

COMPOSITIONS OF MODIFIED TREMS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 63/130,373, U.S. Provisional Application No. 63/130,374, U.S. Provisional Application No. 63/130,375, U.S. Provisional Application No. 63/130,377, U.S. Provisional Application No. 63/130,381, and U.S. Provisional Application No. 63/130,387, each of which was filed on December 23, 2020. The entire contents of each of the foregoing applications is hereby incorporated by reference.

BACKGROUND

tRNAs are complex RNA molecules that possess a number of functions including the ability to initiate and elongate proteins.

SUMMARY

The present disclosure features, *inter alia*, a tRNA-based effector molecule (TREM) entity comprising an asialoglycoprotein receptor (ASGPR) binding moiety, as well as compositions and methods of use thereof. The ASGPR binding moiety may be conjugated to a nucleobase within the TREM entity, or within an internucleotide linkage of the TREM entity, or at a terminus (e.g., the 5' or 3' terminus) of the TREM entity. In an ~~embodiment~~example, the TREM entity comprises a TREM, a TREM Core Fragment, or a TREM Fragment. In an ~~embodiment~~example, the nucleobase comprises adenine, thymine, cytosine, guanosine, or uracil, or a variant or modified form thereof.

In one ~~aspect~~example, the TREM entity (e.g., TREM) described herein comprises the sequence of Formula A: [L1]-[ASt Domain1]-[L2]-[DH Domain]-[L3]-[ACH Domain] -[VL Domain]-[TH Domain]-[L4]-[ASt Domain2] (A), wherein, independently, the TREM comprises an ASGPR binding moiety. In an ~~embodiment~~example, the ASGPR binding moiety comprises an ASGPR carbohydrate and an ASGPR linker. In an ~~embodiment~~example, the ASGPR binding moiety comprises a galactose (Gal) and/or N-acetylgalactosamine (GalNAc) moiety. In an ~~embodiment~~example, the ASGPR binding moiety comprises a plurality of Gal and/or GalNAc moieties (e.g., 2, 3, 4, 5, 6, 7, 8, or more Gal and/or GalNAc moieties). In an ~~embodiment~~example, the ASGPR binding moiety comprises a

triantennary GalNAc moiety. In an embodimentexample, the TREM further comprises a chemical modification (e.g., a phosphothiorate internucleotide linkage, or a 2'-modification on a ribose moiety within the TREM). In another aspect, the present disclosure provides a tRNA effector molecule (TREM) comprising a sequence of Formula A comprising [L1]_y-[ASt Domain1]_x-[L2]_x-[DH Domain]_x-[L3]_x-[ACH Domain]_x-[VL Domain]_y-[TH Domain]_x-[L4]_x-[ASt Domain2]_x, (A); wherein, independently, [L1] and [VL Domain], are optional; each y is independently 0 or 1; and each x is 0 or 1; and an asialoglycoprotein receptor (ASGPR) binding moiety, wherein the ASGPR binding moiety is conjugated to a nucleobase within a nucleotide of the TREM, or at a terminus of the TREM, or within the internucleotide linkage of a TREM.

In an embodimentexample, the ASGPR binding moiety is present on a nucleobase within a nucleotide in the TREM. In an embodimentexample, the ASGPR binding moiety is present on the 5' terminus of the TREM. In an embodimentexample, the ASGPR binding moiety is present on the 3' terminus of the TREM.

In an embodimentexample, the ASGPR binding moiety is present in a TREM domain selected from L1, ASt Domain1, L2, DH Domain, L3, ACH Domain, VL Domain, TH Domain, L4, and ASt Domain2. In an embodimentexample, the ASGPR binding moiety is present in the L1 region. In an embodimentexample, the ASGPR binding moiety is present in the ASt Domain1. In an embodimentexample, the ASGPR binding moiety is present in the L2 region. In an embodimentexample, the ASGPR binding moiety is present in the DH Domain. In an embodimentexample, the ASGPR binding moiety is present in the L3 region. In an embodimentexample, the ASGPR binding moiety is present in the ACH Domain. In an embodimentexample, the ASGPR binding moiety is present in the VL Domain. In an embodimentexample, the ASGPR binding moiety is present in the TH Domain. In an embodimentexample, the ASGPR binding moiety is present in the L4 region. In an embodimentexample, the ASGPR binding moiety is present in the ASt Domain2.

