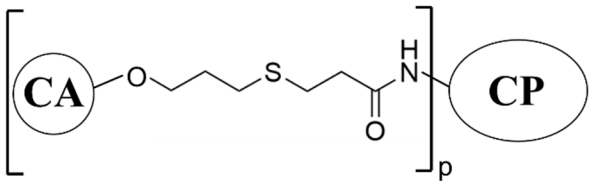
acids) are used for carbohydrate conjugation, far too many carbohydrate antigens become attached to the carrier proteins, thus resulting in masking potentially essential T cell peptide epitopes with the inherent diminution/elimination of immunogenicity. Thus, current strategies for preparing glycoconjugate vaccines are inadequate and face significant regulatory and/or commercial obstacles, since the preparations lack the necessary homogeneity in terms of their carbohydrate distribution and reproducibility (i.e., the attachment points of the sugars onto the proteins are randomly distributed and in various densities from batch to batch). Thus, glycoconjugate vaccines having greater carbohydrate antigen homogeneity, more precisely characterizable structures, and reproducibility from batch to batch would be highly desirable.

SARS-CoV-2, the causative agent of the COVID-19 pandemic that began in late 2019, represents an ongoing threat to global human health that has also crippled global economies. Initial vaccine development efforts have largely focused on protein antigens and epitopes present on the spike (S) glycoprotein, which mediates cell entry and membrane fusion of SARS-CoV-2 into host cells. However, global health experts have strongly recommended that scientists explore different strategies in parallel for developing therapeutic interventions against SARS-CoV-2 to mitigate against potential failures or complications that may arise for a single strategy. Thus, there remains a need for developing vaccines and other therapeutic tools against SARS-CoV-2 in parallel to those focused on the protein antigens present on the S protein of SARS-CoV-2.

SUMMARY

In one aspect, described herein is a neoglycoconjugate vaccine or an adaptive immune response-triggering composition comprising a synthetic neoglycoconjugate and a pharmaceutically acceptable excipient and/or an adjuvant, wherein the synthetic neoglycoconjugate comprises one or more carbohydrate antigens (CA) conjugated to one or more amine groups of a carrier protein or peptide (CP-NH) via a linker, the synthetic neoglycoconjugate having the structure:



, wherein p is 1 to 50 and corresponds to the total number of carbohydrate antigens conjugated to the carrier protein or peptide at said one or more amine groups.

In another aspect, described herein is a use of the neoglycoconjugate vaccine or an adaptive immune response-triggering composition as described herein in the manufacture of a medicament for immunizing, vaccinating, or treating a subject having a disease.

In another aspect, described herein is a neoglycoconjugate vaccine or an adaptive immune response-triggering composition as described herein for use in therapy.

In another aspect, described herein is a use of the neoglycoconjugate vaccine or an adaptive immune response-triggering composition as described herein in detecting the presence of an antibody that specifically binds to the neoglycoconjugate *in vitro.*

In another aspect, described herein is a use of the neoglycoconjugate vaccine or an adaptive immune response-triggering composition as disclosed herein in the manufacture of a medicament for immunizing, vaccinating, or treating a subject having a cancer that expresses said TACA.

In another aspect, described herein is a use of the neoglycoconjugate vaccine or an adaptive immune response-triggering composition as described herein in the manufacture of a medicament for immunizing or vaccinating a subject against a virus that expresses said viral polysaccharide antigen.

In another aspect, described herein is a use of the neoglycoconjugate vaccine or an adaptive immune response-triggering composition as described herein in the manufacture of a medicament for immunizing or vaccinating a subject against a bacteria that expresses said CPS. In a first example, described herein is a method for producing a neoglycoconjugate, the method comprising: (a) providing a neocarbohydrate antigen or neocarbohydrate antigen intermediate comprising a linker having a first end and a second end, wherein the first end is conjugated to a carbohydrate antigen via a thio ether bond and the second end comprises a functional group reactable with a free amine group, the functional group being -COX, $-SO_2X$, -O-C(O)-X, -N=C=O , or -N=C=S, wherein X is a leaving group; (b) providing a carrier material (e.g., carrier protein or peptide) having one or more free amine groups; and (c) performing a coupling reaction to conjugate one or more of the purified neocarbohydrate antigens or neocarbohydrate antigen intermediates to the carrier material (e.g., carrier protein or peptide) at the one or more free amine groups via an amide, a carbamate, a sulfonamide, a urea, or a thiourea bond, thereby producing the neoglycoconjugate.

