a deoxycytidine;
T is a deoxythymidine;
G is a deoxyguanosine;
* is a PS internucleoside linkage;
u is a MsPA internucleoside linkage; and
n is a PN internucleoside linkage (*see* FIG. 14).

D. Linking Group

[0185] The MAPT ASO can be linked to the first Fc polypeptide, the second Fc polypeptide, the first NTF, and/or the second NTF. In some embodiments, the MAPT ASO is covalently linked to the first Fc polypeptide, the second Fc polypeptide, the first NTF, and/or the second NTF. The first Fc polypeptide, the second Fc polypeptide, the first NTF, and/or the second NTF can contain an amino acid substitution (*e.g.*, a cysteine substitution) to facilitate attachment of the MAPT ASO.

[0186] In some embodiments, the MAPT ASO is linked to the first Fc polypeptide. In some embodiments, the MAPT ASO is linked to a cysteine residue in the first Fc polypeptide. In some embodiments, the cysteine residue in the first Fc polypeptide comprises a cysteine substitution at position 239 (according to EU numbering). In some embodiments, the first Fc polypeptide comprises a cysteine at position 239, 442, 330, and 289, according to EU numbering. In some embodiments, the cysteine residue in the first Fab-Fc fusion polypeptide comprises a cysteine substitution at position 114 (according to Kabat numbering) or position 124 (according to EU numbering).

[0187] The MAPT ASO can be linked to the first Fc polypeptide, the second Fc polypeptide, or the first NTF, and/or the second NTF via any linking group available in the art suitable for linking an oligonucleotide (*e.g.*, an ASO, *e.g.*, a gapmer) to a polypeptide (*e.g.*, a Fc polypeptide or a Fab polypeptide). In some embodiments, the linking group comprises any known linking group that is a bifunctional linker capable of covalently linking an oligonucleotide to a polypeptide.

[0188] Oligonucleotide polypeptide conjugates can be generated using well-known chemical cross-linking reagents and protocols that covalently link an oligonucleotide and a polypeptide through a linking group. For example, there are a large number of chemical cross-linking agents that are known to those skilled in the art and useful for cross-linking a protein with an agent of

interest. For example, the cross-linking agents can be heterobifunctional cross-linkers, which can be used to link molecules in a stepwise manner. Heterobifunctional cross-linkers provide the ability to design more specific coupling methods for conjugating proteins, thereby reducing the occurrences of unwanted side reactions such as homo-protein polymers. A wide variety of heterobifunctional cross-linkers are known in the art, including, but not limited to, N-hydroxysuccinimide (NHS) or its water soluble analog N-hydroxysulfosuccinimide (sulfo-NHS), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), N-succinimidyl (4-iodoacetyl)aminobenzoate (SIAB), succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), 4-succinimidylloxycarbonyl- α -methyl- α -(2-pyridyldithio)-toluene (SMPT), N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), and succinimidyl 6-[3-(2-pyridyldithio)propionate]hexanoate (LC-SPDP). Those cross-linking agents having N-hydroxysuccinimide moieties can be obtained as the N-hydroxysulfosuccinimide analogs, which generally have greater water solubility. In addition, those cross-linking agents having disulfide bridges within the linking chain can be synthesized instead as the alkyl derivatives to reduce the amount of linker cleavage *in vivo*. In addition to the heterobifunctional cross-linkers, there exist a number of other cross-linking agents including homobifunctional and photoreactive cross-linkers. Disuccinimidyl suberate (DSS), bismaleimido hexane (BMH) and dimethylpimelimidate.2HCl (DMP) are examples of useful homobifunctional cross-linking agents, and bis-[B-(4-azidosalicylamido)ethyl]disulfide (BASED) and N-succinimidyl-6(4'-azido-2'-nitrophenyl-amino)hexanoate (SANPAH) are examples of useful photoreactive cross-linkers.

[0189] Non-limiting examples of conjugate linkers include those described in WO2023/279099 and WO2023/056388, each of which is incorporated by reference in its entirety. In some embodiments, the linking group comprises a val-cit linker as described in U.S. 6,214,345, which is incorporated herein by reference. In some embodiments, the linking group comprises those described in WO2020/028840 and WO2022/212886, each of which is incorporated by reference in its entirety. Other linkers can include those described in Bioconjugate Chemistry 2023 34 (11), 2096-2111.

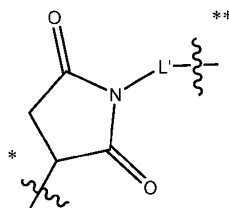
[0190] The linking group may be attached to any region of the polypeptide, (*e.g.*, to the N-terminal region, to the C-terminal region, or to an amino acid within the protein, such as a cysteine residue or a glutamine residue), so long as the oligonucleotide does not prevent binding of the second Fc polypeptide to TfR. Similarly, the linking group may be attached to any region of the oligonucleotide (*e.g.*, the 5' end, the 3' end or to a nucleic acid residue within

the molecule), so long as the polypeptide does not interfere with the functionality of the oligonucleotide (*e.g.*, complementary binding to a target nucleic acid). For example, the linker may be attached to the oligonucleotide through any number of synthetically feasible points located throughout the oligonucleotide, such as at the 3' or 5' terminal residues of the oligo; at a sugar moiety; at a base moiety; or at a residue located within the backbone. In some embodiments, the linker is attached to the oligonucleotide at the 5' terminal residue of the oligonucleotide.

[0191] In some embodiments, the linking group comprises a spacer. The spacer can be, but is not limited to, a hydrophilic spacer. The hydrophilic spacer can be, but is not limited to, polyethylene glycol (PEG).

[0192] The linking group can be a cleavable linking group or a non-cleavable linking group. In some embodiments, the linking group is cleavable. A cleavable linking group contains a cleavable linker. Cleavable linkers include, but are not limited to, nuclease-cleavable linkers, acid-labile linkers, peptidase-sensitive linkers, photolabile linkers, dimethyl linkers, and disulfide-containing linkers (Chari *et al.*, *Cancer Res.* 52:127-131 (1992); U.S. Pat. No. 5,208,020).

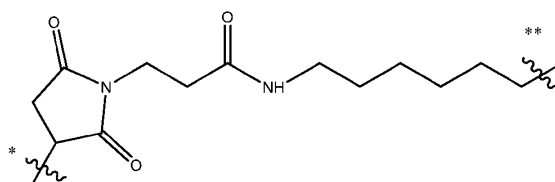
[0193] In some embodiments, the linking group comprises a maleimide. In some embodiments, the linking group has the following structure:



wherein L' is a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 2 to 25 carbon atoms, wherein one or more (*e.g.*, 1, 2, 3, or 4) of the carbon atoms is optionally replaced by $(-O-)$, $(-NH-)$, $(-S-)$, an amino acid, a hydrazone $(-C(R')=N=N(R')-)$, a nucleotide, or a 3-12 membered divalent heterocycle, wherein the chain and any 3-12 membered divalent heterocycle is optionally substituted with one or more (*e.g.*, 1, 2, 3, or 4) substituents independently selected from the group consisting of (C_1-C_6) alkoxy, (C_3-C_6) cycloalkyl, (C_1-C_6) alkanoyl, (C_1-C_6) alkanoyloxy, (C_1-C_6) alkoxycarbonyl, (C_1-C_6) alkylthio, azido, cyano, nitro, halo, hydroxy, oxo ($=O$), a hydrazone ($=N=N(R')-$) carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy; wherein each R' is independently H or (C_1-C_6) alkyl; and wherein the valence marked * is attached to the polypeptide and the valence

marked ** is attached to the MAPT ASO (or a 5' terminal group of the MAPT ASO). In another embodiment, L' is a divalent, branched or unbranched, saturated or unsaturated, chain having from 2 to 25 atoms selected from carbon, oxygen, nitrogen, and sulfur, wherein the chain comprises one or more disulfide linkages. In another embodiment, L' is a divalent, branched or unbranched, saturated or unsaturated, chain having from 2 to 25 atoms selected from carbon, oxygen, nitrogen, and sulfur, wherein the chain comprises one or more hydrazone groups in the chain or appended to a carbon atom of the chain. In another embodiment, L' is a divalent, branched or unbranched, saturated or unsaturated, chain having from 2 to 35 atoms selected from carbon, oxygen, nitrogen, and sulfur, wherein the chain comprises one or more amino acids in the chain. In another embodiment, L' is a divalent, branched or unbranched, saturated or unsaturated, chain having from 2 to 35 atoms selected from carbon, oxygen, nitrogen, and sulfur, wherein the chain comprises a dipeptide in the chain. In another embodiment, L' is a divalent, branched or unbranched, saturated or unsaturated, chain having from 2 to 35 atoms selected from carbon, oxygen, nitrogen, and sulfur, wherein the chain comprises the dipeptide Val-Cit in the chain. In another embodiment, L' comprises one or more nucleotides. In another embodiment, L' comprises two or more nucleotides. In another embodiment, L' comprises a tri-nucleotide group. In another embodiment, L' comprises one or more nucleotides having unmodified bases, unmodified sugar groups and/or unmodified phosphate groups.

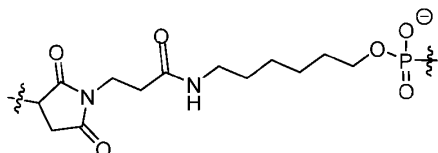
[0194] In some embodiments, the linking group comprises the following structure:



wherein “~” that intersects a bond in a chemical structure indicates the point of attachment of the bond that the wavy bond intersects in the chemical structure to the remainder of a molecule. In some embodiments, the valence marked * is attached to the TfR-targeting Fc polypeptide dimer and the valence marked ** is attached to the MAPT ASO. In some embodiments, the valence marked ** is attached to the MAPT ASO through a 5' terminal group of the MAPT ASO. In some embodiments, the valence marked ** is attached to the MAPT ASO through a 5' terminal PO group of the MAPT ASO. In some embodiments, the valence marked ** is attached to the MAPT ASO through a 5' terminal PS group of the MAPT ASO. In some embodiments, the valence marked ** is attached to the MAPT ASO through a 5' terminal PS2 group of the MAPT ASO. In some embodiments, the valence marked ** is

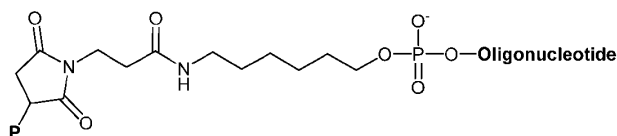
attached to the MAPT ASO through a 5' terminal PN group of the MAPT ASO. In some embodiments, the valence marked ** is attached to the MAPT ASO through a 5' terminal MsPA group of the MAPT ASO. In some embodiments, the valence marked ** is attached to the MAPT ASO through a 5' terminal OiPS group of the MAPT ASO.

[0195] In some embodiments, the linking group comprises:



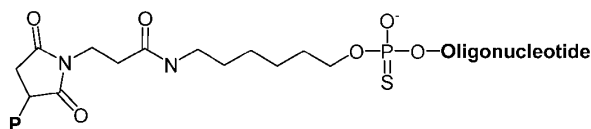
wherein a wavy line “~” that intersects a bond in a chemical structure indicates the point of attachment of the bond that the wavy bond intersects in the chemical structure to the remainder of a molecule.

[0196] In certain embodiments, a stabilized oligonucleotide polypeptide conjugate comprises:



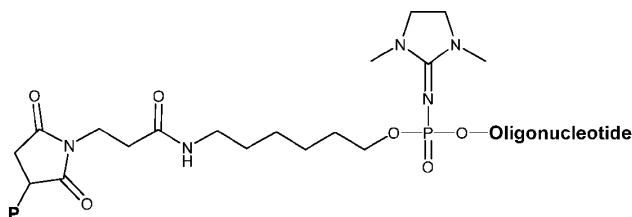
wherein P is the polypeptide, and wherein the phosphodiester (PO) group is a 5' terminal group of the oligonucleotide.

[0197] In certain embodiments, a stabilized oligonucleotide polypeptide conjugate comprises:



wherein P is the polypeptide, and wherein the phosphorothioate (PS) group is a 5' terminal group of the oligonucleotide.

[0198] In certain embodiments, a stabilized oligonucleotide polypeptide conjugate comprises:



wherein P is the polypeptide, and wherein the cyclic phosphoryl guanidine (PN) group is a 5' terminal group of the oligonucleotide.

NUCLEIC ACIDS

[0199] Described herein are nucleic acids encoding any of the heavy and light chains of any of the described Fc peptides, Fc polypeptide dimers, NTFs, and/or Fab-Fc fusion polypeptides. Optionally, such nucleic acids further encode a signal peptide. Coding sequences of nucleic acids can be operably linked to one or more regulatory sequences to facilitate expression of the coding sequences in a host cell. Such regulatory sequence includes, but are not limited to, a promoter, an enhancer, a ribosome binding site, a transcription termination signal, and the like. The nucleic acids encoding heavy and light chains can occur in isolated form or can be cloned into one or more vectors. The nucleic acids can be synthesized by, for example, solid state synthesis or PCR of overlapping oligonucleotides. Nucleic acids encoding heavy and light chains can be joined as one contiguous nucleic acid, *e.g.*, within an expression vector, or can be separate, *e.g.*, each cloned into its own expression vector.

[0200] In some embodiments, nucleic acids encoding the Fc polypeptides, Fc polypeptide dimers, NTFs, or Fab-Fc fusion polypeptides comprise nucleotide sequences encoding the amino acid sequences of any of the described Fc peptides, Fc polypeptide dimers, NTFs, and/or Fab-Fc fusion polypeptides. The nucleic acid sequences can be provided in expression vectors to facilitate expression of the heavy and/or light chains in a host cell. The nucleic acid or expression vector containing the nucleic acid is transformed into a host cell. The host cell can then be used to produce the heavy and/or light chains of the NTF.

[0201] In some embodiments, nucleic acids encoding a TfR-targeting Fc polypeptide dimer comprise a first nucleic acid sequence encoding the first Fc fusion polypeptide, and a second nucleic acid sequence encoding the second Fab-Fc fusion polypeptide. In some embodiments the first nucleic acid sequence encodes any one of SEQ ID NOs:1, 47-48, 51-54, 61-63, 66, 73-78, 80, and 90; and the second nucleic acid sequence encodes any one of SEQ ID NOs:4-29, 36, 43-44, and 49-50.

[0202] In some embodiments, nucleic acids encoding a TfR-targeting Fc polypeptide dimer comprise a first nucleic acid sequence encoding a first Fab-Fc fusion polypeptide, a second nucleic acid sequence encoding a second Fab-Fc fusion polypeptide and a third nucleic acid sequence encoding a NTF light chain. In some embodiments the first nucleic acid sequence encodes any one of SEQ ID NOs:100-101 and 130-131; the second nucleic acid sequence encodes any one of SEQ ID NOs:98-99, and the third nucleic acid sequence encodes any one of SEQ ID NOs:108 and 129.

[0203] In some embodiments, the first nucleic acid sequence comprises SEQ ID NO:133 or a sequence having at least 75% identity to SEQ ID NO:133; the second nucleic acid sequence comprises SEQ ID NO:132 or a sequence having at least 75% identity to SEQ ID NO:132; and the third nucleic acid sequence comprises SEQ ID NO:134 or a sequence having at least 75% identity to SEQ ID NO:134. In some embodiments the first nucleic acid sequence comprises SEQ ID NO:133; the second nucleic acid sequence comprises SEQ ID NO:132, and the third nucleic acid sequence comprises SEQ ID NO:134.

[0204] Methods of preparing the described Fc polypeptides, Fc polypeptide dimers, NTFs, Fab-Fc fusion polypeptides, or antibodies include, but are not limited to, expressing in a plurality of host cells one or more nucleic acids encoding the Fc polypeptide, Fc polypeptide dimer, NTF, Fab-Fc fusion polypeptide, or heavy and light chains of a NTF, propagating the cells under conditions suitable for expression of the Fc polypeptide, Fc polypeptide dimer, NTF, Fab-Fc fusion polypeptide, or heavy and light chains of the NTF in the cells, and purifying the Fc polypeptide, Fc polypeptide dimer, NTF, Fab-Fc fusion polypeptide, or heavy and light chains of the NTF. The Fc polypeptide, Fc polypeptide dimer, Fab-Fc fusion polypeptide, purified NTF can be conjugated to one or more of the described MAPT ASOs.

[0205] Described are cells containing nucleic acids encoding the described Fc polypeptides, Fc polypeptide dimers, NTFs, Fab-Fc fusion polypeptides, or heavy and light chains of the Fab. The cell can be a bacterial cell, a yeast cell, an insect cell, or a mammalian cell. The cell can be used to express the Fc polypeptide, Fc polypeptide dimer, a NTF, or a Fc polypeptide-NTF heavy chain fusion polypeptide. The expressed polypeptides can then be isolated and optionally purified from the cell.

METHODS OF USE

[0206] A MAPT ASO conjugate as described herein may be used for a variety of purposes, including therapeutic indications.

[0207] In some embodiments, the MAPT ASO conjugate is used to deliver a MAPT ASO to a target cell type that expresses the transferrin receptor. In some embodiments, a MAPT ASO conjugate may be used to transport a MAPT ASO across an endothelium, *e.g.*, the blood-brain barrier, to be taken up by the brain.