In an embodimentexample, the TREM comprising an ASGPR binding moiety retains the ability to support protein synthesis, be charged by a synthetase, be bound by an elongation factor, introduce an amino acid into a peptide chain, support elongation, and/or support initiation. In an embodimentexample, the TREM comprising an ASGPR binding moiety comprises at least X contiguous nucleotides without a chemical modification, wherein X is greater than 10. In an embodimentexample, the TREM comprising an ASGPR binding moiety comprises no more than

5, 10, or 15 nucleotides of a type (e.g., A, T, C, G or U) that do not comprise chemical modification. In an ~~embodiment~~example, the TREM comprising an ASGPR binding moiety comprises no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, or 80 nucleotides of a type (e.g., A, T, C, G or U) that do not comprise a chemical modification. In an ~~embodiment~~example, the TREM comprising an ASGPR binding moiety comprises at least X contiguous nucleotides comprising a chemical modification, wherein X is greater than 10. In an ~~embodiment~~example, the TREM comprising an

ASGPR binding moiety comprises more than 5, 10, or 15 nucleotides of a type (e.g., A, T, C, G or U) that comprise a chemical modification. In an embodimentexample, the TREM comprising an ASGPR binding moiety comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, or 80 nucleotides of a type (e.g., A, T, C, G or U) that comprise a chemical modification. In an embodimentexample, the chemical modification is a naturally occurring chemical modification or a non-naturally occurring chemical modification (e.g., a phosphothiorate internucleotide linkage or a 2'-modification on a ribose moiety within the TREM). In an embodimentexample, the chemical modification comprises a fluorophore.

In another aspectexample, a TREM comprising an ASGPR binding moiety, or a composition thereof, described herein may be used to modulate a production parameter (e.g., an expression parameter and/or a signaling parameter) of an RNA corresponding to, or a polypeptide encoded by, a nucleic acid sequence comprising an endogenous open reading frame (ORF) having a premature termination codon (PTC).

In one aspect, the present disclosure provides a pharmaceutical composition comprising a TREM as disclosed herein. In another aspect, the present disclosure provides a lipid nanoparticle formulation comprising a TREM as disclosed herein. In yet another aspect, the present disclosure provides a lipid nanoparticle formulation comprising a pharmaceutical composition as disclosed herein. In one aspect, the present disclosure provides a method of making a TREM as disclosed herein. In another aspectexample, a TREM comprising an ASGPR binding moiety, or a composition thereof, described herein may be used in a method of modulating a production parameter of an mRNA corresponding to, or polypeptide encoded by, an endogenous open reading frame (ORF) in a subject, which ORF comprises a premature termination codon (PTC), contacting the subject with a TREM comprising an ASGPR binding moiety or a composition thereof in an amount and/or for a time sufficient to modulate the production parameter of the mRNA or polypeptide, wherein the TREM comprising an ASGPR binding moiety has an anticodon that pairs with the codon having the first sequence, thereby modulating the production parameter in the subject. In an embodimentexample, the production parameter comprises a signaling parameter and/or an expression parameter, e.g., as described herein.