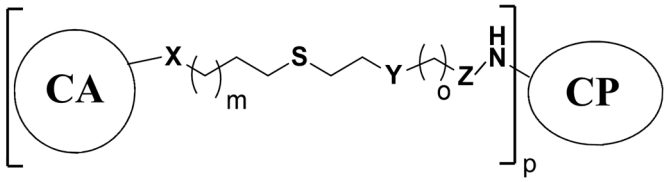
In some examples, prior to step (a), the neocarbohydrate antigen or neocarbohydrate antigen intermediate in (a) is prepared by a method comprising: (i) providing a carbohydrate antigen covalently linked to a terminal alkene (alkenyl carbohydrate antigen), the terminal alkene being directly conjugatable to a thiol group via a thiol-ene reaction; (ii) providing a thio-linker comprising a first functional group at a first end and a second functional group at a second end, the first functional group being a free thiol group

and the second functional group being a carboxyl group, sulfinic acid group, carbonic acid group, isocyanate group, or thiocyanate group; (iii) performing a photocatalytic thiol-ene reaction to directly conjugate the alkenyl carbohydrate antigen to the thio-linker at the first end, thereby producing a neocarbohydrate antigen comprising the carbohydrate antigen at the first end and the second functional group at a second end; (iv) when the second functional group is a carboxyl group, sulfinic acid group, or carbonic acid group, converting the neocarbohydrate antigen to a neocarbohydrate antigen intermediate by replacing the carboxyl group's, sulfinic acid group's, or carbonic acid group's terminal hydroxyl group with a better leaving group for conjugation to a free amine group of a polypeptide; and (v) purifying the neocarbohydrate antigen or the neocarbohydrate antigen intermediate.

In a further example, described herein is a neocarbohydrate antigen or neocarbohydrate antigen intermediate comprising a linker having a first end and a second end, wherein the first end is conjugated to a carbohydrate antigen via a thio ether bond and the second end comprises a functional group reactable with a free amine group, the functional group being -COX, $-SO_2X$, -O-C(O)-X, -N=C=O , or -N=C=S, wherein X is a leaving group.

In a further example, described herein is a synthetic neoglycoconjugate comprising a linker having a first end and a second end, wherein the first end is conjugated to a carbohydrate antigen via a thio ether bond and the second end is conjugated to a carrier protein or peptide at one or more free amine groups therein via an amide, a carbamate, a sulfonamide, a urea, or a thiourea bond.

In a further example, described herein is a synthetic neoglycoconjugate comprising one or more carbohydrate antigens (CA) conjugated to one or more amine groups of a carrier protein or peptide (CP-NH) via a linker, the synthetic neoglycoconjugate having the structure:



wherein: X is O, S, NR_1, or CH_2 ; R_1 is H, COH (formamide), COMe, or COEt ; m is 1, 2, 3, 4, or 5 ; Y is $-(CH_2)_n-$ or $-(OCH_2CH_2O)_n-$; n is 0, 1, 2, 3, 4, or 5 ; o is 0, 1, 2, 3, 4, or 5 ; or o is 0 and Z is -CO- and Y is $-(OCH_2CH_2O)_n-$; or o is 0 and Z is $-SO_2-$ and Y is $-(OCH_2CH_2O)_n-$; Z is -CO- , $-NR_2SO_2-$, -OCO- , $-NR_2CO-$, or $-NR_2CS-$, R_2 is H, Me, or Et ; and p is an integer corresponding to the total number of carbohydrate antigens conjugated to the carrier protein or peptide at said one or more amine groups (e.g., p = 1 to 50). In examples, the carrier protein or peptide in the synthetic neoglycoconjugate has a native or non-denatured conformation, and conjugation of the carbohydrate antigen to the carrier protein or peptide increases the immunogenicity of the carbohydrate antigen upon administration to the subject as compared to a corresponding administration of the unconjugated carbohydrate antigen.

In a further example, described herein is a method for producing a neoglycoconjugate vaccine or an immune response-triggering composition. The method may comprise formulating a neoglycoconjugate as described herein or prepared by a method as described herein with a pharmaceutically acceptable excipient, and/or an adjuvant.

In a further example, described herein is a neoglycoconjugate vaccine or an adaptive immune response-triggering composition produced by a method described herein and/or comprising a neoglycoconjugate as described herein and a pharmaceutically acceptable excipient and/or adjuvant as described herein.

In some examples, described herein is a method of immunizing, vaccinating, or treating a subject comprising administering to the subject a neoglycoconjugate produced by a method as described herein, a synthetic neoglycoconjugate as described herein, a neoglycoconjugate vaccine or an adaptive immune response-triggering composition produced by a method as described herein, or a neoglycoconjugate vaccine as described herein.