[0208] In some embodiments, methods of reducing the expression of a MAPT gene in a subject are described, the methods comprising administering an effective amount of a MAPT ASO conjugate or composition thereof as described herein to the subject. In some embodiments, MAPT ASO conjugates or compositions thereof for use in reducing the expression of a MAPT

gene in a cell of subject are provided. In certain embodiments, the MAPT ASO binds to a MAPT transcript and recruits RNase H, which degrades the transcript. In certain embodiments, administration of a described MAPT ASO conjugate or composition thereof to a cell or subject reduces expression of the MAPT gene in the cell or subject. Expression can be reduced by more than 10%, more than 20%, more than 30%, more than 40%, more than 50%, more than 60%, more than 70%, more than 80%, more than 90%, or more than 95%, compared to the expression in a control (*e.g.*, a cell or subject that was not administered the MAPT ASO, MAPT ASO conjugate or composition as described herein) or compared to the level of expression of MAPT in the cell or subject prior to administration of the MAPT ASO.

[0209] For example, certain embodiments provide a method for transcytosis of a MAPT ASO across an endothelium, the method comprising contacting the endothelium (*e.g.*, blood-brain barrier (BBB)) with a MAPT ASO conjugate as described herein. Thus, certain embodiments provide a method of transporting a MAPT ASO across the BBB of a subject in need thereof, comprising administering a MAPT ASO conjugate as described herein to the subject. In certain embodiments, MAPT ASO conjugates as described herein for use in transporting a MAPT ASO across the BBB of a subject in need thereof are provided. In some embodiments, provided herein are methods of delivering MAPT ASOs to the CNS. In some embodiments, provided herein are methods of delivering a MAPT ASO to deep brain regions (*e.g.*, cortex, brainstem, hippocampus, striatum, cerebellum, thalamus, caudate putamen, and substantia nigra). In some embodiments, provided herein are methods of delivering a MAPT ASO to deep brain regions and spinal cord (*e.g.*, cervical spinal cord, lumbar spinal cord). In some embodiments, provided herein are methods of delivering a MAPT ASOs to the CNS and muscle (*e.g.*, cardiac and skeletal). In some embodiments, provided herein are methods of delivering a MAPT ASOs to the CNS, peripheral nerves (*e.g.*, retina, sciatic nerve), muscle (*e.g.*, quadricep), and other peripheral organs (*e.g.*, heart, diaphragm, spleen, intestine, lung, liver, and kidney).

[0210] Certain embodiments also provide methods of modulating the expression MAPT in a subject in need thereof, comprising administering an effective amount of a MAPT ASO conjugate as described herein to the subject. In some embodiments, the MAPT ASO conjugates as described herein are for use in modulating the expression of MAPT in a subject. In certain embodiments, the MAPT is expressed in a cell in the brain of a subject.

[0211] A MAPT ASO or a MAPT ASO conjugate as described herein may be administered to a subject at a therapeutically effective amount or dose. The dosages, however, may be varied according to several factors, including the chosen route of administration, the formulation of the composition, patient response, the severity of the condition, the subject's weight, and the

judgment of the prescribing physician. The dosage can be increased or decreased over time, as required by an individual patient.

[0212] In various embodiments, a MAPT ASO or a MAPT ASO conjugate as described herein is administered parenterally. In some embodiments, the MAPT ASO or MAPT ASO conjugate is administered intravenously. Intravenous administration can be by infusion, *e.g.*, over a period of from about 10 to about 30 minutes, or over a period of at least 1 hr, 2 hr, or 3 hr. In some embodiments, the MAPT ASO or MAPT ASO conjugate is administered as an intravenous bolus. Combinations of infusion and bolus administration may also be used.

[0213] In some parenteral embodiments, a MAPT ASO or a MAPT ASO conjugate is administered intraperitoneally, subcutaneously, intradermally, or intramuscularly. In some embodiments, the MAPT ASO or MAPT ASO conjugate is administered intradermally or intramuscularly. In some embodiments, the MAPT ASO or MAPT ASO conjugate is administered intrathecally, such as by epidural administration, or intracerebroventricularly.

[0214] In other embodiments, a MAPT ASO or MAPT ASO conjugate as described herein may be administered orally, by pulmonary administration, intranasal administration, intraocular administration, or by topical administration. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0215] The MAPT ASOs and MAPT ASO conjugates described herein may also be used to treat, prevent, or ameliorate of diseases, disorders, and conditions associated with Tau. Thus, in some embodiments, provided herein are methods of treatment, prevention, or amelioration of diseases, disorders, and conditions associated with Tau in a subject in need thereof. In certain embodiments, tau associated disease is a tau associated neurodegenerative disorder. In certain embodiments, tau associated diseases include, but are not limited to, tauopathies, Alzheimer's Disease, Fronto-temporal Dementia (FTD), FTDP-17, Progressive Supranuclear Palsy (PSP), Chronic Traumatic Encephalopathy (CTE), Corticobasal Ganglionic Degeneration (CBD), Epilepsy, and Dravet's Syndrome.

[0216] Accordingly, certain embodiments provide methods of treating a tau-associated neurodegenerative disorder in a subject in need thereof, the method comprising administering to the subject a MAPT ASO, a MAPT ASO conjugate or a composition as described herein.

[0217] In certain embodiments, the tau-associated neurodegenerative syndrome is Alzheimer's Disease. Thus, certain embodiments provide a method of treating Alzheimer's disease, the method comprising administering to a subject in need thereof, a MAPT ASO, a MAPT ASO conjugate or a composition as described herein. The subject may be diagnosed with Alzheimer's disease, diagnosed with one or more symptoms of Alzheimer's disease, or be at

risk of developing Alzheimer's disease or one or more symptoms associated with Alzheimer's disease.

[0218] Described are methods of targeting delivery of an MAPT ASO to CNS tissue in a patient comprising administering to the subject any of the described MAPT ASO conjugates or pharmaceutical compositions. In some embodiments, the MAPT ASO is distributed throughout the CNS. In some embodiments, the MAPT ASO is distributed across brain regions. Brain regions include, but are not limited to, frontal lobe, parietal lobe, temporal lobe, occipital lobe, and cerebellum. In some embodiments, the MAPT ASO is distributed to a deep brain region. In some embodiments, the MAPT ASO is distributed to the spinal cord. In some embodiments, the MAPT ASO modulates the expression of a target gene. In some embodiments, modulation of target gene expression is inhibition of gene expression (*i.e.*, gene knockdown).

[0219] In certain embodiments, the subject is a human subject.

PHARMACEUTICAL COMPOSITIONS AND KITS

[0220] In some embodiments, pharmaceutical compositions and kits comprising a MAPT ASO conjugate as described herein are provided.

A. Pharmaceutical compositions

[0221] Guidance for preparing formulations for use as described herein can be found in any number of handbooks for pharmaceutical preparation and formulation that are known to those of skill in the art.

[0222] In some embodiments, a pharmaceutical composition comprises a MAPT ASO conjugate as described herein and further comprises one or more pharmaceutically acceptable carriers and/or excipients.

[0223] In certain embodiments, the composition comprises a plurality of MAPT ASO conjugates as described herein, which can be the same or different (*e.g.*, a mixture of different conjugates).

[0224] In some embodiments, an MAPT ASO conjugate as described herein comprises 1-8 (1, 2, 3, 4, 5, 6, 7, or 8) MAPT ASOs linked to one or more amino acids of the MAPT ASO conjugate. In some embodiments, an MAPT ASO conjugate comprises one (1) MAPT ASO linked to an amino acid of the MAPT ASO conjugate. In some embodiments, an MAPT ASO conjugate comprises two (2) MAPT ASOs linked to two amino acids of the MAPT ASO conjugate. In some embodiments, an MAPT ASO conjugate comprises two (2) MAPT ASOs

linked to one amino acid of the MAPT ASO conjugate. In some embodiments, an MAPT ASO conjugate comprises four (4) MAPT ASOs linked to four amino acids of the MAPT ASO conjugate. In some embodiments, an MAPT ASO conjugate comprises four (4) MAPT ASOs linked to two amino acids of the MAPT ASO conjugate (2 MAPT ASOs linked to each of two amino acids). In some embodiments, an MAPT ASO conjugate comprises four (4) MAPT ASOs linked to one amino acid of the MAPT ASO conjugate.

[0225] In some embodiments, a single MAPT ASO is attached to the TfR-targeting Fc dimer or Fab-Fc dimer. In some embodiments, two or more MAPT ASOs are attached to the TfR-targeting Fc dimer or Fab-Fc dimer. Two or more MAPT ASOs can be linked to a single linking group or two or more MAPT ASOs can also be linked to the TfR-targeting Fc dimer or Fab-Fc dimer via two or more linking groups. The two or more linked groups can be the same or different. In some embodiments, two MAPT ASOs are attached to the TfR-targeting Fc dimer or Fab-Fc dimer. In some embodiments, four MAPT ASOs are attached to the TfR-targeting Fc dimer or Fab-Fc dimer.

[0226] In some embodiments, 1 MAPT ASO is attached to a single linking group (L). In some embodiments, 2 MAPT ASOs are attached to a single linking group (L). In some embodiments, two or more MAPT ASOs are linked in tandem to a single linker (L). For tandem linkage, L may be linked to the 5' end of a first MAPT ASO and a second MAPT ASO is linked to the 3' end of the first MAPT ASOs. Alternatively, for tandem linkage, L may be linked to the 3' end of a first MAPT ASO and a second MAPT ASO is linked to the 5' end of the first MAPT ASOs. The first and second MAPT ASO may be linked to each other via a nucleic acid linker or a non-nucleic acid cleavable linker.

[0227] In some embodiments, two or more MAPT ASOs are linked to a single branched linker (L). The linker can be a branched linking group wherein 2 or more oligonucleotides are attached separately to a single linking group (L) (*i.e.*, y is 2 or more).

[0228] In certain embodiments, the ratio of oligonucleotide to protein in the composition is about 1:1 to about 4:1. In certain embodiments, the ratio of oligonucleotide to protein in the composition is about 1:1 to about 2:1. In certain embodiments, the ratio of oligonucleotide to protein in the composition is about 1.23. In certain embodiments, the ratio of oligonucleotide to protein in the composition is about 2:1 to about 3:1. In certain embodiments, the ratio of oligonucleotide to protein in the composition is about 2.5.

[0229] As used herein, the term pharmaceutically acceptable carrier includes any solvents, dispersion media, or coatings that are physiologically compatible and that preferably does not interfere with or otherwise inhibit the activity of the active agent. Various pharmaceutically

acceptable excipients are well-known. In some embodiments, the carrier is suitable for intravenous, intrathecal, intracerebroventricular, intramuscular, oral, intraperitoneal, transdermal, topical, or subcutaneous administration. Pharmaceutically acceptable carriers can contain one or more physiologically acceptable compounds that act, for example, to stabilize the composition or to increase or decrease the absorption of the MAPT ASO conjugate. Physiologically acceptable compounds can include, for example, carbohydrates, such as glucose, sucrose, dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins, compositions that reduce the clearance or hydrolysis of the active agents, or excipients or other stabilizers and/or buffers. Other pharmaceutically acceptable carriers and their formulations are also available in the art.

[0230] The pharmaceutical compositions described herein can be manufactured in a manner that is known to those of skill in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, emulsifying, encapsulating, entrapping, or lyophilizing processes. The following methods and excipients are merely exemplary and are in no way limiting.

[0231] For administration to a subject, a MAPT ASO conjugate as described herein can be formulated by combining it with pharmaceutically acceptable carriers that are well-known in the art. Such carriers enable the compounds to be formulated as tablets, pills, dragees, capsules, emulsions, lipophilic and hydrophilic suspensions, liquids, gels, syrups, slurries, suspensions and the like. Pharmaceutical preparations for administration to a subject use can be obtained by mixing the MAPT ASO conjugates with a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include, for example, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone. If desired, disintegrating agents can be added, such as a cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0232] As disclosed above, a MAPT ASO conjugate as described herein can be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. For injection, the MAPT ASO conjugates can be formulated into preparations by dissolving, suspending, or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers, and preservatives. In some

embodiments, MAPT ASO conjugates can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks’s solution, Ringer’s solution, or physiological saline buffer. Formulations for injection can be presented in unit dosage form, e.g., in ampules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing, and/or dispersing agents.

[0233] Typically, a pharmaceutical composition for use in in vivo administration is sterile. Sterilization can be accomplished according to methods known in the art, e.g., heat sterilization, steam sterilization, sterile filtration, or irradiation.

[0234] Dosages and desired drug concentration of pharmaceutical compositions as described herein may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of one in the art. Suitable dosages are also described above.

B. Kits

[0235] In some embodiments, kits comprising a MAPT ASO conjugate as described herein are provided. In some embodiments, the kits are for use in modulating the expression of a target gene or sequence (e.g., a target gene expressed in the brain or central nervous system (CNS)). In some embodiments, the kits are for use in in modulating the expression of a target gene.

[0236] In some embodiments, the kit further comprises one or more additional therapeutic agents. For example, in some embodiments, the kit comprises a MAPT ASO conjugate as described herein and further comprises one or more additional therapeutic agents. In some embodiments, the kit further comprises instructional materials containing directions (i.e., protocols) for the practice of the methods described herein (e.g., instructions for using the kit for administering a composition across the blood-brain barrier). While the instructional materials typically comprise written or printed materials, they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated herein. Such media include, but are not limited to, electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD-ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

Table 1. Sequences

SEQ ID NO.	Sequence	Description
1	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE	Wild-type human Fc sequence;

	KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	amino acids 1-3 (PCP) are from a hinge region
2	NSVITVDKNGRLVYLVENPGGYVAYSKAATVTGKLVHANFGTKKDFEDLYTPV NGSIVIVRAGKITFAEKVANAESLNAIGVLIYMDQTKFPIVNAELSFEGHAHL GTGDPYTPGFPSFNHTQFPSSRSSLNIPVQTISSRAAEKLFNGMEGDCPSD WKT DSTCRMVTSSEKNVKLTVS	Human TfR apical domain
3	MMDQARSFAFSLFGGEP LSYTRFSLARQVDGDN SHVEMKLAIVDEENADNNTK ANVT KPKRCSGSICYGTIAVIVFFLIGFMIGYLG YCKGVEPKTECERLAGTES PVREEPGEDFPAARRLYWDDLKRKLEKLDSTDFGTGTLKLLNENS YVPREAGS QKDENLALYVENQFREPKLSKVWRDQHFKIQVKDSAQNSVITVDKNGRLVYL VENPGGYVAYSKAATVTGKLVHANFGTKKDFEDLYTPVNGSIVIVRAGKITFA EKVANAESLNAIGVLIYMDQTKFPIVNAELSFEGHAHLGTGDPYTPGFPSFNH TQFPSSRSSLNIPVQTISSRAAEKLFNGMEGDCPSDWKT DSTCRMVTSSEK NVKLT VSNVLKEIKILNIFGVIKGFVEPDHYVVVGAQRDAWGPGAAGSGVGTA LLLKLAQMFS DMVLKDFQPSRSIIIFASWSAGDFGSVGATEWLEGYLSSSLHLK AFTYINLDKAVLGTSNFKVSASPLLYTLIEKTMQNVKHPVTGQFLYQDSNWS KVEKLTLDNAAFPFLAYSGIPAVSFCFCEDTDYPYLGTTMDTYKELIERIPEL NKVARAAAEVAGQFVIKLT HDVELNLDYERYNSQLLSFVRDLNQYRADIKEMG LSLQWLYSARGDFFRATSRLTTDFGNAEKTDREVMKLNDRVMRVEYHFLSPY VSPKESPF RHVFWGSGSHTLPALLENLKLKRNQNGAFNETLFRNQLALATWTI QGAANALSGDVWDIDNEF	Human transferrin receptor protein 1 (TFR1)
4	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLYSKLTVTKKEEWQQGFVFSVMHEALHNHYTQKSLS LSPGK	Clone CH3C.35.23.2
5	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGT EWANYKTTTPVLDSDGSFFLYSKLTVTKKEEWQQGFVFSVMHEALHNHYTQK SLSLSPGK	Clone CH3C.35.23.2 with knob mutation amino acids 1-3 (PCP) are from a hinge region
6	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLYSKLTVTKKEEWQQGFVFSVMHEALHNHYTQKSLS LSPG	Clone CH3C.35.23.2 with knob mutation
7	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLYSKLTVTKKEEWQQGFVFSVMHEALHNHYTQKSLS LSPGK	Clone CH3C.35.23.2 with knob and LALA mutations
8	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLYSKLTVTKKEEWQQGFVFSVMHEALHNHYTQKSLS LSPG	Clone CH3C.35.23.2 with knob and LALA mutations
9	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLYSKLTVTKKEEWQQGFVFSVMHEALHNHYTQKSLS LSPGK	Clone CH3C.35.23.2 with knob and LALAPG mutations
10	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLYSKLTVTKKEEWQQGFVFSVMHEALHNHYTQKSLS LSPG	Clone CH3C.35.23.2 with knob and LALAPG mutations
11	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALSAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA	Clone CH3C.35.23.2 with knob and LALAPS mutations