In one aspect, the present disclosure provides a use of a TREM comprising an ASGPR binding moiety as disclosed herein or a pharmaceutical composition as disclosed herein or a lipid

nanoparticle formulation as disclosed herein, in the manufacture of a medicament for treating a disease or disorder associated with a PTC. In another aspect~~example~~, a TREM comprising an ASGPR binding moiety, or a composition thereof, described herein may be used in a method of treating a subject having an endogenous open reading frame (ORF) which comprises a premature termination codon (PTC), comprising providing a TREM comprising an ASGPR binding moiety, or a composition thereof, wherein the TREM comprising an ASGPR binding moiety comprises an anticodon that pairs with the PTC in the ORF; contacting the subject with the TREM comprising an ASGPR binding moiety or a

composition thereof in an amount and/or for a time sufficient to treat the subject, thereby treating the subject. In an ~~embodiment~~example, the PTC comprises UAA, UGA or UAG.

In another ~~aspect~~example, a TREM comprising an ASGPR binding moiety, or a composition thereof, described herein may be used in a method of treating a subject having a disease or disorder associated with a premature termination codon (PTC), comprising providing a TREM comprising an ASGPR binding moiety or a composition described herein; contacting the subject with the TREM comprising an ASGPR binding moiety or a composition thereof in an amount and/or for a time sufficient to treat the subject, thereby treating the subject. In an ~~embodiment~~example, the PTC comprises UAA, UGA or UAG. In an ~~embodiment~~example, the disease or disorder associated with a PTC is a disease or disorder described herein, e.g., a cancer or a monogenic disease.

Additional features of any of the aforesaid TREM entities (e.g., TREMs, TREM core fragments, TREM Fragments, TREM compositions, preparations, methods of making TREM compositions and preparations, and methods of using TREM compositions and preparations include one or more of the following enumerated ~~examples~~embodiments).

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific ~~embodiments~~examples of the ~~invention~~disclosure described herein. Such equivalents are intended to be encompassed by the following enumerated ~~embodiments~~examples.

BRIEF DESCRIPTIONS OF THE DRAWINGS

FIGS. 1A-1J are images that depict ASGPR-expressing U2OS cells transfected with exemplary TREMs comprising an ASGPR binding moiety described herein. In this experiment, uptake of the TREMs comprising a SEQ ID NO. 650 backbone with ASGPR binding moieties at various positions along the sequence and conjugated to Cy3 was monitored and visualized by fluorescent microscopy.

FIG. 2 is a graphical representation of the fluorescent microscopy results of FIGs. 1A-1J. The results are depicted as the average intensity over the concentration of oligo (nM) given to the cells.

FIGs. 3A-3H are images that depict ASGPR-expressing U2OS cells transfected with exemplary TREMs comprising an ASGPR binding moiety described herein. In this experiment, uptake of the TREMs comprising a SEQ ID NO. 650 backbone with ASGPR binding moieties at

CLAIMS

1. A tRNA effector molecule (TREM) comprising:

(i) a sequence of Formula A comprising:

[L1]_y-[ASt Domain1]_x-[L2]_x-[DH Domain]_x-[L3]_x-[ACH Domain]_x-[VL Domain]_y-[TH Domain]_x-[L4]_x-[ASt Domain2]_x, (A);

wherein, independently, [L1] and [VL Domain], are optional;

each y is independently 0 or 1; and

each x is 0 or 1; and

(ii) an asialoglycoprotein receptor (ASGPR) binding moiety ~~(e.g., a GalNAc moiety, e.g., GalNAc),~~

wherein the ASGPR binding moiety is conjugated to a nucleobase within a nucleotide of the TREM, or at a terminus ~~(e.g., the 5' or 3' terminus)~~ of the TREM, or within the internucleotide linkage of a TREM.

2. The TREM of claim 1, wherein each x is 1.

3. The TREM of any one of claims 1-2, wherein the ASGPR moiety is present within the L1, ASt Domain1, L2, DH Domain, L3, ACH Domain, VL Domain, TH Domain, L4, or ASt Domain.