	NYKTT PPVLDSDGSFFLYSKLTVTKKEWQQGFVFSCSVMEALHNHYTQKSLS LSPGK	
12	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALSAPIEKT SKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTT PPVLDSDGSFFLYSKLTVTKKEWQQGFVFSCSVMEALHNHYTQKSLS LSPG	Clone CH3C.35.23.2 with knob and LALAPS mutations, w/lysine truncation
13	APELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTT PPVLDSDGSFFLYSKLTVTKKEWQQGFVFSCSVMEALHNHYTQKSLS LSPGK	Clone CH3C.35.23.2 with knob and YTE mutations
14	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTT PPVLDSDGSFFLYSKLTVTKKEWQQGFVFSCSVLHEALHSHYTQKSLS LSPGK	Clone CH3C.35.23.2 with knob and M201L and N207S mutations
15	APEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTT PPVLDSDGSFFLYSKLTVTKKEWQQGFVFSCSVMEALHNHYTQKSLS LSPGK	Clone CH3C.35.23.2 with knob, LALA, and YTE mutations
16	APEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKT SKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTT PPVLDSDGSFFLYSKLTVTKKEWQQGFVFSCSVMEALHNHYTQKSLS LSPGK	Clone CH3C.35.23.2 with knob, LALAPG, and YTE mutations
17	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTT PPVLDSDGSFFLYSKLTVTKKEWQQGFVFSCSVLHEALHSHYTQKSLS LSPGK	Clone CH3C.35.23.2 with knob, LALA, and M201L and N207S mutations
18	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKT SKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTT PPVLDSDGSFFLYSKLTVTKKEWQQGFVFSCSVLHEALHSHYTQKSLS LSPGK	Clone CH3C.35.23.2 with knob, LALAPG, and M201L and N207S mutations
19	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESYGTEWA NYKTT PPVLDSDGSFFLVSKLTVTKKEWQQGFVFSCSVMEALHNHYTQKSLS LSPGK	Clone CH3C.35.23.2 with hole mutations
20	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESYGTEWA NYKTT PPVLDSDGSFFLVSKLTVTKKEWQQGFVFSCSVMEALHNHYTQKSLS LSPGK	Clone CH3C.35.23.2 with hole and LALA mutations
21	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKT SKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESYGTEWA NYKTT PPVLDSDGSFFLVSKLTVTKKEWQQGFVFSCSVMEALHNHYTQKSLS LSPGK	Clone CH3C.35.23.2 with hole and LALAPG mutations
22	APELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESYGTEWA NYKTT PPVLDSDGSFFLVSKLTVTKKEWQQGFVFSCSVMEALHNHYTQKSLS LSPGK	Clone CH3C.35.23.2 with hole and YTE mutations
23	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESYGTEWA	Clone CH3C.35.23.2 with hole and M201L and N207S mutations

	NYKTTTPVLDSDGSFFLVSKLTVTKKEWQQGFVFSCSVLHEALHSHYTQKSLS LSPGK	
24	APEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLVSKLTVTKKEWQQGFVFSCSVLHEALHSHYTQKSLS LSPGK	Clone CH3C.35.23.2 with hole, LALA, and YTE mutations
25	APEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLVSKLTVTKKEWQQGFVFSCSVLHEALHSHYTQKSLS LSPGK	Clone CH3C.35.23.2 with hole, LALAPG, and YTE mutations
26	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLVSKLTVTKKEWQQGFVFSCSVLHEALHSHYTQKSLS LSPGK	Clone CH3C.35.23.2 with hole, LALA, and M201L and N207S mutations
27	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLVSKLTVTKKEWQQGFVFSCSVLHEALHSHYTQKSLS LSPGK	Clone CH3C.35.23.2 with hole, LALAPG, and M201L and N207S mutations
28	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLYSKLTVTKKEWQQGFVFSCSVLHEALHSHYTQKSLS LSPGK	Clone CH3C.35.23.2 with M201L and N207S mutations
29	APEAAGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLYSKLTVTKKEWQQGFVFSCSVLHEALHSHYTQKSLS LSPGK	Clone CH3C.35.23.2 with knob, LALA, and S239C mutations
36	APEAAGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLYSKLTVTKKEWQQGFVFSCSVLHEALHSHYTQKSLS LSPGK	Clone CH3C.35.23.2 with knob, LALAPG, and S239C mutations
43	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLYSKLTVTKKEWQQGFVFSCSVLHEALHSHYTQKSLS LSPG	Clone CH3C.35.23.2
44	APEAAGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTEWA NYKTTTPVLDSDGSFFLYSKLTVTKKEWQQGFVFSCSVLHEALHSHYTQKSLS LSPG	Clone CH3C.35.23.2 with knob, LALA, and S239C mutations
47	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALHSHYTQKSLS LSPG	Wild-type human Fc sequence
48	DKTHTCPPCPAPEAAGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVLHEAL HSHYTQKSLSLSPG	Fc with portion of human IgG1 hinge sequence and hole, JAI.A, and S239C mutations
49	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAV	Clone 35.23.2 with portion of human IgG1 hinge sequence

	EWESYGTWANYKTTTPVLDSDGSFFLYSKLTVTKEEWQQGFVFSVCSVMHEALHNHYTQKSLSLSPG	and knob and LALA mutations
90	DKTHTCPPCPAPEAAGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK	Fc sequence with portion of human IgG1 hinge sequence and hole, LALA, and S239C mutations
50	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESYGTWANYKTTTPVLDSDGSFFLYSKLTVTKEEWQQGFVFSVCSVMHEALHNHYTQKSLSLSPGK	Clone 35.23.2 with portion of human IgG1 hinge sequence and knob and LALA mutations
51	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK	Fc sequence with knob and LALA mutations
52	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK	Fc sequence with hole mutations
53	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK	Fc sequence with hole and LALA mutations
54	APEAAGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK	Fc sequence with hole, LALA, and S239C mutations
61	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK	Fc sequence with hole and LALAPG mutations
62	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALSAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK	Fc sequence with hole and LALAPS mutations
63	APEAAGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALSAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK	Fc sequence with hole, LALAPS, and S239C mutations
66	APEAAGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK	Fc sequence with hole, LALAPG, and S239C mutations
73	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPG	Fc sequence with hole mutations
74	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN	Fc sequence with hole and LALA mutations

	NYKTT PPVLDS DGSFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLS LSPG	
75	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTT PPVLDS DGSFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLS LSPG	Fc sequence with hole and LALAPG mutations
76	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALSAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTT PPVLDS DGSFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLS LSPG	Fc sequence with hole and LALAPS mutations
77	APEAAGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALSAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTT PPVLDS DGSFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLS LSPG	Fc sequence with hole, LALA, and S239C mutations
78	APEAAGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTT PPVLDS DGSFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLS LSPG	Fc sequence with hole, LALAPG, and S239C mutations w/ lysine truncation
80	APEAAGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALSAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTT PPVLDS DGSFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLS LSPG	Fc sequence with hole, LALAPS, and S239C mutations w/ lysine truncation
86	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWNSGALTSKVH PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWE SYGTEWANYKTTPPVLDSDGSFFLYSKLTVTKEEWQQGFVFSQSVMEALHNH YTQKSLSLSPGK	Heavy constant region sequence with 35.23.2, knob, and LALA, mutations
87	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWNSGALTSKVH PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALG APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWE SYGTEWANYKTTPPVLDSDGSFFLYSKLTVTKEEWQQGFVFSQSVMEALHNH YTQKSLSLSPGK	Heavy constant region sequence with 35.23.2, knob, and LALAPG, mutations
90	DKTHTCPPEAPEAAGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTT PPVLDS DGSFFLVSKLTVDKSRWQQGNVFSCSVMEAL HNHYTQKSLSLSPGK	Fc with portion of human IgG1 hinge sequence and hole, LALA, and S239C mutations
121	TCPPCP	Hinge region sequence
91	DKTHTCP	Portion of human IgG1 hinge sequence
92	DKTHTCPPE	Portion of human IgG1 hinge sequence
93	EPKSCDKTHTCPPCP	Human IgG1 hinge amino acid sequence
94	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWNSGALTSKVH PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALG APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWE	Heavy constant region sequence with 35.23.2, knob, and LALAPG, mutations

	SYGTEWANYKTTPPVLDSDGSFFLYSKLTVTKEEWQQGFVFSVMEALHNNH YTQKSLSLSPG	
97	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKSDSTYSLSSLTLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C	Kappa constant region sequence
98	QVQLQESGPGLVKPSSETLSLTCAVSGYSITSDYAWGWIRQPPGKLEWIGSMS YSGSTYYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCARGWPLAYWG QGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSKVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKHTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALSAPIEKTIKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFY PSDIAVEWESYGTEWANYKTTPPVLDSDGSFFLYSKLTVTKEEWQQGFVFSV VMEALHNNHYTQKSLSLSPGK	Fab-Fc polypeptide 35.23.2 LALAPS Knob
132	caagtgcactgcaggaaagtgggcctggtttggtcaaacccagtgaaacact tagtcttacttgcgctgtatctggatactctattacaagcgattatgcctggg gggtgattcggcagccccctggaaaaggcttggagtgatcggttcaatgagt tatagtgggtagatataactacaacccttccctgaagtctcgcgtcacataaag cgtagatacaagtaagaatcaattttccctgaaacttagcagtgtaactgccg ctgataccgcagctctactattgtgccagaggatggccccctggcctactgggg cagggcaccctcgtgacccgtatcatcagctagcaccaaaggctcccagcgtgtt cccactgcgcccgagttcaaaaatcaacttctggaggcaccgccgcctgggtt gcctggttaaaggactacttcccagagcccgtagccgtgagctggaactccggg gcactgacatctggcgcttcatactttcccgccgctgctccagctcttcaggtct gtatagtctctcctctgtggtcactgtcccatctagctctctgggaccccaaa cctacatatgcaacgtaataacacaagccgagcaataactaaagtgcacaaaag gtggaaccccaagctctgtgacaagaccacacgtgtccccctgcccggctcc tgaggctgcagggcccccagcgtcttctcttcccccaaaagccaaaagata ccttgatgatcagcagaactcccagagtgacatgcgtcgctggtgacgtaagc catgaagatcccaggttaagttaactggtatgtcgatggcggtggaagtcca taagtctaagactaaacctcgcgaagagcagtaacaattcaacttaccgggtcg tttccgcttctgaccgtgctgcatcaggactggctgaatggtaaagagtacaag tgcaaaagtgtctaacaaggcactcagcgcccccaattgagaagactatctccaa agctaaagggcaaccaagagagccccaggtctacaccctgccccctcaaggg atgagcttaagaaaccaggttagtctctggtgcttgggttaaaggattttat ccaagcgataattgctgtggagtgggagtcctatggcacggagtgggccaacta taaaaccacccccctgttcttgacagtgacggtagtttcttctctgtattcca aactgaccgtcacaaaggaggagtggcaacagggatttgtgttcagctgtctc gtgatgcataaggcgctccataatcattacacacaaaaaagtttgtccctgag cccaggcaag	Fab-Fc polypeptide 35.23.2 LALAPS Knob nucleic acid sequence
99	QVQLQESGPGLVKPSSETLSLTCAVSGYSITSDYAWGWIRQPPGKLEWIGSMS YSGSTYYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCARGWPLAYWG QGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSKVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKHTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALSAPIEKTIKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFY PSDIAVEWESYGTEWANYKTTPPVLDSDGSFFLYSKLTVTKEEWQQGFVFSV VMEALHNNHYTQKSLSLSPG	Fab-Fc polypeptide 35.23.2 LALAPS Knob w/lysine truncation
100	QVQLQESGPGLVKPSSETLSLTCAVSGYSITSDYAWGWIRQPPGKLEWIGSMS YSGSTYYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCARGWPLAYWG QGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSKVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKHTHTCPPCPAPEAAGGPCVFLFPPKPKDTLMISRTPEVTCVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALSAPIEKTIKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSV VMEALHNNHYTQKSLSLSPGK	Fab-Fc polypeptide LALAPS S239C Hole
133	caagtgcactgcaggaaagtgggcctggtttggtcaaacccagtgaaacact tagtcttacttgcgctgtatctggatactctattacaagcgattatgcctggg gggtgattcggcagccccctggaaaaggcttggagtgatcggttcaatgagt	Fab-Fc polypeptide LALAPS S239C

	tatagtggttagtacataactacaacccttcctgaagtctcgcgtcacataag cgtagatacaagtaagaatcaattttccctgaaacttagcagtgtaactgccg ctgataccgcagctctactattgtgccagaggatggcccttgccctactggggt cagggcaccctcgtgaccgtatcatcagctagcaccaaaggtcccagcgtgtt cccactcgcccgagttcaaaatcaacttctggaggcaccgcccctgggtt gcctggttaaaggactacttcccagagcccgtagccgtgagctggaactccggg gcactgacatctggcgttcatactttcccgccgtgctccagctcttcaggtct gtatagtctctcctctgtggtcactgtcccatctagctctctgggcacccaaa cctacatatgcaacgttaatcacaagccgagcaataactaaagtgacaaaaag gtggaacccaagtcttgtgacaagaccacacgtgtccccctgcccggctcc tgaggctgcagggcccccctgcgtctttctctttcccccacccaaaagata ccttgatgatcagcagaactcccaggtgacatgcgtcgtcgtgacgtaagc catgaagatcccaggttaagttcaactggtatgtcgatggcgtggaagtcca taatgcttaagactaaacctcgccgaagagcagtaacaattcaacttaccgggtcg tttccgttctgaccgtgctgcatcaggactggctgaatggtaaagagtacaag tgcaagtgcttaacaaggcactcagcgccccaattgagaagactatctccaa agctaaagggcaaccaagagagcccgaggtctacaccctgccccctcaaggg atgagcttactaagaaccaggttagtctcagctgcggttaaggaattttat ccaagcgataattgtgtggagtgggagtcacggccagcctgagaacaatta taaaaccacccccctgttcttgacagtgacggtagtttcttctggtttcca aactgaccgtcgataagagcagatggcaacagggaaatgtgttcagctgctcc gtgatgcatagaggcgctccataatcattacacacaaaaagttgtccctgag cccaggcaag	Hole nucleic acid sequence
101	QVQLQESGPGLVKPSSETLSLTCAVSGYSITSDYAWGWIRQPPGKLEWIGSMS YSGSTYYNPSLKSRTVISVDTSKNQSLKLSSVTAADTAVYYCARGWPLAYWG QGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKHTHTCPPCPAPEAAGGPGCVLFPPKPKDTLMISRTPEVTCVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALSAPIEKTKSKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSS VMHEALHNHYTQKSLSLSPG	Fab-Fc polypeptide LALAPS S239C Hole w/lysine truncation
102	RASQSVSSYLAWY	NTF CDR-L1
103	DASNLAT	NTF CDR-L2
104	QQRSNWPPIT	NTF CDR-L3
105	GYSITSDYAWG	NTF CDR-H1
106	SMSYSGSTYYNPSLKS	NTF CDR-H2
107	ARGWPLAY	NTF CDR-H3
122	SASSSVSYMYWY	NTF2 CDR-L1
123	DTSNLAS	NTF2 CDR-L2
124	QQWSSYPPIIT	NTF2 CDR-L3
125	GYSITSDYAWN	NTF2 CDR-H1
126	YMSYSGSTRYNPSLRS	NTF2 CDR-H2
127	ARGWPLAY	NTF2 CDR-H3
128	X ₁ MSYSGSTX ₂ YNPSLX ₃ S Wherein: X ₁ =Y or S; X ₂ = R or Y; and X ₃ = R or K	NTF CDR-H2 consensus
108	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASN LATGIPARFSGSGSDFTLTITSSLEPEDFAVYYCQQRSNWPPITFGQGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC	NTF light chain
134	gaaattgtcctcaccagagccagccactctttccctctctccaggggaaag agcaacactgagttgccgggctcccagagtgtaagtctacctcgcagtggt atcaacagaagcctgtgtaagcaccggccttctgatttacgatgccagcaat ctcgccaccggaatccccgcaaggttttcaggagcggttcagggactgactt taccctgaccataagcagctcttgagcctgaggattttgcagtgattactgcc aacagagaagtaactggccccccataacctttggacaggggactaaggtggag attaaacgtacgggtggcagctccatcagttttatcttccaccaagcgacga gcaattgaagtccggcactgcctctgtggtgtgccttctgaacaactctatc caagggaggccaaggtccagtggaaggtcgataatgcgctgcagagcggaac	NTF1 light chain nucleic acid sequence

	agccaagagtcagtgaccgagcaggactcaaaagatagcacatactctctgag ttccaccctgaccctgtcaaaggctgactacgaaaagcataaggtatacgcat gccaagtgacccatcagggctctctcatctcccgtacacaaatcttttaataga ggagaatgc	
109	QVQLQESGPGLVKPSSETLSLTCAVSGYSITSDYAWGWIRQPPGKGLEWIGSMS YSGSTYYNPSLRSRVTSVDTSKNQFSLKLSSVTAADTAVYYCARGWPLAYWG QGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKTH	NTF heavy chain
110	QVQLQESGPGLVKPSSETLSLTCAVSGYSITSDYAWGWIRQPPGKGLEWIGSMS YSGSTYYNPSLRSRVTSVDTSKNQFSLKLSSVTAADTAVYYCARGWPLAYWG QGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKTHTCP	NTF heavy chain with portion of hinge region
129	EIVMTQSPATLSLSPGERATLSCASSSVSYMYWYQQKPGQAPRLLIYDTSNL ASGIPARFSGSGSGTDFTLTISLQPEDFAVYYCQQWSSYPITFGQGTKEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC	NTF2 light chain
130	QVQLQESGPGLVKPSSETLSLTCAVSGYSITSDYAWNIRQPPGKGLEWIGYMS YSGSTRYNPSLRSRVTSVDTSKNQFSLKLSSVTAADTAVYYCARGWPLAYWG QGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPK	NTF2 heavy chain
131	QVQLQESGPGLVKPSSETLSLTCAVSGYSITSDYAWNIRQPPGKGLEWIGYMS YSGSTRYNPSLRSRVTSVDTSKNQFSLKLSSVTAADTAVYYCARGWPLAYWG QGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKTHTCP	NTF2 heavy chain with portion of hinge region
111	ACCTTAAGTATTACTTGC	MAPT ASO
112	CTGTTAGACATTCTCTC	MAPT ASO
30	GGTTGACATCGTCTGCCTGT	Control ASO
113	CCGTTTCTCTTACCACCCT	Control ASO
115	refseq NM_001123066	MAPT mRNA

EXAMPLES

Example 1. Construction of OTV:MAPT ASO Conjugates

[0237] *i. Fab-Fc dimer fusions design, cloning.* Fab-Fc dimer fusions were designed that contain a first Fc polypeptide comprising a S239C cysteine substitution and hole and LALAPS mutations (SEQ ID NO:63), a second Fc polypeptide that forms an Fc dimer with the first Fc polypeptide and comprises a modified constant domain that specifically binds to a transferrin receptor (35.23.2), knob and LALAPS mutations (SEQ ID NO:11), and wherein the first and second Fc polypeptide are each fused to a non-targeting Fab (SEQ ID NO:108 and 109). Therefore, the first Fab-Fc polypeptide fusion has a sequence of SEQ ID NO:100 and the second Fab-Fc polypeptide fusion has a sequence of SEQ ID NO:98.