~~4. The TREM of any one of claims 1-3, wherein the ASGPR binding moiety is present within L1.~~

~~5. The TREM of any one of claims 1-3, wherein the ASGPR binding moiety is present within ASt Domain1.~~

~~6. The TREM of any one of claims 1-3, wherein the ASGPR binding moiety is present within L2.~~

7. — ~~The TREM of any one of claims 1-3, wherein the ASGPR binding moiety is present within DH Domain.~~

8. — ~~The TREM of any one of claims 1-3, wherein the ASGPR binding moiety is present within L3.~~

9. — ~~The TREM of any one of claims 1-3, wherein the ASGPR binding moiety is present within the ACH Domain.~~

10. — ~~The TREM of any one of claims 1-3, wherein the ASGPR binding moiety is present within VL Domain.~~

11. — ~~The TREM of any one of claims 1-3, wherein the ASGPR binding moiety is present within TH Domain.~~

12. — ~~The TREM of any one of claims 1-3, wherein the ASGPR binding moiety is present within L4.~~

13. — ~~The TREM of any one of claims 1-3, wherein the ASGPR binding moiety is present within ASt Domain2.~~

14. The TREM of any one of claims 1-3, wherein the ASGPR binding moiety is present within one of L1, ASt Domain1, or ASt Domain2.

15. — ~~The TREM of any one of claims 1-14, wherein the ASGPR binding moiety is covalently bound to a nucleobase within a nucleotide of the TREM, or at a terminus (e.g., the 5' or 3' terminus) of the TREM, or within the internucleotide linkage of a TREM (e.g., at a nitrogen or carbon atom in the nucleobase moiety).~~

~~16. The TREM of any one of claims 1-15, wherein the ASGPR binding moiety (e.g., a GalNAc moiety, e.g., GalNAc) is bound to a nucleobase within a nucleotide of the TREM.~~

~~175. The TREM of any one of claims 1-416, wherein the nucleobase is a naturally occurring nucleobase or a non-naturally occurring nucleobase.~~

~~186. The TREM of any one of claims 1-517, wherein the nucleobase comprises uracil, adenine, guanine, cytosine, thymine, or a variant thereof.~~

~~197. The TREM of any one of claims 1-415, wherein the ASGPR binding moiety (e.g., a GalNAc moiety, e.g., GalNAc) is bound to the 5' terminus, the 3' terminus, or both the 5' and 3' termini one or both of the termini within the TREM.~~

~~20. The TREM of claim 19, wherein the ASGPR binding moiety (e.g., a GalNAc moiety, e.g., GalNAc) is bound to the 5' terminus of the TREM.~~

~~21. The TREM of claim 19, wherein the ASGPR binding moiety (e.g., a GalNAc moiety, e.g., GalNAc) is bound to the 3' terminus of the TREM.~~

~~22. The TREM of any one of claims 1-15, wherein the ASGPR binding moiety is present within an internucleotide linkage of the TREM.~~

~~238. The TREM of any one of claims 1-227, wherein the TREM comprises the sequence of any one of the TREMs provided in Table 12 or a sequence that is at least 60%, 65%, 70%, 75%, 80%, 82%, 85%, 87%, 88%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to a sequence of a TREM provided in Table 12, e.g., SEQ ID NOs. 622-654.~~

~~24. The TREM of any one of claims 1-23, wherein the TREM comprises a sequence that is at least 60%, 65%, 70%, 75%, 80%, 82%, 85%, 87%, 88%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to a sequence of a TREM provided in Table 12, e.g., any one of SEQ ID NOs. 622-654 provided in Table 12.~~

~~25. The TREM of any one of claims 1-24, wherein the TREM comprises a sequence that is at least 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to a sequence of a TREM provided in Table 12, e.g., any one of SEQ ID NOs. 622-654 provided in Table 12.~~

~~269. The TREM of any one of claims 1-258, wherein the TREM comprises at least 5 ribonucleotides (nt), 10 nt, 15 nt, 20 nt, 25 nt, 30 nt, 35 nt, 40 nt, 45 nt, 50 nt, 55 nt, 60 nt, 65 nt, 70 nt, or 75 nt (but less than the full length) of a TREM provided in Table 12, e.g., any one of SEQ ID NOs. 622-654 disclosed in Table 12.~~