[0238] Additionally, as described herein, the C-terminal lysine residue of an Fc polypeptide may be fully or partially removed by the cellular machinery during protein production. Therefore, a first Fc polypeptide may comprise SEQ ID NO:80 and a second Fc may comprise

SEQ ID NO:12. Similarly, the first Fab-Fc polypeptide fusion may have a sequence of SEQ ID NO:101 and the second Fab-Fc polypeptide fusion may have a sequence of SEQ ID NO:99.

[0239] Constructs were cloned by gene synthesis and Gibson assembly into a mammalian expression vector pRK5.

[0240] *ii. Fab-Fc dimer fusions protein expression, and purification.* Vectors were co-transfected to Expi293 cells along with the corresponding light chain vector in the ratio knob:hole:light chain of 1:1:2. The expressed protein was purified from conditioned media by loading the supernatant over a Protein A column. The column was washed with 10 column volumes of PBS, pH 7.4. The proteins were eluted with 50 mM sodium citrate, pH 3.0 containing 150 mM NaCl, and immediately neutralized with 200 mM arginine, 137 mM succinic acid, pH 5.0. The proteins were further purified by size-exclusion chromatography (SEC) (GE Superdex200) using 200 mM arginine, 137 mM succinic acid, pH 5.0 as running buffer. The purified proteins were confirmed by intact mass LC/MS, and purity of > 95% was confirmed by SDS-PAGE and analytical HPLC-SEC.

[0241] *iii. Synthesis of 5'-maleimide modified ASO*

[0242] *Preparation of 5'-amino-modified ASO*

[0243] *Solid phase oligonucleotide synthesis.* Oligonucleotide synthesis was performed on a MerMade 12 (LGC) DNA/RNA synthesizer at 100 μ mol scale following standard solid-phase oligonucleotide synthesis protocols. All locked nucleic acid (LNA) and deoxyribonucleic acid (DNA) phosphoramidites were purchased from Hongene Biotech Corporation, including LNA-A(Bz), LNA-5MeC(Bz), LNA-T, LNA-G(dmf), and dA(Bz), dC(Ac), dT, dG(dmf). LNA-5MeC(Bz) was dissolved in a mixed solvent of DCM/acetonitrile (1:1, v/v), while all other phosphoramidites were dissolved in acetonitrile and molecular sieves (3 Å) were added. The parent antisense oligonucleotide (ASO) sequences were first assembled on UnyLinker CPG solid support, followed by attachment of amino linker on 5'-end using 6-(Trifluoroacetyl-amino)-hexyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite (CAS: 133975-85-6; Glen Research Cat. # 10-1916). The synthesis cycle for adding one nucleotide (or non-nucleic acid) unit consists of four individual steps, detritylation, coupling, oxidation (or sulfurization), and capping. 5-Ethylthio-1H-tetrazole (ETT, 0.25 M in acetonitrile) was used as activator solution. A 0.2 M solution of PADS (phenylacetyl disulfide) in 50% pyridine/50% acetonitrile was employed to introduce phosphorothioate linkages. Detailed protocols for 100 μ mol scale ASO synthesis are summarized in **Table 2A-2D below**.

Table 2A. Generic 100 μmol scale ASO synthesis parameters.

Process	Reagents	Parameters
Solid support	UnyLinker CPG, 500 Å, 80 $\mu\text{mol/g}$	100 μmol scale
Wash	Anhydrous acetonitrile (moisture content < 10 ppm)	10 mL, 20 s
Detritylation	3% Trichloroacetic acid (TCA) in DCM	10 mL, 45 s
Coupling	0.1 M phosphoramidites in ACN or ACN/DCM or DCE (1:1, v/v)	2.5 mL amidite,
	0.25 M ETT in acetonitrile	5 mL ETT, 6 min
Oxidation	0.02 M I_2 in THF/Pyridine/ H_2O (7:2:1)	10 mL, 2 min
Sulfurization	0.2 M PADS in 50% pyridine/50% acetonitrile	10 mL, 4 min
	or ADTT (0.2 M, 120 eq.) in pyridine, 10 min	
Capping	Cap Mix A: THF/Pyridine/ Ac_2O (8:1:1)	2.5 mL Cap A,
	Cap Mix B: 16% Methyl imidazole in THF	2.5 mL Cap B, 40 s

Table 2B. Example synthesis of oligo containing MsPA backbone modifications.

Synthesizer	ÄKTA oligopilot plus 100 (CV=6.3 mL)
CPG	Universal CPG (1000 Å, 40 $\mu\text{mol/g}$)
Scale	100 μmol
Coupling 1 (recycle)	0.2 M amidite, 6.0 eq.; 1.0 M DCI/0.1 M NMI, 45 eq., 17 min (cycled twice for the 1 st monomer; one time for all other amidites)
Oxidation for PS bonds	0.02 M I_2 in MeCN/pyridine/ H_2O , 75/20/5 (v/v/v), 5 eq., 5 min
Oxidation for MsPA bonds	methanesulfonyl azide (0.5 M, 75 eq.) in MeCN, 30 min; repeated 2 times, 60 min in total
Sulfurization	ADTT (0.2 M, in pyridine), 10 CV, 126 eq., 10 min

Table 2C. Example synthesis of oligo containing PN backbone modifications.

Synthesizer	ÄKTA oligopilot plus 100 (CV=6.3 mL)
CPG	Universal CPG (1000 Å, 40 $\mu\text{mol/g}$)
Scale	100 μmol
Coupling 1 (recycle)	0.2 M amidite, 6.0 eq.; 1.0 M DCI/0.1 M NMI, 45 eq., 17 min (cycled twice for the 1 st monomer; one time for all other amidites)
Oxidation for PS bonds	0.02 M I_2 in MeCN/pyridine/ H_2O , 75/20/5 (v/v/v), 5 eq., 5 min
Oxidation for PN bonds	2-Azido-1,3-dimethylimidazolinium Hexafluorophosphate (0.5 M, in MeCN), 10 mL, 50 eq., 120 min
Sulfurization	ADTT (0.2 M, in pyridine), 10 CV, 126 eq., 10 min

Table 2D. Example synthesis of oligo containing PS2 and PN backbone modifications.

Synthesizer	ÄKTA oligopilot plus 100 (CV=6.3 mL)
CPG	Universal CPG (500 Å, 24 $\mu\text{mol/g}$)
Scale	100 μmol
Coupling for PS and PN	LNA-C in 4:1 MeCN/DCM, other amidites in MeCN at 0.2 M, 6.0 eq.; 10.6 M ETT, 27 eq., 19 min (cycled twice for the 1 st monomer; one time for all other amidites)

Oxidation for PS bonds	0.02 M I ₂ in MeCN/pyridine/H ₂ O, 75/20/5 (v/v/v), 5 eq., 5 min
Oxidation for PN bonds	2-Azido-1,3-dimethylimidazolinium Hexafluorophosphate (0.5 M, in MeCN), 10 mL, 50 eq., 120 min
Coupling for PS2 monomers	PS2 amidite 0.2 M in DCE, 6 eq.; 0.6 M ETT, 27 eq., 19 min; one time
Sulfurization	ADTT (0.2 M, in pyridine), 10 CV, 126 eq., 10 min

Table 2E. Example synthesis of oligo containing OiPr-PS and PN backbone modifications

Synthesizer	ÅKTA oligopilot plus 100 (CV=6.3 ml)
CPG	Universal CPG (500 Å, 12 µmol/g)
Scale	12 µmol
Coupling for PS and PN	LNA-C in 4:1 MeCN/DCM, other amidites in MeCN at 0.05 M, 2.0 eq.; 0.25 M ETT, 20 eq., 2.1 min (cycled twice for the 1 st monomer; one time for all other amidites)
Oxidation for PS bonds	0.02 M I ₂ in MeCN/pyridine/H ₂ O, 75/20/5 (v/v/v), 5 eq., 5 min
Oxidation for PN bonds	2-Azido-1,3-dimethylimidazolinium Hexafluorophosphate (0.2 M, in MeCN), 10 mL, 50 eq., 60 min
Sulfurization for PS and OiPS bonds	ADTT (0.2 M, in pyridine), 10 CV, 126 eq., 10 min

[0244] *Post-synthetic manipulations.* Upon completion of solid-phase oligonucleotide synthesis, the phosphate protecting group (2-cyanoethyl group) was removed by a 20% solution of diethylamine (DEA) in acetonitrile for 1 hr. Cleavage from solid support and nucleobase deprotection (C&D) were performed in NH₄OH/EtOH (3:1) at 45 °C for 20 hr. The crude oligonucleotide solution was concentrated by centrifugal evaporation under reduced pressure, and the solid residue was reconstituted in water for prep-HPLC purification.

[0245] *Purification of 5'-amino ASO by prep-HPLC.* The crude 5'-amino ASO was purified by ion-pairing reverse phase HPLC. The detailed parameters were summarized in **Table 3**. The appropriate fractions were pooled and lyophilized to give the purified 5'-amino ASO.

Table 3. Prep-HPLC purification conditions.

Column	Waters Xbridge BEH C18 19*150 mm
Mobile phase A	240 mM HFIP and 7 mM TEA in 95% water/5% MeOH
Mobile phase B	240 mM HFIP and 7 mM TEA in MeOH
Flow rate	15 mL/min
Column temperature	50 °C
Gradient	0–3 min (10% B), 3–25 min (10% - 30%), 100% B hold time: 2 min
Amount per injection	100 mg crude ASO

[0246] *Synthesis of 5'-amino-modified ASO.* 5'-amino modified ASOs shown in **Tables 4** and **5** below were synthesized in accordance with the general methods mentioned above.

[0247] *Preparation of 5'-maleimide modified ASO.* General procedure: To a solution of ASO amine TEA salt (30 mg, by OD) in PBS buffer (TEKNOVA 10× PBS stock solution, pH 6.0, 2.5 mL) was added a solution of 3-maleimidopropionic acid N-hydroxysuccinimide ester (MCOSu, 10 eq.) in DMF (2.5 mL) at room temperature. The resulting solution was shaken at room temperature for 18 hr. Upon completion, the solution was desalted by passing through a sephadex G25 column (2.5×40 cm) on AKTA pure 25 M, eluting with Milli Q water at 3 mL/min, monitored by UV 260/280 and conductivity. The appropriate fractions were pooled and lyophilized to give desired 5'-maleimide ASO.

[0248] *iv. Bioconjugation.* The Fab-Fc dimer fusions generated above containing the S239C cysteine modification for conjugation was first reduced using 30 molar equivalents of TCEP and 2 mM EDTA, 37 °C for 1 hr. Reduction was confirmed by LC/MS. Post reduction, remaining TCEP was removed by dialysis using 1× PBS, pH 6.8 with 2 mM EDTA (purification by *e.g.*, dialysis) and the Fab-Fc dimer fusions were reoxidized with 50 molar equivalents of dHAA at room temperature for 3 hr. Oxidation was confirmed by LC/MS of dHAA. For the bioconjugation, 1.2 molar equivalents of the 5'-maleimide modified ASOs generated above were added to the oxidized Fab-Fc dimer fusions at room temperature for 1 hr. The resulting MAPT ASO conjugates were purified by anion exchange chromatography using Resource Q column (equilibration buffer: 50 mM Tris, pH 7.5, elution buffer: 50 mM Tris, pH 7.5 + 2 M NaCl) to remove unwanted and unconjugated products. Purity of the MAPT ASO conjugates were determined by LC/MS and SEC. The resulting conjugates are referred to as OTV or OTV:MAPT.

Example 2. *In Vitro* Screening of ASOs to the MAPT gene

[0249] Selection of ASO sequences specific for a given target sequence is based upon analysis of the chosen target sequence and determination of a number of factors, including *in vitro* and *in vivo* potency, and liver toxicity profile.

[0250] *Initial MAPT ASO Screening.* Initial identification of 393 MAPT ASO sequences 100% complementary to the MAPT pre-mRNA (Ensembl ENST0000034429) and or MAPT mRNA (refseq NM_001123066) SEQ ID NO:115 were made based on computational predictions of target binding, specificity, efficacy, and safety.

[0251] Knockdown efficacy of the MAPT-specific ASOs in human MiaPaCa-2 and HDLM2 cells was tested. The cells were treated with MAPT-specific ASO or control oligonucleotide at a concentration of 5 µM without the use of a transfection reagent. After three days treatment, cells were lysed. MAPT and HPRT1 mRNA expression was analyzed using the QuantiGene

Singleplex assay (ThermoFisher). MAPT expression values were normalized to HPRT1 values and the degree of knockdown in comparison to mock-treated cells was determined.

[0252] *Two-concentration screen.* 55 MAPT-specific ASOs with a knockdown efficacy in both tested cell lines of >99.9% (at 5 μ M), were tested in a two- concentration screen (100 nM, 1000 nM) in MiaPaCa-2 cells. After three days treatment, cells were lysed. MAPT and HPRT1 mRNA expression was analyzed using the QuantiGene Singleplex assay (ThermoFisher). MAPT expression values were normalized to HPRT1 values and the degree of knockdown in comparison to mock-treated cells was determined. 22 ASOs were selected for investigation of the TLR9 activating capacity and concentration response relations ship (IC₅₀ determination).

[0253] *Investigation of the TLR9 activating capacity.* The 22 ASOs were tested for their TLR9-dependent proinflammatory potential. A reporter cell line (HEK-Blue-hTLR9 cells, Invivogen) was treated with MAPT-specific ASOs, a positive and a negative control without the use of transfection reagent at a concentration of 5 μ M. After 20h, QUANTI-Blue™ Solution (Invivogen) was added to the cells. Optical density was measured 2 hr later in order to determine the TLR9 activating capacity of the tested ASOs.

Example 3. In Vivo Knockdown Screening

[0254] An ASO comprising SEQ ID NO:111 was synthesized according to the procedures described below for *in vivo* dosing. The ASO was diluted in sterile saline and administered to hTau mice (mTau^{-/-}) via intracerebroventricular (ICV) injection at a single dose of 50 μ g or 100 μ g in 10 μ L total volume per mouse. The 50 μ g or 100 μ g dose was determined based on *in vivo* tolerability.

[0255] Two weeks post dose, tissues (brain and spinal cord) were harvested and terminal blood was collected. MAPT expression was measured in the brain and spinal cord as follows. A piece of the frontal lobe and cervical spinal cord was homogenized with a bead homogenizer in Trizol for bulk RNA isolation. Briefly, homogenized tissues were incubated with chloroform for 3-5 minutes to allow for phase separation after centrifugation. The aqueous phase was then incubated with isopropanol for 10 minutes to allow for RNA precipitation followed by a 75% ethanol wash and resuspension in nuclease-free water. MAPT expression was then measured by qPCR using the Express One-Step Superscript Kit and normalized to expression of the housekeeping gene Gapdh. A panel of glial activation markers (Aif1, Gfap, Tlr9, Itgax) were also quantified by qPCR using the Express One-Step Superscript Kit to determine if the ASO triggered a chronic neuroinflammatory response.

[0256] ASO6 knocked down human tau expression 76% at a dose of 50 μ g.

Example 4. *In Vivo* Liver Toxicity Screening

[0257] Liver safety was assessed for ASO6. Alanine transaminase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) are enzymes found in high quantities in liver. When liver cells are damaged, they release these enzymes into the bloodstream. Therefore, serum ALT, AST, and LDH levels were measured to determine if the ASO induced liver damage.

[0258] The ASO was diluted in sterile saline and administered to wild-type mice subcutaneously at a dose of 20 mpk daily for five consecutive days. The mice were weighed daily for 12 days, and blood was drawn on Day 5, Day 8, and Day 12. Serum was prepared by allowing the blood samples to clot followed by centrifugation. ALT, AST, and LDH levels were then measured in the serum sample. ALT, AST and LDH levels were low following administration of ASO6. There was also little change in bodyweight.