~~27. The TREM of any one of claims 1-26, wherein the TREM comprises at least 60 nt, 65 nt, 70 nt, or 75 nt of a TREM provided in Table 12, e.g., any one of SEQ ID NOs. 622-654 disclosed in Table 12.~~

~~28. The TREM of any one of claims 1-27, wherein the TREM at least 5 ribonucleotides (nt), 10 nt, 15 nt, 20 nt, 25 nt, 30 nt, 35 nt, 40 nt, 45 nt, 50 nt, 55 nt, 60 nt, 65 nt, 70 nt, or 75 nt (but less than the full length) of a TREM which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to TREM provided in Table 12, e.g., any one of SEQ ID NOs. 622-654 disclosed in Table 12.~~

~~29. The TREM of any one of claims 1-28, wherein the TREM comprises at least 60 nt, 65 nt, 70 nt, or 75 nt of a TREM which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to TREM provided in Table 12, e.g., any one of SEQ ID NOs. 622-654 disclosed in Table 12.~~

~~30. The TREM of any one of claims 1-29, wherein the TREM comprises a sequence that differs no more than 1 ribonucleotide (nt), 2 nt, 3 nt, 4 nt, 5 nt, 6 nt, 7 nt, 8 nt, 9 nt, 10 nt, 12 nt, 14 nt, 16 nt, 18, nt, or 20 nt from a TREM provided in Table 12, e.g., any one of SEQ ID NOs. 622-652 provided in Table 12.~~

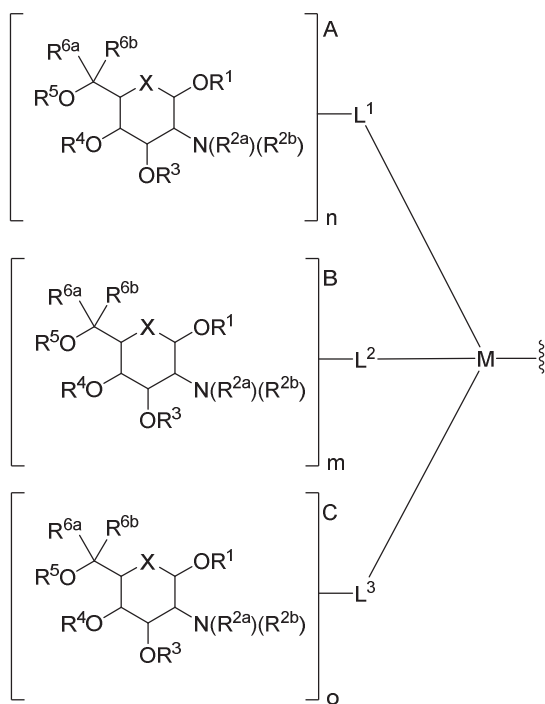
~~3410.~~ The TREM of any one of claims 1-~~930~~, wherein the TREM is selected from SEQ ID NO. 622, SEQ ID NO. 650, and SEQ ID NO. 653.

~~32.~~ The TREM of any one of claims 1-31, wherein the TREM is at least 60%, 65%, 70%, 75%, 80%, 82%, 85%, 87%, 88%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO. 622.

~~33.~~ The TREM of any one of claims 1-31, wherein the TREM is at least 60%, 65%, 70%, 75%, 80%, 82%, 85%, 87%, 88%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO. 650.

~~34.~~ The TREM of any one of claims 1-31, wherein the TREM is at least 60%, 65%, 70%, 75%, 80%, 82%, 85%, 87%, 88%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO. 653.