[0259] To determine if internal cytosine methylation, *i.e.*, methylating the cytosines in the gap region of the ASO, had an impact on the liver safety profile of an ASO, the internal cytosines in ASO 6 were replaced with 5-methylcytosines. Liver safety was analyzed using the same protocols described above. The addition of 5-methylcytosine had only a minor beneficial effect on the liver safety profile of ASO6, *i.e.*, the minor increase in liver enzyme levels were ameliorated by cytosine methylation.

Example 5. *In Vivo* ED50 Screening

[0260] ASO6 was diluted in sterile saline and administered to hTau (mTau^{-/-}) mice via ICV injection at several doses ranging, including 5 and 15 µg (0 and 50 µg data were used from the previous study) at a final volume of 10 µL.

[0261] Two weeks post dose, tissues were harvested and MAPT expression was measured in the brain and spinal cord via bulk RNA isolation followed by qPCR of MAPT and Gapdh according to the protocol described above. In *in vivo* ED50s of 17.7 µg and 13.1 µg were observed.

Example 6. *In Vivo* ASO Half Life Determination

[0262] ASO6 was diluted in sterile saline and administered to hTau (mTau^{-/-}) mice via ICV injection at a moderate dose of 25 µg in 10 µL total volume. Tissues were harvested at 5 and 9 weeks post-dose to determine the duration of tau knockdown. MAPT expression was measured in the brain and spinal cord via bulk RNA isolation followed by qPCR of MAPT and Gapdh

according to the protocol described above. MAPT knockdown persisted in the brain and spinal cord for at least 9 weeks.

Example 7. *In Vivo* Rat Renal Toxicity Determination

[0263] The rat is considered the most sensitive species for detecting ASO-mediated kidney toxicity and are the preferred species to use for risk assessment because they can overpredict susceptibility of renal toxicity in humans. The objective of this study was to determine the effects, if any, on kidney function and morphology (gross and histologic evaluation) following repeat dose administration of five ASO6, in a 2-week rat study.

[0264] Five Male Wistar Hannover rats (7-8 weeks old) were administered two 40 mg/kg subcutaneous injections of Control ASO A, ASO6, A or saline (vehicle control) on Days 1 and 8; animals were euthanized on Day 15. Body weight measurements were performed prior to dosing on Days 1 and 8, and on Days, 5, 10, and 15. Clinical observations were performed following dosing on Days 1 and 8. Blood samples were collected for measurement of blood urea nitrogen (BUN) and creatinine as markers of kidney function on Days 5, 10, and 15. On Day 15, kidneys were collected for microscopic evaluation, and urine was collected for measurement of KIM-1:creatinine ratio as an index for renal injury.

[0265] ASO6 was well tolerated in Wistar Hannover rat with no changes in clinical signs or body weight throughout the treatment period. Serum BUN and creatinine levels were within normal historical range in all treatment groups and comparable to vehicle controls. On Day 15, changes in urine KIM-1: creatinine ratio were considered below critical limit and of a magnitude of change commonly observed in rats under similar study conditions.

[0266] Microscopic minimal to mild proximal tubular epithelial degeneration associated with minimal regeneration and minimal to mild interstitial, perivascular, and/or periglomerular mononuclear cellular infiltrates in the kidney were observed. These microscopic changes were considered non-adverse, based on minimal to mild severity and the absence of correlating functional changes in clinical signs, body weight, and serum and urinary kidney biomarkers (BUN, creatinine, and KIM-1). In conclusion, no evidence of adverse effects on kidney function or histopathology was detected in Wistar Han rats.

Table 4

Name	SEQ ID NO	Corresponding Modified Sequence**
ASO 6 with LNAs and PS linkages	111	L1+A*+[%C]*+[%C]*T*T*A*A*G*T*A*T*T*A*C*T*+T*+G*+[%C]

ASO 6 with LNAs, PS linkages, and internal 5-methyl C	111	L1+A*+[%C]*+[%C]*T*T*A*A*G*T*A*T*T*A*[%C]*T*+T*+G*+[%C]
**wherein recited base sequence is modified as follows: “+” indicates LNA nucleosides, all others are DNA nucleosides, [%C] = 5-methylcytosine nucleoside * indicates a phosphorothioate internucleoside linkage		

Example 8: In Vivo Potency and Stability Assessment of MAPT ASOs

[0267] ASO SEQ ID NO:111 was further modified as shown in **Table 5** below:

Table 5

ASO ID	Sequence**	Conjugated Molecule ID
ASOM_unmod	L1+A*+[%C]*+[%C]*T*T*A*A*G*T*A*T*T*A*C*T*+T*+G*+[%C]	OTVM_Unmod/SEQ ID NO:111
ASOM1	L1+A*+[%C]*+[%C]*T*T*A*AnGnT*A*T*T*A*C*T*+Tn+Gn+[%C]	OTVM1/SEQ ID NO:111
ASOM2	L1+An+[%C]n+[%C]*T*T*AuAuG*T*A*T*T*A*C*T*+Tn+Gn+[%C]	OTVM2/SEQ ID NO:111
ASOM3	L1+An+[%C]n+[%C]*T*T*AtAtG*T*A*T*T*A*C*T*+Tn+Gn+[%C]	OTVM3/SEQ ID NO:111
ASOM4	L1+A*+[%C]*+[%C]*T*T*AuAuGuT*A*T*T*A*C*Tn+Tn+Gn+[%C]	OTVM4/SEQ ID NO:111
ASOM5	L1+An+[%C]n+[%C]nT*T*AtAtG*T*A*T*T*A*C*Tn+Tn+Gn+[%C]	OTVM5/SEQ ID NO:111
Control ASO 1	L1mG*mG*mT*mT*mG*A*[%C]*A*T*[%C]*G*T*[%C]*T*G*m[%C]*m[%C]*mT*mG*mT (GGTTGACATCGTCTGCCTGT)	SEQ ID NO:30
Control ASO 2	L1m[%C]*m[%C]PmG*mT*mT*mT*T*[%C]*T*T*A*[%C]*[%C]*mA*m[%C]Pm[%C]*m[%C]*mT (CCGTTTCTTACCACCT)	SEQ ID NO:113
**wherein recited base sequence is modified as follows: “+” indicates LNA nucleosides, mG, mA, mT, and m[%C] indicate 2'MOE modified nucleosides, all others are DNA nucleosides [%C] = 5-methylcytosine nucleoside * indicates a phosphorothioate internucleoside linkage n indicates a PN linkage u indicates a MsPA internucleoside linkage t indicates a PS2 internucleoside linkage L1 indicates a 5' terminal PO group		

[0268] ASOs were diluted in sterile saline and administered to hTau mice (mTau^{-/-}) via intracerebroventricular (ICV) injection at a single dose of either 10 or 25 µg (according to **Table 6** below) in 10 µL total volume per mouse. 10-14 days post dose (according to **Table 6** below), brain tissues were harvested to measure relative MAPT RNA levels (according to the

tissue homogenization and qPCR procedures below) and total ASO concentration (according to the tissue homogenization and total ASO assay procedures below).

Table 6.

ASO	Dose (μ g)	Takedown (days post dose)
ASOM_unmod	10	14
ASOM_unmod	25	14
Control ASO 1	10	14
Control ASO 1	25	14
Control ASO 2	10	14
Control ASO 2	25	14
ASOM_unmod	10	10
ASOM_unmod	10	28
ASOM4	10	10
ASOM4	10	28
ASOM5	10	10
ASOM5	10	28

[0269] Results are shown in FIG. 8 (relative MAPT RNA levels) and FIG. 9 (total ASO). These results suggest enhanced potency of the ASO base sequence compared to existing control MAPT ASOs, and enhanced tissue stability of the modified ASOs (e.g., ASOM4 and ASOM5) compared to the unmodified version (ASOM_unmod), which should extend the duration of action of the modified ASOs in vivo.

Example 9: *In Vivo* OTV Brain Uptake and MAPT Knockdown after modified OTV:MAPT

[0270] *Peripheral Dosing.* The five modified ASOs above selected for potency were synthesized and bioconjugated to TV proteins according to the procedures described above to make five MAPT OTV molecules (OTVM1, OTVM2, OTVM3, OTVM4, OTVM5) for assessing tau knockdown in the brain following peripheral intravenous administration.

[0271] Tfr^{ms/hu} knock-in mice (*see*, US Patent No. 10,143,187 herein incorporated by reference) were dosed once at 25mpk with each of the OTVs. The MAPT OTVs were diluted in sterile saline and administered intravenously. Mice were weighed prior to each dose to determine appropriate dosage. Plasma was collected 4, 24, and 72 hr post dose, while terminal plasma and tissue samples (*i.e.*, brain and liver) were collected one week post dose to determine

the level of total full length ASO deposited to the brain (FIG. 1 and FIG. 2) according to the procedures below.

[0272] Additionally, $TfR^{ms/hu}$ knock-in mice (*see*, US Patent No. 10,143,187 herein incorporated by reference) were crossed to mice transgenic for $hTau^{+/-}$ (human Tau) and $mTau^{-/-}$ (mouse Tau knockout) and resulting mice were administered either 4 doses (Day 0, 1 week, 2 week, 3 week) or 8 doses (Day 0, Day 3, Day 7, Day 10, Day 14, Day 17, Day 21, Day 24) of each of the MAPT OTVs. The MAPT OTVs were diluted in sterile saline and administered to the mice intravenously. Anti-CD4 was administered prior to the initial and 3rd week of dosing to prevent anti-drug antibody responses in the mice. Mice were weighed prior to each dose to determine appropriate dosage. Brain samples were collected one week after the final dose to determine ASO concentration in the brain using the total ASO assay described below (FIG. 3), and the level of human Tau knockdown relative to Gapdh and saline-dosed mice (FIG. 4 and FIG. 5) according to the procedures below.

[0273] These values were then compared to a separate study in which the same mouse line was used to assess brain uptake and target knockdown an OTV molecule conjugated to an unmodified ASO. In this study, mice were administered either 1 (Day 0) or 4 doses (Day 0, 1 week, 2 week, 3 week) of the unmodified MAPT OTV. The MAPT OTV was diluted in sterile saline and administered to the mice intravenously. Anti-CD4 was administered prior to the initial and 3rd week of dosing to prevent anti-drug antibody responses in the mice. Mice were weighed prior to each dose to determine appropriate dosage. Brain samples were collected one week after the final dose to determine ASO concentration in the brain using the total ASO assay (FIG. 6), and the level of human Tau knockdown relative to Gapdh and saline-dosed mice (FIG. 7) according to the procedures below.

[0274] Taken together, these results demonstrate superior ASO brain uptake and target knockdown with modified MAPT OTVs compared to unmodified MAPT OTV. We observed 20-27nM ASO brain uptake (modified sequences) one week after a 25mpk IV dose (FIG. 1 and FIG. 2) compared to 18nM (unmodified) (FIG. 6), 33-53nM ASO brain uptake (modified sequences) one week after four 25mpk IV doses (FIG. 3) compared to 18nM (unmodified) (FIG. 6), 53-75% MAPT knockdown in the brain (modified sequences) one week after four 25mpk IV doses (FIG. 4) compared to 21% (unmodified) (Fig. 7), and 137nM ASO brain uptake along with 84% MAPT knockdown in the brain one week after eight 25mpk IV doses (FIG. 3 and FIG. 5).

In Vivo Methods

Methods

[0275] *Mouse handling and tissue collection.* Mice were peripherally administered therapeutic treatment via intravenous (IV) tail vein injection (~200 μ L total volume). For in-life plasma collection, blood was collected via submental puncture and transferred to EDTA coated tubes, then spun down at 12,700 rpm for 7 min at 4 °C before collecting the top plasma layer. For tissue collection, animals were anesthetized with tribromoethanol and whole blood was collected via cardiac puncture into EDTA coated tubes for plasma drug concentration assessment. Following transfer to EDTA coated tubes, whole blood was spun down at 12,700 rpm for 7 min at 4 °C before collecting the top plasma layer. Mice were then perfused with ice-cold PBS transcardially at a rate of 5 mL/min for 5 min. For biochemical analysis, tissues were collected, weighed, snap frozen on dry ice, and then stored at -80°C .

[0276] *Intracerebroventricular Bolus (ICV) surgery.* Procedures were performed as described in DeVos SL (“Direct intraventricular delivery of drugs to the rodent central nervous system” J Vis Exp 2013 May 12;(75):e50326; incorporated herein by reference) and summarized in brief as follows. In preparation for the surgery, the surgical area was sterilized with 70% ethanol. Mice were brought under anesthesia with 4% isoflurane. Hair was shaved between from the shoulder region to between the eyes prior to placing mouse on stereotax surface. With a maintenance level of 2% isoflurane, an incision was made from the base of the neck up to between the eyes. Following cleaning with hydrogen peroxide, a needle was slowly driven through the skull at a rate of 1 mm/s. After a 2-3 min period to allow for brain sealing around the needle, a dose of 10 μ L ASO at 1 μ L per second was administered. With a cotton swab held against the skull at the base of the needle, the needle was raised at a rate of 1 mm per second. The cotton swab was held at the site of injection for 1 min to limit drug leakage. Following ICV bolus, the incision was sutured and treated with an antibiotic ointment. The mouse was transferred to a heated recovery pad and observed for full recovery. Mice were monitored daily after surgery to check for pain, discomfort, or infections.

[0277] *Tissue homogenization for drug concentration measurement and protein assays.* Weighed frozen tissue samples were processed for biochemical assays by adding 10 $\times$ volume chilled 1% NP40 + PBS homogenization buffer with added cOmplete Protease Inhibitor (Roche #04693132001) and PhosStop (Roche 04906837001) phosphatase inhibitors. Samples were homogenized using 3 mm tungsten carbide beads in 1.5 mL Eppendorf tubes, shaken using the Qiagen TissueLyzer II (Cat No./ID: 85300) (2 $\times$ 3 min at 27 Hz). For protein assays, samples were then centrifuged for 15 min at 17000 $\times$ g, and the supernatant was removed and used for assays.

[0278] *Tissue homogenization for RNA measurements.* Weighed frozen tissue samples were processed for RNA assays by adding 10× volume Qiazol reagent. Samples were homogenized using 5 mm tungsten carbide beads in 2 mL Eppendorf tubes, shaken using the Qiagen TissueLyzer II (Cat No./ID: 85300) (2× 3 min at 27 Hz). After lysis, samples were incubated for 5 min at room temperature, then chloroform was added. Samples were vortexed, incubated at room temperature for 3 min, then centrifuged for 15 min at 12000×g at 4°C. The aqueous phase was then isolated. RNA was then isolated by adding isopropanol, vortexing, incubating for 10 min at room temperature, then centrifuging for 10 min at 12000×g at 4°C. The resulting pellet was then resuspended in 75% ethanol, vortexed and centrifuged for 5 min at 7500×g at 4°C. The final pellet was resuspended in water.

[0279] *huIgG Assay.* Quantification of humanized antibodies in mouse plasma and tissue lysates were measured using a generic electrochemiluminescence immunoassay (ECLIA). Briefly, to the wells of an MSD GOLD 96-well streptavidin-coated microtiter plate (Meso Scale Discovery, Rockville, MD), a working concentration of biotinylated goat anti-human IgG polyclonal primary antibody (Southern Biotech, Birmingham, AL) prepared in assay diluent was incubated for approximately 1 hr. Following this incubation and a plate wash step, prepared test samples (with sample pre-dilution, where appropriate) and relevant standards were added to the assay plate and allowed to incubate for approximately 1 hr. Following test sample incubation and a plate wash step, secondary ruthenylated (SULFO-TAG) goat anti-human IgG antibody (Meso Scale Discovery, Rockville, MD) at a working concentration in assay diluent was added to the assay plate and incubated for approximately 1 hr. Following a plate wash, a 1× MSD Read Buffer T (Meso Scale Discovery, Rockville, MD) was then added to generate the electrochemiluminescence (ECL) assay signal, which was then expressed in ECL units (ECLU). All of the assay reaction steps were performed at ambient temperature with shaking on a plate shaker (where appropriate); and all test samples were pre-diluted at the assay MRD of 1:20 prior to analyzing in the assay plate. Sample ECLU signals generated in the assay subsequently were processed into concentrations by back-calculating off the assay calibration (CS) curve. The assay CS curve was fitted with a weighted four-parameter nonlinear logistic regression for use in calculating concentrations for unknown/test samples.

[0280] *Total ASO Assay.* Quantification of total ASO (in conjugated and free forms) in mouse plasma and tissue homogenates were measured using a hybridization-based electrochemiluminescence immunoassay (ECLIA). Briefly, custom biotinylated and digoxigenin-conjugated antisense probes (synthesized by Integrated DNA Technologies, Coralville, IA) at working concentrations were combined with prepared test samples (with

sample pre-dilution, where appropriate) and relevant standards in TE Buffer (10 mM Tris-HCL containing 1 mM EDTA). Prepared samples in TE buffer were added, in a 1:1 mix, into 1× SSC Buffer (Sigma-Aldrich, St. Louis, MO) containing a working concentration of recombinant proteinase K enzyme (ThermoFisher, Waltham, MA). Hybridization/Enzyme mixture was then digested, denatured, annealed, and cooled in a thermal cycler instrument. Following hybrid product incubation, samples were added to the wells of an MSD GOLD 96-well streptavidin-coated microtiter plate (Meso Scale Discovery, Rockville, MD) and incubated for approximately 30 min. Following incubation and a plate wash step, secondary ruthenylated (SULFO-TAG) sheep anti-digoxigenin antibody (Novus Biologicals, Littleton, CO) at a working concentration in assay diluent was added to the plate and incubated for approximately 30 min. Following a plate wash, a 1× MSD Read Buffer T (Meso Scale Discovery, Rockville, MD) was then added to generate the electrochemiluminescence (ECL) assay signal, which was then expressed in ECL units (ECLU). All of the assay reaction steps were performed at ambient temperature with shaking on a plate shaker (where appropriate); and all test samples were pre-diluted at the assay MRD of 1:20 prior to analyzing in the assay plate. Sample ECLU signals generated in the assay subsequently were processed into concentrations by back-calculating off the assay calibration (CS) curve. The assay CS curve was fitted with a weighted four-parameter nonlinear logistic regression for use in calculating concentrations for unknown/test samples.

[0281] *qPCR analysis.* To evaluate target mRNA levels, qRT-PCR was run on RNA extracted from tissue lysates. Target mRNA levels were evaluated using Taqman probes (hMAPT, mGapdh) and the Express One-Step Kit. For each sample, hMAPT mRNA levels were normalized to the housekeeping gene Gapdh. qRT-PCR was performed using a QuantStudio 6 Flex system (Applied Biosystems) and average CT values were measured for each probe using technical duplicates. Next, the delta delta CT ($\Delta\Delta CT$) values were calculated relative to the non-ASO treated group and plotted as relative expression levels.

Example 10. Soft Spot Identification Assay

[0282] In order to determine the catabolism and biotransformation of oligonucleotides (ASOs) and to further characterize the oligonucleotides and oligonucleotide polypeptide conjugates described herein, a stability assay to identify likely sites of catabolism (clipping sites) of ASOs (referred to herein as "soft spots") had to be developed. The catabolism assay, described in detail below, was used to identify soft spots for potential modification in order to design and provide more stable molecules. Previous assay methods for in vitro of oligonucleotides are

described, for example, by Basiri et al. in *Molecular Therapy: Nucleic Acids*; 21: 725-736 (2020).

[0283] Tissue Homogenization and Incubation—Frozen mouse livers were homogenized at 200 mg/ml tissue concentration in pbs buffer (pH 7.4) containing 1% NP-40. 200 µL of the homogenate was spiked with ASOs at 1-2 mM concentration and incubated at 37° C for 48 hr (at 300 g shaking).

[0284] Sample Preparation—200 mL of 10% phosphoric acid was added to 200 mL incubated tissue homogenates and were vortexed for 5 min. 600 mL of the Clarity OTX Lysis-Loading Buffer (Phenomenex, PN AL0-8579) was added to each tube and were vortexed for 5 min followed by centrifugation at 3200 rpm at 4°C for 10 min. Clarity OTX SPE plates were used. Table 5 shows the detailed procedure.

Table 7. Sample Preparation

SPE Step	Solvent
Condition	1 mL Methanol
Equilibrate	1mL 50mM NaH ₂ PO ₄ , 2 mM NaN ₃ , 10 mM K ₂ EDTA in water (pH = 5.5)
Load	0.4 mL pretreated sample
Equilibrate	1mL 50mM NaH ₂ PO ₄ , 2 mM NaN ₃ , 10 mM K ₂ EDTA in water (pH = 5.5)
Wash 1	1mL 50 mM NaH ₂ PO ₄ in 50:50 (v:v) water: acetonitrile (pH = 5.5)
Wash 2	1mL 50 mM NH ₄ HCO ₃ in 50:50 (v:v) water: acetonitrile (pH = 5.5)
Elute	2x0.5 mL 100 mM NH ₄ HCO ₃ , 10 mM TCEP in 50:40:10 (v:v:v) water:acetonitrile:tetrahydrofuran (pH = 9.8)
Evaporation	Under nitrogen dryer (60° C) until dry
Reconstitute	Reconstitute with 100 mL 5% MeOH in water
Analysis	10-20 mL samples injected into the LC-HRMS for analysis

[0285] Sample Analyses were conducted by liquid chromatography and mass spectrometry.

[0286] Liquid chromatography (LC): The ASO separation was carried out by ion pairing chromatography at 70C with a Waters BEH oligonucleotide 2.1×50 mm column. Mobile phase buffer A and B are water and method with 100 mM hexafluoroisopropanol (HFIP) and 15 mM N,N-diisopropylethylamine (DIEA). Chromatography was performed at 0.3 mL/min under the following gradient condition (min-%B) 0-5, 1-5, 6-50, 6.1-95, 7.1-95, 7.2-5, 10-5. The total run time was 10 min and the LC output was diverted to waste from 0-1 min and 7-10min.

[0287] Mass spectrometry (MS): A high resolution MS with IDA method was used to identify catabolites. Identified Soft Spots—The table below shows soft spots that were identified according the methods provided herein. Once one or more soft spot is identified, the relevant internucleoside linkage is replaced by a PS2, MsPA, OiPS, or PN group. More than one

internucleoside linkage adjacent to the soft spot may be replaced. The process may be repeated as necessary after stability testing in order to improve the stability of an ASO.

Table 8. Soft Spots Identified in Exemplary ASO Sequences.

ASOs	ASO Sequences: listed from 5' to 3' ("+" indicates a LNA, "[%C]" indicates 5-methylC, "*" indicate a PS linkage, "n" indicates a PN linkage, "\$" indicates a soft spot)	SEQ ID NO.
2	+A*+[%C]*+[%C]*T*T*A\$A\$G*T*A*T*T*A*C*T*+T*+G*+[%C]	111
6	+A*+[%C]*+[%C]*T*T*A*AnGnT*A*T*T\$A*C*T*+nT+nG+[%C]	111

Example 11. In Vivo Plasma Pharmacokinetics

[0288] ASO SEQ ID NOs:111 and 112 were further modified as shown in **Table 9** below.

Table 9. Stabilized ASO sequences.

ASO ID	ASO Sequence	Conjugated Molecule ID	SEQ ID NO.
ASOM6	L1+A*+[%C]n+[%C]ndT*dT*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM6	111
ASOM7	L1+A*+[%C]*+[%C]*dT*dT*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM7	111
ASOM8	L1+A*+[%C]*+[%C]*dT*dT*dA*dA*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM8	111
ASOM9	L2+An+[%C]n+[%C]*dT*dT*dA*dA*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM9	111
ASOM10	L1+An+[%C]n+[%C]ndT*dT*dA*dA*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM10	111
ASOM11	L1+An+[%C]n+[%C]ndT*dT*dA*dA*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM11	111
ASOM12	L1+An+[%C]n+[%C]ndT*dT*dA*dA*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM12	111
ASOM13	L1+An+[%C]n+[%C]*dT*dT*dA*dA*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM13	111
ASOM14	L1+An+[%C]n+[%C]*dT*dT*dA*dA*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM14a	111
ASOM14	L2+An+[%C]n+[%C]*dT*dT*dA*dA*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM14b	111
ASOM15	L1+An+[%C]n+[%C]ndT*dT*dA*dA*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM15	111
ASOM16	L3+An+[%C]*+[%C]*dT*dT*dA*dA*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM16	111
ASOM17	L1+A*+[%C]*+[%C]*dT*dT*dA*dA*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM17	111
ASOM18	L1+An+[%C]n+[%C]*dT*dT*dA*dA*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM18	111
ASOM19	L1+An+[%C]n+[%C]ndT*dT*dA*dA*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM19	111
ASOM20	L1+An+[%C]n+[%C]ndT*dT*dA*dA*dA*dT*dT*dA*dC*dT*+Tn+Gn+[%C]	OTVM20	111

ASOM21	L1+An+[%C]n+[%C]ndT*dT*dAtdAtdG*dT*dA*dT*dT*dA*dC*dTP+Tn+Gn+[%C]	OTVM21	111
ASOM22	L1+An+[%C]n+[%C]PdT*dT*dAtdAtdG*dT*dA*dT*dT*dA*dC*dTn+Tn+Gn+[%C]	OTVM22	111
ASOM23	L1+An+[%C]P+[%C]ndT*dT*dAtdAtdG*dT*dA*dT*dT*dA*dC*dTn+Tn+Gn+[%C]	OTVM23	111
ASOM24	L1+AP+[%C]n+[%C]ndT*dT*dAtdAtdG*dT*dA*dT*dT*dA*dC*dTn+Tn+Gn+[%C]	OTVM24	111
ASOM25	L1+[%C]n+Tn+G*dT*dTndAndG*dA*dC*dA*dT*dT*dCndAndT*dT*+[%C]*+T*+[%C]	OTVM25	112
ASOM26	L1+[%C]*+T*+G*dT*dTndAndG*dA*dC*dA*dT*dT*dCndAndT*dT*+[%C]n+Tn+[%C]	OTVM26	112
ASOM27	L1+[%C]n+Tn+GndTndT*dAtdG*dA*dC*dA*dT*dT*dC*dAtdT*dTn+[%C]n+Tn+[%C]	OTVM27	112
ASOM28	L1+[%C]n+Tn+G*dT*dT*dAtdGtdA*dC*dA*dT*dT*dCndAndT*dT*+[%C]n+Tn+[%C]	OTVM28	112
+A, +[%C], +T, +G indicate adenine 5-methylcytosine, thymine, and guanine locked nucleosides, respectively. dA, dC, dT, dG indicate deoxyadenosine, deoxycytidine, deoxythymidine, and deoxyguanosine, respectively. * indicates phosphorothioate internucleoside linkage n indicates cyclic phosphoryl guanidine (PN) internucleoside linkage t indicates phosphorodithioate (PS2) internucleoside linkage P indicates phosphodiester internucleoside linkage o indicates OiPS (O-(isopropyl) phosphorothioate L1 indicates a 5' terminal phosphodiester (PO) group L2 indicates a 5' terminal phosphorothioate (PS) group L3 indicates a 5' terminal cyclic phosphoryl guanidine (PN) group			

[0289] To assess the pharmacokinetics of additional oligonucleotide polypeptide conjugates (see Table 7B), the additional conjugates were administered to Sprague Dawley rats at a concentration of 10 mg/kg by intravenous injection as described above. All oligonucleotide polypeptide conjugates were administered at a dose volume of 2 mL/kg. Plasma was collected at 0.25, 4, 24, 48, and 72 h after injection. huIgG and ASO concentrations were measured in plasma as described above.

[0290] Results are shown in Table 10. These results demonstrate that adding stabilizing modifications in various placements and formats consistently improves huIgG clearance, and modifying the region at or near the soft spot with backbone modifications can adjust the ASO clearance by protecting or shifting the clipping site.

Table 10. Rat PK data including ASO CL and IgG CL (all conjugated to S239C site).

Conj. Mol. ID	huIgG clearance (mL/d/kg)	ASO clearance (mL/d/kg)
OTVM6	9.2	70.8

OTVM7	15	86.9
OTVM9	13	89.1
OTVM8	21.3	85.8
OTVM13	18.1	76.3
OTVM12	13.6	72.2
OTVM19	22.7	43.9
OTVM20	28	50
OTVM21	20.6	42.4
OTVM22	28.2	56.6
OTVM23	30.3	68.6
OTVM24	33.7	82.7
OTVM14a	17.3	70.7
OTVM17	26.2	98.0
OTVM18	17.8	
OTVM15	13.4	49.5
OTVM16	12.3	58.3
OTVM14b	8.8	55.1
OTVM25	17.1	56.8
OTVM26	16.2	27.9
OTVM27	16.5	51.8
OTVM28	44.7	67.2

[0291] ASOs (unconjugated) were administered to hTau mice (mTau^{-/-}) via intracerebroventricular (ICV) injection at a single dose in 10 µL total volume per mouse. Brain tissues were harvested to measure relative MAPT RNA levels. The results, which demonstrate significant knockdown of MAPT expression across the tested ASOs, are shown in FIG. 12.

Claims:

1. A Microtubule-associated protein tau (MAPT) antisense oligonucleotide (ASO) conjugate comprising a transferrin receptor (TfR)-targeting Fc polypeptide dimer and a MAPT ASO, wherein:

(a) the TfR-targeting Fc polypeptide dimer comprises

(i) a first Fc polypeptide;

(ii) a second Fc polypeptide, wherein the second Fc polypeptide comprises a modified constant domain that specifically binds to human transferrin receptor 1 (TfR) and comprises a sequence having at least 90% sequence identity to SEQ ID NO:12, wherein second Fc polypeptide forms an Fc dimer with the first Fc polypeptide,

(b) the MAPT ASO comprises:

5'-AL_xd^mCL_xe^mCL_xfTTAp_aAp_bGp_cTATTACT_xgTL_xhGL_xi^mCL-3' (SEQ ID NO:111) or

5'-CL_xdTL_xeG_xfTp_jTp_kAp_iGp_mA^{*}C^{*}A^{*}T^{*}T^{*}Cp_nAp_oT^{*}T_xgCL_xhTL_xiCL-3' (SEQ ID NO:112)

wherein

AL, ^mCL, GL, and TL are adenine, 5-methylcytosine, guanine, and thymine locked nucleosides, respectively;

A, C, G, and T are deoxyadenosine, deoxycytidine or 5-methyldeoxycytidine, deoxyguanine, and deoxythymidine nucleosides, respectively;

each **p** is independently a phosphorothioate (PS) internucleoside linkage or a stabilizing internucleoside linkage;

each **x** is independently a PS internucleoside linkage, a phosphodiester (PO) internucleoside linkage, or a stabilizing internucleoside linkage; and

any internucleoside linkage that is not a stabilizing internucleoside linkage is a PS internucleoside linkage or a phosphodiester internucleoside linkage; and

wherein the first Fc polypeptide is linked to the MAPT ASO.

2. The MAPT ASO conjugate of claim 1, wherein the stabilizing internucleoside linkages are independently selected from the group consisting of: phosphorodithioate (PS2) internucleoside linkage, a phosphorylguanidine (PN) internucleoside linkage, a mesylphosphoramidate (MsPA) internucleoside linkage, and an O-isopropyl phosphorothioate (OiPS) internucleoside linkage.

3. The MAPT ASO conjugate of claim 1, wherein each **p** and **x** is a PS internucleoside linkage.
4. The MAPT ASO conjugate of claim 1 or 2, wherein
 - (a) **x_g**, **x_h**, and **x_i** are each PS internucleoside linkages;
 - (b) **x_g** is a PS internucleoside linkage, and **x_h** and **x_i** are each PN internucleoside linkages;
 - (c) **x_g**, **x_h**, and **x_i** are each PN internucleoside linkages;
 - (d) **x_g** and **x_h** are each PN internucleoside linkages, and **x_i** is a PO internucleoside linkage;
 - (e) **x_g** and **x_i** are each PN internucleoside linkages, and **x_h** is a PO internucleoside linkage; or
 - (f) **x_h** and **x_i** are each PN internucleoside linkages, and **x_g** is a PO internucleoside linkage.
5. The MAPT ASO conjugate of any one of claims 1, 2 and 4, wherein:
 - (a) **x_d**, **x_e**, and **x_f** are each PS internucleoside linkages;
 - (b) **x_d** and **x_e** are PN internucleoside linkages, and **x_f** is a PS internucleoside linkage;
 - (c) **x_d** is a PS internucleoside linkage, and **x_e** and **x_f** are each PN internucleoside linkages;
 - (d) **x_d** is a PN internucleoside linkage, and **x_e** and **x_f** are each PS internucleoside linkages;
 - (e) **x_d**, **x_e**, and **x_f** are each PN internucleoside linkages;
 - (d) **x_d** and **x_e** are each PN internucleoside linkages, and **x_f** is a PO internucleoside linkage;
 - (e) **x_d** and **x_f** are each PN internucleoside linkages, and **x_e** is a PO internucleoside linkage; or
 - (f) **x_e** and **x_f** are each PN internucleoside linkages, and **x_d** is a PO internucleoside linkage.
6. The MAPT ASO conjugate of claim 4 or 5, wherein:
 - (a) **x_d**, **x_e**, **x_f**, **x_g**, **x_h**, and **x_i** are each PS internucleoside linkages

- (b) x_d , x_e , and x_f are each PS internucleoside linkages, x_g is a PS internucleoside linkage, and x_h and x_i are each PN internucleoside linkages;
- (c) x_d , x_e , and x_f are each PS internucleoside linkages, and x_g , x_h , and x_i are each PN internucleoside linkages;
- (d) x_d and x_e are PN internucleoside linkages, x_f is a PS internucleoside linkage, and x_g , x_h , and x_i are each PS internucleoside linkages;
- (e) x_d and x_e are PN internucleoside linkages, x_f is a PS internucleoside linkage, x_g is a PS internucleoside linkage, and x_h and x_i are each PN internucleoside linkages;
- (f) x_d is a PS internucleoside linkage, x_e and x_f are each PN internucleoside linkages, x_g is a PS internucleoside linkage, and x_h and x_i are each PN internucleoside linkages;
- (g) x_d is a PN internucleoside linkage, and x_e and x_f are each PS internucleoside linkages, x_g is a PS internucleoside linkage, and x_h and x_i are each PN internucleoside linkages;
- (h) x_d , x_e , x_f , x_g , x_h , and x_i are each PN internucleoside linkages;
- (i) x_d , x_e , and x_f are each PN internucleoside linkages, x_g and x_h are each PN internucleoside linkages, and x_i is a PO internucleoside linkage;
- (j) x_d , x_e , and x_f are each PN internucleoside linkages, x_g and x_i are each PN internucleoside linkages, and x_h is a PO internucleoside linkage;
- (k) x_d , x_e , and x_f are each PN internucleoside linkages, x_h and x_i are each PN internucleoside linkages, and x_g is a PO internucleoside linkage;
- (l) x_d and x_e are each PN internucleoside linkages, and x_f is a PO internucleoside linkage, and x_g , x_h , and x_i are each PN internucleoside linkages;
- (m) x_d and x_f are each PN internucleoside linkages, and x_e is a PO internucleoside linkage, and x_g , x_h , and x_i are each PN internucleoside linkages; or
- (n) x_e and x_f are each PN internucleoside linkages, and x_d is a PO internucleoside linkage, and x_g , x_h , and x_i are each PN internucleoside linkages.