~~3511.~~ The TREM of any one of claims 1-~~1034~~, wherein the ASGPR binding moiety comprises a structure of Formula (III-b):



(III-b), or a salt thereof, wherein:

each X is independently O, N(R⁷), or S;

each of R¹, R³, R⁴, and R⁵ are independently hydrogen, alkyl, alkenyl, alkynyl, heteroalkyl, haloalkyl, aryl, heteroaryl, cycloalkyl, heterocyclyl, C(O)-alkyl, C(O)-alkenyl, C(O)-alkynyl, C(O)-heteroalkyl, C(O)-haloalkyl, C(O)-aryl, C(O)-heteroaryl, C(O)-cycloalkyl, or C(O)-heterocyclyl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, haloalkyl, aryl, heteroaryl, cycloalkyl, and heterocyclyl is optionally substituted with one or more R⁸;

or R³ and R⁴ are taken together with the oxygen atoms to which they are connected to form a heterocyclyl ring optionally substituted with one or more R⁸;

R^{2a} is hydrogen or alkyl;

R^{2b} is -C(O)alkyl (e.g., C(O)CH₃);

each of R^{6a} and R^{6b} is hydrogen, alkyl, alkenyl, alkynyl, heteroalkyl, haloalkyl, halo, cyano, nitro, -OR^A, aryl, heteroaryl, cycloalkyl, or heterocyclyl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, haloalkyl, aryl, heteroaryl, cycloalkyl, and heterocyclyl is optionally substituted with one or more R⁹;

R⁷ is hydrogen, alkyl, or C(O)-alkyl;

each of R⁸ and R⁹ is independently hydrogen, halo, cyano, alkyl, alkenyl, alkynyl, heteroalkyl, haloalkyl, cycloalkyl, or heterocyclyl;

R^A is hydrogen, or alkyl, alkenyl, alkynyl;

each of L¹, L², and L³ is independently a linker;

each of m, n, and o is independently an integer between 1 and 100;

M is a linker,

wherein “ ξ ” represents an attachment point to a branching point, additional linker, or TREM, e.g., a linker, a nucleobase, internucleotide linkage, or terminus within the TREM sequence.

3612. The TREM of claim 1135, wherein X is O.

3713. The TREM of any one of claims 3511-1236, each of R¹, R³, R⁴, and R⁵ are independently hydrogen or alkyl (e.g., CH₃).

3814. The TREM of any one of claims 1135-1337, R^{2a} is hydrogen and R^{2b} is C(O)CH₃.

~~39~~15. The TREM of any one of claims ~~1135-1438~~, wherein each of R^{6a} and R^{6b} is hydrogen.

~~40~~16. The TREM of any one of claims ~~1135-1539~~, wherein each of m, n, and o is independently an integer between 1 and 10.

~~41. The TREM of any one of claims 35-40, wherein each of m, n, and o is independently 1.~~

~~42~~17. The TREM of any one of claims ~~1135-1641~~, wherein each of L¹, L², and L³ independently:

(a) comprises an alkylene, alkenylene, alkynylene, heteroalkylene, or haloalkylene group;

(b) is cleavable or non-cleavable; and/or

(c) comprises a polyethylene glycol group.

~~43. The TREM of any one of claims 35-42, wherein each of L¹, L², and L³ independently is cleavable or non-cleavable.~~

~~44. The TREM of any one of claims 35-43, wherein each of L¹, L², and L³ independently comprises a polyethylene glycol group.~~

~~45~~18. The TREM of any one of claims ~~3511-1744~~, wherein M is cleavable or non-cleavable and comprises an alkylene, alkenylene, alkynylene, heteroalkylene, or haloalkylene, ester, amide, disulfide, ether, carbonate, aryl, heteroaryl, cycloalkyl, or heterocyclyl group.