7. The MAPT ASO conjugate of any one of claims 1-2 and 4-6, wherein the MAPT ASO comprises SEQ ID NO:111, and

- (a) p_a , p_b , and p_c are PS internucleoside linkages;
- (b) p_a is a stabilizing internucleoside linkage, and p_b and p_c are PS internucleoside linkages;

- (c) p_b is a stabilizing internucleoside linkage, and p_a and p_c are PS internucleoside linkages;
 - (d) p_a and p_b are stabilizing internucleoside linkages and p_c is a PS internucleoside linkage;
 - (e) p_b and p_c are stabilizing internucleoside linkages and p_a is a PS internucleoside linkage; or
 - (f) p_a , p_b , and p_c are stabilizing internucleoside linkages.
8. The MAPT ASO conjugate of claim 7, wherein
- (a) p_a is a PS2 linkage, and p_b and p_c are PS internucleoside linkages;
 - (b) p_b is a PS2 linkage, and p_a and p_c are PS internucleoside linkages;
 - (c) p_b is a PN linkage, and p_a and p_c are PS internucleoside linkages;
 - (d) p_a and p_b are PN internucleoside linkages and p_c is a PS internucleoside linkage;
 - (e) p_a and p_b are PS2 internucleoside linkages and p_c is a PS internucleoside linkage;
 - (f) p_a and p_b are MsPA internucleoside linkages and p_c is a PS internucleoside linkage;
 - (g) p_a and p_b are OiPS internucleoside linkages and p_c is a PS internucleoside linkage;
 - (h) p_b and p_c are PN internucleoside linkages and p_a is a PS internucleoside linkage;
 - (i) p_a , p_b , and p_c are PN internucleoside linkages; or
 - (j) p_a , p_b , and p_c are MsPA internucleoside linkages.
9. The MAPT ASO conjugate of claim 1, wherein the MAPT ASO comprises the oligonucleotide:
- (a) $A_L^{*m}C_L^{*m}C_L^{*m}T^*T^*A^*A^*G^*T^*A^*T^*T^*A^{*m}C^*T^*T_L^*G_L^{*m}C_L$;
 - (b) $A_L^{*m}C_L^{*m}C_L^{*m}T^*T^*A^*S^*A^*S^*G^*T^*A^*T^*T^*A^*C^*T^*T^*L^*G_L^{*m}C_L$;
 - (c) $A_L^{*m}C_L^{*m}C_L^{*m}T^*T^*A^*n^*n^*G^*n^*T^*A^*T^*T^*A^*C^*T^*T_L^*G_L^{*m}C_L$;
 - (d) $A_L^{*m}C_L^{*m}C_L^{*m}T^*T^*A^*A^*n^*G^*n^*T^*A^*T^*T^*A^*C^*T^*T_L^*n^*G_L^*n^*C_L$;
 - (e) $A_L^{*m}C_L^{*m}C_L^{*m}T^*T^*A^*n^*n^*G^*n^*T^*A^*T^*T^*A^*C^*T^*T_L^*n^*G_L^*n^*C_L$;
 - (f) $A_L^{*m}C_L^{*m}C_L^{*m}T^*T^*A^*u^*u^*G^*u^*T^*A^*T^*T^*A^*C^*T^*T_L^*n^*G_L^*n^*C_L$;
 - (g) $A_L^{*m}C_L^{*m}C_L^{*m}T^*T^*A^*n^*n^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^*n^*G_L^*n^*C_L$;
 - (h) $A_L^{*m}C_L^{*m}C_L^{*m}T^*T^*A^*n^*n^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^*n^*G_L^*n^*C_L$;

- (i) $A_L n^m C_L n^m C_L * T * T * A n A n G * T * A * T * T * A * C * T * T_L * G_L * m^m C_L$;
- (j) $A_L n^m C_L n^m C_L * T * T * A * A n G n T * A * T * T * A * C * T * T_L * G_L * m^m C_L$;
- (k) $A_L n^m C_L n^m C_L * T * T * A * A n G * T * A * T * T * A * C * T * T_L n G_L n^m C_L$;
- (l) $A_L n^m C_L n^m C_L * T * T * A u A u G * T * A * T * T * A * C * T * T_L n G_L n^m C_L$;
- (m) $A_L n^m C_L n^m C_L * T * T * A t A t G * T * A * T * T * A * C * T * T_L n G_L n^m C_L$;
- (n) $A_L n^m C_L n^m C_L * T * T * A * A u G u T * A * T * T * A * C * T * T_L n G_L n^m C_L$;
- (o) $A_L n^m C_L n^m C_L n T * T * A * A * G * T * A * T * T * A * C * T n T_L n G_L n^m C_L$;
- (p) $A_L n^m C_L n^m C_L n T * T * A t A * G * T * A * T * T * A * C * T n T_L n G_L n^m C_L$;
- (q) $A_L n^m C_L n^m C_L n T * T * A * A t G * T * A * T * T * A * C * T n T_L n G_L n^m C_L$;
- (r) $A_L n^m C_L n^m C_L n T * T * A t A t G * T * A * T * T * A * C * T n T_L n G_L n^m C_L$;
- (s) $A_L n^m C_L n^m C_L n T * T * A o A o G * T * A * T * T * A * C * T n T_L n G_L n^m C_L$;
- (t) $A_L n^m C_L n^m C_L n T * T * A t A t G * T * A * T * T * A * C * T n T_L n G_L P^m C_L$;
- (u) $A_L n^m C_L n^m C_L n T * T * A t A t G * T * A * T * T * A * C * T n T_L P G_L n^m C_L$;
- (v) $A_L n^m C_L n^m C_L n T * T * A t A t G * T * A * T * T * A * C * T P T_L n G_L n^m C_L$;
- (w) $A_L n^m C_L n^m C P T * T * A t A t G * T * A * T * T * A * C * T n T_L n G_L n^m C_L$;
- (x) $A_L n^m C_L P^m C n T * T * A t A t G * T * A * T * T * A * C * T n T_L n G_L n^m C_L$; or
- (y) $A_L P^m C_L n^m C n T * T * A t A t G * T * A * T * T * A * C * T n T_L n G_L n^m C_L$;

wherein:

A_L is an adenine locked nucleoside;

$m^m C_L$ is a 5-methylcytosine locked nucleoside;

T_L is a thymine locked nucleoside;

G_L is a guanine locked nucleoside;

A is a deoxyadenosine;

C is a deoxycytidine;

$m^m C$ is a 5-methyldeoxycytidine;

T is a deoxythymidine;

G is a deoxyguanosine;

$*$ is a PS internucleoside linkage;

n is a PN internucleoside linkage;

t is a PS2 internucleoside linkage;

u is a MsPA internucleoside linkage;

o is an OiPS internucleoside linkage; and

P is a PO internucleoside linkage.

10. The MAPT ASO conjugate of any one of claims 1-2 and 4-6, wherein the MAPT ASO comprises SEQ ID NO:112, and

- (a) p_k , p_l , p_n , and p_o are stabilizing internucleoside linkages;
- (b) p_k , p_l , p_n , and p_o are stabilizing internucleoside linkages, and p_j and p_m are PS internucleoside linkages;
- (c) p_j , p_l , and p_o are stabilizing internucleoside linkages;
- (d) p_j , p_l , and p_o are stabilizing internucleoside linkages, and p_k , p_m , and p_n are PS internucleoside linkages;
- (e) p_l , p_m , p_n , and p_o are stabilizing internucleoside linkages; or
- (d) p_l , p_m , p_n , and p_o are stabilizing internucleoside linkages, and p_j and p_k are PS internucleoside linkages.

11. The MAPT ASO conjugate of claim 10, wherein

- (a) p_k , p_l , p_n , and p_o are PN internucleoside linkages;
- (b) p_k , p_l , p_n , and p_o are PN internucleoside linkages, and p_j and p_m are PS internucleoside linkages;
- (c) p_j is a PN linkage, and p_l and p_o are PS2 internucleoside linkages;
- (d) p_j is a PN linkage, p_l and p_o are PS2 internucleoside linkages, and p_k , p_m , and p_n are PS internucleoside linkages;
- (e) p_l and p_m are PS2 internucleoside linkages, and p_n , and p_o are PN internucleoside linkages; or
- (d) p_l and p_m are PS2 internucleoside linkages, p_n , and p_o are PN internucleoside linkages, and p_j and p_k are PS internucleoside linkages.

12. The MAPT ASO conjugate of claim 1, wherein the MAPT ASO comprises the oligonucleotide:

- (a) ${}^mC_LnT_LnG_L*T*TnAnG*A*C*A*T*T*CnAnT*T*{}^mC_L*T_L*{}^mC_L$;
- (b) ${}^mC_L*T_L*G_L*T*TnAnG*A*C*A*T*T*CnAnT*T*{}^mC_LnT_Ln{}^mC_L$;
- (c) ${}^mC_LnT_LnG_LnTnT*AtG*A*C*A*T*T*C*AtT*Tn{}^mC_LnT_Ln{}^mC_L$; or
- (d) ${}^mC_LnT_LnG_L*T*T*AtGtA*A*T*T*CnAnT*T*{}^mC_LnT_Ln{}^mC_L$

wherein:

mC_L is a 5-methylcytosine locked nucleoside;

T_L is a thymine locked nucleoside;

G_L is a guanosine locked nucleoside;

A is a deoxyadenosine;
C is a deoxycytidine;
T is a deoxythymidine;
G is a deoxyguanosine;
* is a PS internucleoside linkage;
n is a PN internucleoside linkage; and
t is a PS2 internucleoside linkage.

13. The MAPT ASO conjugate of any one of claims 1-12, wherein the modified constant domain that specifically binds to TfR comprises a glutamate at position 380, a Y at position 384, a T at position 386, a glutamate at position 387, a tryptophan at position 388, an alanine at position 389, an N at position 390, a T at position 413, a glutamate at position 415, a glutamate at position 416, and a phenylalanine at position 421, according to EU numbering.

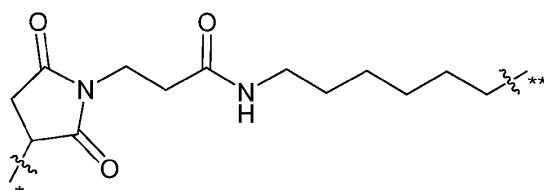
14. The MAPT ASO conjugate of any one of claims 1-13, wherein the first Fc polypeptide and/or the second Fc polypeptide comprises at least one cysteine substitution.

15. The MAPT ASO conjugate of claim 14, wherein at least one cysteine substitution is selected from the group consisting of a S239C substitution, a S442C substitution, a A330C substitution, a K149C substitution, or a T289C substitution.

16. The MAPT ASO conjugate of claim 15, wherein the first Fc polypeptide comprises the at least one cysteine substitution.

17. The MAPT ASO of any one of claims 1-16, wherein the MAPT ASO comprises a 5' terminal group selected from the group consisting of: a phosphodiester group, a phosphorothioate group, a phosphorodithioate group, a mesylphosphoramidate group, a cyclophosphoryl guanine group, and an *O*-isopropyl phosphorothioate group.

18. The MAPT ASO conjugate of any one of claims 14-17, wherein Fc dimer is linked to the MAPT ASO via a linking group attached to the at least one cysteine substitution and the 5' end of the MAPT ASO, wherein the linking group is



wherein valence marked * is attached to the TfR-targeting Fc polypeptide dimer and the valence marked ** is attached to the MAPT ASO.

19. The MAPT ASO conjugate of any one of claims 1-18, wherein the first and/or the second Fc polypeptide further one or more mutations that modulate effector function.
20. The MAPT ASO conjugate of claim 19, wherein the first Fc polypeptide and/or the second Fc polypeptide comprises an alanine at position 234, an alanine at position 235, and a serine or glycine at position 329, each according to EU numbering.
21. The MAPT ASO conjugate any one of claims 1-20, wherein the first and/or the second Fc polypeptide further comprises one or more mutations that increase serum stability.
22. The MAPT ASO conjugate of any one of claims 1-21, wherein the second Fc polypeptide further comprises a tryptophan at position 366, according to EU numbering.
23. The MAPT ASO conjugate of claim 22, wherein the first Fc polypeptide further comprises a serine a position 366, an alanine at position 368, and a valine at position 407, according to EU numbering.
24. The MAPT ASO conjugate of any one of claims 1-21, wherein the second Fc polypeptide comprises a serine a position 366, an alanine at position 368, and a valine at position 407, according to EU numbering.
25. The MAPT ASO conjugate of claim 24, wherein the first Fc polypeptide further comprises a tryptophan at position 366, according to EU numbering.
26. The MAPT ASO conjugate of any one of claims 1-12, wherein the first Fc polypeptide comprises the amino acid sequence of any of SEQ ID NOs:1, 47-48, 51-54, 61-63, 66, 73-78, 80, and 90; and the second Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOs:4-29, 36, 43-44, 49, and 50.

27. The MAPT ASO conjugate of claim 26, wherein the first Fc polypeptide comprises the amino acid sequence of SEQ ID NO:63 or 80; and the second Fc polypeptide comprised the amino acid sequence of SEQ ID NO:11 or 12.
28. The MAPT ASO conjugate of any one of claims 1-27, wherein the first Fc polypeptide is fused to a first non-targeting Fab (NTF) via a first hinge region to form a first Fab-Fc fusion polypeptide
29. The MAPT ASO conjugate of any one of claims 1-27, wherein the second Fc polypeptide is fused to second NTF via a second hinge region to form a second Fab-Fc fusion polypeptide.
30. The MAPT ASO conjugate of any one of claims 1-27, wherein the first Fc polypeptide is fused to a NTF via a first hinge region to form a first Fab-Fc fusion polypeptide and the second Fc polypeptide is fused to second NTF via a second hinge region to form a second Fab-Fc fusion polypeptide.
31. The MAPT ASO conjugate of any one of claims 28-30, wherein the first Fab-Fc fusion polypeptide and/or the second Fab-Fc fusion polypeptide comprises at least one cysteine substitution, optionally wherein the cysteine substitution is selected from the group consisting of: position 114 of the (according to Kabat numbering) of the heavy chain, position 124 (according to EU numbering) of the heavy chain; position 149 (according to EU numbering) of the light chain, or position 156 (according to EU numbering) of the light chain.
32. The MAPT ASO conjugate claim 30 or 31, wherein the first and second NTFs each comprise a heavy chain comprising SEQ ID NO:109 or 130 and a light chain comprising SEQ ID NO:108 or 129; optionally wherein the heavy chain further comprises a hinge region comprising SEQ ID NO:91, 92, 93, or 121.
33. The MAPT ASO conjugate of claim 30 or 31, wherein the first Fab-Fc fusion polypeptide comprises SEQ ID NO:100 or 101 and the second Fab-Fc fusion polypeptide comprises SEQ ID NO:98 or 99.
34. A MAPT ASO conjugate comprising a TfR-targeting Fc polypeptide dimer and at least one MAPT ASO, wherein:

- (a) the TfR-targeting Fc polypeptide dimer comprises
- (i) a first Fc polypeptide,
 - (ii) a second Fc polypeptide, wherein the second Fc polypeptide comprises an alanine at position 234, an alanine at position 235, and serine at position 329, each according to EU numbering, and a modified constant domain that specifically binds to human transferrin receptor 1 (TfR) and comprises a sequence having at least 90% sequence identity to SEQ ID NO:12, wherein second Fc polypeptide forms an Fc dimer with the first Fc polypeptide,
 - (iii) a first non-targeting Fab (NTF) fused to the first Fc polypeptide via a first hinge region to form a first Fab-Fc fusion polypeptide and a second NTF fused to the second Fc polypeptide via a second hinge region to form a second Fab-Fc fusion polypeptide,
- (b) the at least one MAPT ASO comprises
- 5'-AL_{x_d}^mCL_{x_e}^mCL_{x_f}TTAp_aAp_bGp_cTATTACT_{x_g}T_Lx_hGL_{x_i}^mCL-3' (SEQ ID NO:111) or
- 5'-CL_{x_d}T_Lx_eGx_fTp_jTp_kAp_iGp_mA*C*A*T*T*Cp_nAp_oT*T_{x_g}CL_{x_h}T_Lx_iCL-3' (SEQ ID NO:112)
- wherein
- AL, ^mCL, GL, and TL are adenine, 5-methylcytosine, guanine, and thymine locked nucleosides, respectively;
 - A, C, G, and T are deoxyadenosine, deoxycytidine or 5-methyldeoxycytidine, deoxyguanine, and deoxythymidine nucleosides, respectively;
 - each **p** is independently a phosphorothioate (PS) internucleoside linkage or a stabilizing internucleoside linkage;
 - each **x** is independently a PS internucleoside linkage, a phosphodiester (PO) internucleoside linkage, or a stabilizing internucleoside linkage; and
 - any internucleoside linkage that is not a stabilizing internucleoside linkage is a PS internucleoside linkage or a phosphodiester internucleoside linkage; and
 - wherein the first Fab-Fc fusion polypeptide and/or the second Fab-Fc fusion polypeptide comprises at least one cysteine substitution, and
 - wherein the at least one MAPT ASO is conjugated to the first Fab-Fc fusion polypeptide and/or the second Fab-Fc fusion polypeptide to the at least one cysteine substitution via a linker.