~~46. The TREM of any one of claims 35-45, wherein M comprises an ester, amide, disulfide, ether, carbonate, aryl, heteroaryl, cycloalkyl, or heterocyclyl group.~~

~~47. The TREM of any one of claims 35-46, wherein M is cleavable or non-cleavable.~~

~~48~~19. The TREM of any one of claims ~~1-4718~~, wherein the ASGPR binding moiety comprises a galactose (Gal), galactosamine (GalNH₂), or N-acetylgalactosamine (GalNAc) moiety or a

plurality of galactose (Gal), galactosamine (GalNH₂), or N-acetylgalactosamine (GalNAc) moieties.

~~49. The TREM of any one of claims 1-486, wherein the ASGPR binding moiety comprises a plurality of galactose (Gal), galactosamine (GalNH₂), or N-acetylgalactosamine (GalNAc) moieties.~~

~~5020.~~ The TREM of any one of claims 1-~~1949~~, wherein the ASGPR binding moiety comprises an ASGPR carbohydrate and an ASGPR linker.

~~5121.~~ The TREM of any one of claims 1-~~5020~~, wherein the ASGPR binding moiety comprises a triantennary GalNAc moiety.

~~5222.~~ The TREM of any one of claims 1-~~5121~~, wherein the TREM is a compound provided in Table 12, ~~e.g., any one of Compound Nos. 99-131.~~

~~5323.~~ The TREM of any of claims 1-~~2252~~, wherein the TREM retains the ability to:

- ~~(a) support protein synthesis, e.g., relative to a TREM that does not comprise an ASGPR binding moiety or a naturally occurring tRNA;~~
- ~~(b) be charged by a synthetase;~~
- ~~(c) be bound by an elongation factor;~~
- ~~(d) introduce an amino acid into a peptide chain; and~~
- ~~(e) support elongation or support initiation.~~

~~54. The TREM of any of claims 1-53, wherein the TREM retains the ability to be charged by a synthetase, e.g., relative to a TREM that does not comprise an ASGPR binding moiety or a naturally occurring tRNA.~~

~~55. The TREM of any of claims 1-54, wherein the TREM retains the ability to be bound by an elongation factor, e.g., relative to a TREM that does not comprise an ASGPR binding moiety or a naturally occurring tRNA.~~

~~56. The TREM of any of claims 1-55, wherein the TREM retains the ability to introduce an amino acid into a peptide chain, e.g., relative to a TREM that does not comprise an ASGPR binding moiety or a naturally occurring tRNA.~~

~~57. The TREM of any of claims 1-56, wherein the TREM retains the ability to support elongation or support initiation, e.g., relative to a TREM that does not comprise an ASGPR binding moiety or a naturally occurring tRNA.~~

~~5824. The TREM of any of claims 1-2357, wherein the TREM has a binding affinity to an ASGPR of between 0.01 nM and 100 mM.~~

~~5925. The TREM of any one of claims 1-2458, wherein the TREM further comprises a chemical modification (e.g., a naturally occurring modification or a non-naturally occurring chemical modification).~~

~~6026. A pharmaceutical composition comprising a TREM of any one of claims 1-2559.~~

~~6127. The pharmaceutical composition of claim 6026, further comprising a pharmaceutically acceptable component, e.g., an excipient.~~

~~6228. A lipid nanoparticle formulation comprising a TREM of any one of claims 1-2559.~~

~~6329. A lipid nanoparticle formulation comprising a pharmaceutical composition of any one of claim 6026-6127.~~

~~6430. A method of making a TREM of any one of claims 1-5925.~~

~~6531. Use of a TREM comprising an ASGPR binding moiety of any one of claims 1-25 A composition for use in treating a subject having a disease or disorder associated with a PTC comprising administering to the subject a TREM comprising an ASGPR binding moiety~~

described herein (e.g., a TREM of any one of claims 1-59) or a pharmaceutical composition of any one of claims ~~2660-61~~27 or a lipid nanoparticle formulation of any one of claims ~~2862-63~~29, in the manufacture of a medicament for treating a disease or disorder associated with a PTC ~~thereby treating the subject having the disease or disorder.~~