35. A MAPT ASO comprising

5'-AL_{x_d}^mCL_{x_e}^mCL_{x_f}TTAp_aAp_bGp_cTATTACT_{x_g}T_Lx_hGL_{x_i}^mCL-3' (SEQ ID NO:111) or

5'-CL_{x_d}TL_{x_e}Gx_fTp_jTp_kAp_iGp_mA^{*}C^{*}A^{*}T^{*}T^{*}Cp_nAp_oT^{*}T_gCL_{x_h}TL_{x_i}CL-3' (SEQ ID NO:112)

wherein

A_L, ^mCL, G_L, and T_L are adenine, 5-methylcytosine, guanine, and thymine locked nucleosides, respectively;

A, C, G, and T are deoxyadenosine, deoxycytidine or 5-methyldeoxycytidine, deoxyguanine, and deoxythymidine nucleosides, respectively;

each **p** is independently a phosphorothioate (PS) internucleoside linkage or a stabilizing internucleoside linkage;

each **x** is independently a PS internucleoside linkage, a phosphodiester (PO) internucleoside linkage, or a stabilizing internucleoside linkage; and

any internucleoside linkage that is not a stabilizing internucleoside linkage is a PS internucleoside linkage or a phosphodiester internucleoside linkage, and

wherein the first Fc polypeptide is linked to the MAPT ASO.

36. The MAPT ASO of claim 29, wherein the stabilizing internucleoside linkages are independently selected from the group consisting of: phosphorodithioate (PS2) internucleoside linkage, a phosphorylguanidine (PN) internucleoside linkage, a mesylphosphoramidate (MsPA) internucleoside linkage, and an O-isopropyl phosphorothioate (OiPS) internucleoside linkage.

37. The MAPT ASO of claim 35, wherein each **p** and **x** is a PS internucleoside linkage.

38. The MAPT ASO of claim 35 or 36, wherein

- (a) **x_g**, **x_h**, and **x_i** are each PS internucleoside linkages;
- (b) **x_g** is a PS internucleoside linkage, and **x_h** and **x_i** are each PN internucleoside linkages;
- (c) **x_g**, **x_h**, and **x_i** are each PN internucleoside linkages;
- (d) **x_g** and **x_h** are each PN internucleoside linkages, and **x_i** is a PO internucleoside linkage;
- (e) **x_g** and **x_i** are each PN internucleoside linkages, and **x_h** is a PO internucleoside linkage; or
- (f) **x_h** and **x_i** are each PN internucleoside linkages, and **x_g** is a PO internucleoside linkage.

39. The MAPT ASO of any one of claim 35, 36, and 38, wherein:
- (a) x_d , x_e , and x_f are each PS internucleoside linkages;
 - (b) x_d and x_e are PN internucleoside linkages, and x_f is a PS internucleoside linkage;
 - (c) x_d is a PS internucleoside linkage, and x_e and x_f are each PN internucleoside linkages;
 - (d) x_d is a PN internucleoside linkage, and x_e and x_f are each PS internucleoside linkages;
 - (e) x_d , x_e , and x_f are each PN internucleoside linkages;
 - (d) x_d and x_e are each PN internucleoside linkages, and x_f is a PO internucleoside linkage;
 - (e) x_d and x_f are each PN internucleoside linkages, and x_e is a PO internucleoside linkage; or
 - (f) x_e and x_f are each PN internucleoside linkages, and x_d is a PO internucleoside linkage.
40. The MAPT ASO of claim 38 or 39, wherein:
- (a) x_d , x_e , x_f , x_g , x_h , and x_i are each PS internucleoside linkages
 - (b) x_d , x_e , and x_f are each PS internucleoside linkages, x_g is a PS internucleoside linkage, and x_h and x_i are each PN internucleoside linkages;
 - (c) x_d , x_e , and x_f are each PS internucleoside linkages, and x_g , x_h , and x_i are each PN internucleoside linkages;
 - (d) x_d and x_e are PN internucleoside linkages, x_f is a PS internucleoside linkage, and x_g , x_h , and x_i are each PS internucleoside linkages;
 - (e) x_d and x_e are PN internucleoside linkages, x_f is a PS internucleoside linkage, x_g is a PS internucleoside linkage, and x_h and x_i are each PN internucleoside linkages;
 - (f) x_d is a PS internucleoside linkage, x_e and x_f are each PN internucleoside linkages, x_g is a PS internucleoside linkage, and x_h and x_i are each PN internucleoside linkages;
 - (g) x_d is a PN internucleoside linkage, and x_e and x_f are each PS internucleoside linkages, x_g is a PS internucleoside linkage, and x_h and x_i are each PN internucleoside linkages;
 - (h) x_d , x_e , x_f , x_g , x_h , and x_i are each PN internucleoside linkages;

- (i) x_d , x_e , and x_f are each PN internucleoside linkages, x_g and x_h are each PN internucleoside linkages, and x_i is a PO internucleoside linkage;
- (j) x_d , x_e , and x_f are each PN internucleoside linkages, x_g and x_i are each PN internucleoside linkages, and x_h is a PO internucleoside linkage;
- (k) x_d , x_e , and x_f are each PN internucleoside linkages, x_h and x_i are each PN internucleoside linkages, and x_g is a PO internucleoside linkage;
- (l) x_d and x_e are each PN internucleoside linkages, and x_f is a PO internucleoside linkage, and x_g , x_h , and x_i are each PN internucleoside linkages;
- (m) x_d and x_f are each PN internucleoside linkages, and x_e is a PO internucleoside linkage, and x_g , x_h , and x_i are each PN internucleoside linkages; or
- (n) x_e and x_f are each PN internucleoside linkages, and x_d is a PO internucleoside linkage, and x_g , x_h , and x_i are each PN internucleoside linkages.

41. The MAPT ASO of any one of claims 35-36 and 38-40, wherein the MAPT ASO comprises SEQ ID NO:111, and

- (a) p_a , p_b , and p_c are PS internucleoside linkages;
- (b) p_a is a stabilizing internucleoside linkage, and p_b and p_c are PS internucleoside linkages;
- (c) p_b is a stabilizing internucleoside linkage, and p_a and p_c are PS internucleoside linkages;
- (d) p_a and p_b are stabilizing internucleoside linkages and p_c is a PS internucleoside linkage;
- (e) p_b and p_c are stabilizing internucleoside linkages and p_a is a PS internucleoside linkage;
- (f) p_a , p_b , and p_c are stabilizing internucleoside linkages.

42. The MAPT ASO of any one of claims 41, wherein

- (a) p_a is a PS2 linkage, and p_b and p_c are PS internucleoside linkages;
- (b) p_b is a PS2 linkage, and p_a and p_c are PS internucleoside linkages;
- (c) p_b is a PN linkage, and p_a and p_c are PS internucleoside linkages;
- (d) p_a and p_b are PN internucleoside linkages and p_c is a PS internucleoside linkage;
- (e) p_a and p_b are PS2 internucleoside linkages and p_c is a PS internucleoside linkage;

- (f) p_a and p_b are MsPA internucleoside linkages and p_c is a PS internucleoside linkage;
- (g) p_a and p_b are OiPS internucleoside linkages and p_c is a PS internucleoside linkage;
- (h) p_b and p_c are PN internucleoside linkages and p_a is a PS internucleoside linkage;
- (i) p_a , p_b , and p_c are PN internucleoside linkages; or
- (j) p_a , p_b , and p_c are MsPA internucleoside linkages.

43. The MAPT ASO of claim 35, wherein the MAPT ASO comprises the oligonucleotide:

- (a) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^{*m}C^*T^*T_L^*G_L^{*m}C_L$;
- (b) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^*G_L^{*m}C_L$;
- (c) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^*G_L^{*m}C_L$;
- (d) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (e) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (f) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (g) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (h) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (i) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^*G_L^{*m}C_L$;
- (j) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^*G_L^{*m}C_L$;
- (k) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (l) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (m) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (n) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (o) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (p) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (q) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (r) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (s) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (t) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (u) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (v) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (w) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$;
- (x) $A_L^{*m}C_L^{*m}C_L^{*T}T^*A^*A^*G^*T^*A^*T^*T^*A^*C^*T^*T_L^{*n}G_L^{*m}C_L$; or

(y) $A_L P^m C_L n^m C n T^* T^* A t A t G^* T^* A^* T^* T^* A^* C^* T n T_L n G_L n^m C_L$;

wherein:

A_L is an adenosine locked nucleic acid;

$^m C_L$ is a 5-methylcytosine locked nucleic acid;

T_L is a thymidine locked nucleic acid;

G_L is a guanosine locked nucleic acid;

A is a deoxyadenosine;

C is a deoxycytidine;

$^m C$ is a 5-methyldeoxycytidine;

T is a deoxythymidine;

G is a deoxyguanosine;

$*$ is a PS internucleoside linkage;

n is a PN internucleoside linkage;

t is a PS2 internucleoside linkage;

u is a MsPA internucleoside linkage;

o is an OiPS internucleoside linkage; and

P is a PO internucleoside linkage.

44. The MAPT ASO conjugate of any one of claims 35-36 and 38-40, wherein the MAPT ASO comprises SEQ ID NO:112, and

- (a) p_k , p_l , p_n , and p_o are stabilizing internucleoside linkages;
- (b) p_k , p_l , p_n , and p_o are stabilizing internucleoside linkages, and p_j and p_m are PS internucleoside linkages;
- (c) p_j , p_l , and p_o are stabilizing internucleoside linkages;
- (d) p_j , p_l , and p_o are stabilizing internucleoside linkages, and p_k , p_m , and p_n are PS internucleoside linkages;
- (e) p_l , p_m , p_n , and p_o are stabilizing internucleoside linkages; or
- (d) p_l , p_m , p_n , and p_o are stabilizing internucleoside linkages, and p_j and p_k are PS internucleoside linkages.

45. The MAPT ASO conjugate of claim 44, wherein

- (a) p_k , p_l , p_n , and p_o are PN internucleoside linkages;
- (b) p_k , p_l , p_n , and p_o are PN internucleoside linkages, and p_j and p_m are PS internucleoside linkages;

- (c) p_j is a PN linkage, and p_l and p_o are PS2 internucleoside linkages;
- (d) p_j is a PN linkage, p_l and p_o are PS2 internucleoside linkages, and p_k , p_m , and p_n are PS internucleoside linkages;
- (e) p_l and p_m are PS2 internucleoside linkages, and p_n , and p_o are PN internucleoside linkages; or
- (d) p_l and p_m are PS2 internucleoside linkages, p_n , and p_o are PN internucleoside linkages, and p_j and p_k are PS internucleoside linkages.

46. The MAPT ASO conjugate of claim 35, wherein the MAPT ASO comprises the oligonucleotide:

- (a) ${}^mC_L n T_L n G_L * T * T n A n G * A * C * A * T * T * C n A n T * T * {}^m C_L * T_L {}^m C_L$;
- (b) ${}^m C_L * T_L * G_L * T * T n A n G * A * C * A * T * T * C n A n T * T * {}^m C_L n T_L n {}^m C_L$;
- (c) ${}^m C_L n T_L n G_L n T n T * A t G * A * C * A * T * T * C * A t T * T n {}^m C_L n T_L n {}^m C_L$; or
- (d) ${}^m C_L n T_L n G_L * T * T * A t G t A * C * A * T * T * C n A n T * T * {}^m C_L n T_L n {}^m C_L$

wherein:

${}^m C_L$ is a 5-methylcytosine locked nucleoside;

T_L is a thymine locked nucleoside;

G_L is a guanosine locked nucleoside;

A is a deoxyadenosine;

C is a deoxycytidine;

T is a deoxythymidine;

G is a deoxyguanosine;

$*$ is a PS internucleoside linkage;

n is a PN internucleoside linkage; and

t is a PS2 internucleoside linkage.

47. A pharmaceutical composition comprising the MAPT ASO conjugate of any one of claims 1-34 or the MAPT ASO of any one of claims 35-46 and a pharmaceutically acceptable carrier or excipient.

48. A method of generating a neuron cell with decreased tau expression, the method comprising delivering to the neuron cell the MAPT ASO conjugate of any one of claims 1-34, the MAPT ASO of any one of claims 35-46, or the pharmaceutical composition of claim 47, wherein the MAPT ASO decreases the expression level of an endogenous MAPT gene in the neuron cell.

49. A method of modifying a neuron cell to decrease tau expression, the method comprising delivering to the neuron cell the MAPT ASO conjugate of any one of claims 1-34, the MAPT ASO of any one of claims 35-46, or the pharmaceutical composition of claim 47, wherein the MAPT ASO decreases the expression level of an endogenous MAPT gene.
50. A method of modifying a neuron cell to decrease tau expression, the method comprising delivering to the neuron cell the MAPT ASO conjugate of any one of claims 1-34, the MAPT ASO of any one of claims 35-46, or the pharmaceutical composition of claim 47, wherein the MAPT ASO specifically reduces the expression level of a MAPT transcript in the cell.
51. The method of claim 50, wherein decreasing the expression level of an endogenous MAPT comprises specifically reducing the expression level of a MAPT transcript in the cell.
52. A method of reducing expression of tau in a cell of the spinal cord of a subject comprising administering the MAPT ASO conjugate of any one of claims 1-34, the MAPT ASO of any one of claims 35-46, or the pharmaceutical composition of claim 47 by intrathecal administration.
53. A method of reducing tau expression in a subject comprising administering the MAPT ASO conjugate of any one of claims 1-34, the MAPT ASO of any one of claims 35-46, or the pharmaceutical composition of claim 47 to the subject.
54. The method of claim 53, wherein tau expression is reduced in the CNS of the subject.
55. The method of claim 53, wherein the MAPT ASO conjugate, the MAPT ASO, or the pharmaceutical composition is administered to the subject by intrathecal administration or intravenous injection or intravenous infusion.
56. The method of any one of claims 48-55, wherein the MAPT ASO decreases the expression level of an endogenous MAPT gene or reduces the level of a MAPT mRNA transcript by at least about 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% as compared to the level without administering the pharmaceutical composition.
57. The method of claim 56, wherein the expression of the endogenous MAPT gene or the level of the MAPT transcript is reduced by at least about 50%.

58. The method of claim 56, wherein the expression of an the endogenous MAPT gene or the level of the MAPT transcript is reduced by at least about 70%.
59. A method of treating a tau-associated neurodegenerative disorder in a human subject in need thereof, the method comprising administering to the human subject the MAPT ASO conjugate of any one of claims 1-34, the MAPT ASO of any one of claims 35-46, or the pharmaceutical composition of claim 47.
60. The method of claim 59, wherein the tau-associated neurodegenerative syndrome is Alzheimer's Disease.
61. A method of treating Alzheimer's disease, the method comprising administering to a human subject in need thereof, the MAPT ASO conjugate of any one of claims 1-34, the MAPT ASO of any one of claims 35-46, or the pharmaceutical composition of claim 47.
62. A method of reducing MAPT messenger ribonucleic acid (mRNA) expression in a human subject in need thereof, the method comprising administering to the human subject the MAPT ASO conjugate of any one of claims 1-34, the MAPT ASO of any one of claims 35-46, or the pharmaceutical composition of claim 47.
63. The pharmaceutical composition as described in claim 47, for use in delivering a MAPT ASO to the CNS of a human subject in need thereof, wherein said MAPT ASO decreases the expression level of an endogenous MAPT gene.
64. The pharmaceutical composition as described in claim 47 for use in treating a tau-associated neurodegenerative disorder in a human subject in need thereof.
65. A pharmaceutical composition as claim 64, wherein the tau-associated neurodegenerative disorder is Alzheimer's.
66. The pharmaceutical composition as described in claim 47 for use in reducing MAPT mRNA expression in a human subject in need thereof.
67. Nucleic acid sequence encoding an Fc polypeptide dimer comprising a first nucleic acid encoding any of SEQ ID NOs: 1, 47-48, 51-54, 61-63, 66, 73-78, 80, and 90; and a second nucleic acid sequence encoding any one of SEQ ID NOs: 4-29, 36, 43-44, and 49-50.

68. The nucleic acid sequences of claim 67, wherein the first nucleic acid sequence encodes a first Fab-Fc fusion polypeptide and the second nucleic acid sequence encoding a second Fab-Fc fusion polypeptide.

69. The nucleic acid sequences of claim 68, wherein the first nucleic acid sequence encodes any one of SEQ ID NOs:100-101 and 130-131; the second nucleic acid sequence encodes any one of SEQ ID NOs:98-99; and wherein the nucleic acid sequence sequences further comprise a third nucleic acid sequence encoding SEQ ID NO:108 or 129.

70. The nucleic acid sequences of claim 69, wherein the first nucleic acid sequence comprises SEQ ID NO:133 or a sequence having at least 75% identity to SEQ ID NO:133; the second nucleic acid sequence comprises SEQ ID NO:132 or a sequence having at least 75% identity to SEQ ID NO:132; and the third nucleic acid sequence comprises SEQ ID NO:134 or a sequence having at least 75% identity to SEQ ID NO:134.