In some examples, described herein is a neoglycoconjugate produced by a method as described herein, a synthetic neoglycoconjugate as described herein, a neoglycoconjugate vaccine or an adaptive immune response-triggering composition produced by a method as described herein, or a neoglycoconjugate vaccine as described herein, for use in immunizing, vaccinating, or treating a subject having a disease, or for detecting the presence of an antibody that specifically binds to the neoglycoconjugate or for detecting said immunization, vaccination, or treatment (e.g., in a biological sample from the subject).

In some examples, described herein is a neoglycoconjugate produced by a method as described herein, a synthetic neoglycoconjugate as described herein, or an adaptive immune response-triggering composition produced by a method as described herein, for the manufacture of a vaccine for immunizing or treating a subject having a disease, or for detecting the presence of an antibody that specifically binds to the neoglycoconjugate or for detecting said immunization or treatment (e.g., in a biological sample from the subject).

In some examples, described herein is a neoglycoconjugate produced by a method as described herein, a synthetic neoglycoconjugate as described herein, a neoglycoconjugate vaccine or an adaptive immune response-triggering composition produced by a method as described herein, or a neoglycoconjugate vaccine as described herein, for use in the treatment of a subject having a disease associated with increased expression of said carbohydrate antigen.

In some examples, described herein is a neoglycoconjugate produced by a method as described herein, a synthetic neoglycoconjugate as described herein, a neoglycoconjugate vaccine or an adaptive immune response-triggering composition produced by a method as described herein, or a

neoglycoconjugate vaccine as described herein, for detecting or screening for the presence of an antibody that specifically binds to the carbohydrate antigen or a tumor-circulating cell comprising the carbohydrate antigen, or for detecting the presence of antibodies resulting from an immunization or vaccination with the carbohydrate antigen.

In further examples, described herein is a method of treating a subject comprising administering a neoglycoconjugate or neoglycoconjugate immunogen as defined herein or produced by a method as described herein, to generate an immune response in said subject to a carbohydrate antigen, and optionally screening a biological sample from said subject for the presence of antibodies that specifically binds to the carbohydrate antigen.

In further examples, described herein is a glycoconjugate for use in immunizing a subject against SARS-CoV-2, for use in triggering the production of anti-SARS-CoV-2 antibodies in a subject, or for use in detecting the presence of anti-SARS-CoV-2 antibodies in a sample from a subject, the glycoconjugate comprising carbohydrate antigens conjugated to a suitable carrier material (e.g., a carrier protein or peptide), wherein the carbohydrate antigens comprise or consist of sialylated Thomsen-Friedenreich (TF) antigen, unsialylated TF antigen, sialylated Tn antigen, unsialylated Tn antigen, or any combination thereof.

In further examples, described herein is a SARS-CoV-2 vaccine comprising one or more glycoconjugates as described herein, and a pharmaceutically acceptable excipient and/or an adjuvant.

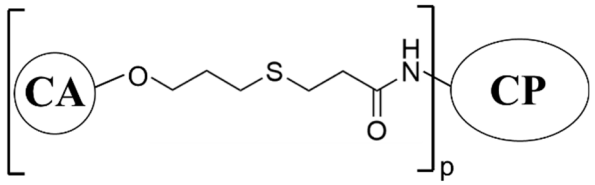
In further examples, described herein is a method for vaccinating a subject for SARS-CoV-2 or for triggering the production of anti-SARS-CoV-2 antibodies in a subject, the method comprising administering the glycoconjugates or the SARS-CoV-2 vaccine as described herein.

In further examples, described herein is a composition for protecting a subject from infection by a SARS-CoV-2 virus, or for treating COVID-19, the composition comprising one or more ligands (e.g., an antibody, antibody fragment, or lectin) that bind to an O-linked glycan expressed on the SARS-CoV-2 S protein, the O-linked glycan comprising sialylated TF antigen (mono- or di-sialylated TF antigen), unsialylated TF antigen, sialylated Tn antigen, unsialylated Tn antigen, or any combination thereof.

In further examples, described herein is a complex comprising: (a) a SARS-CoV-2 S protein, or fragment thereof, expressing an O-linked glycan comprising sialylated TF antigen (mono- or di-sialylated TF antigen), unsialylated TF antigen, sialylated Tn antigen, unsialylated Tn antigen, or any combination thereof; and (b) a ligand as described herein that is bound to the SARS-CoV-2 S protein, or fragment thereof, at the O-linked glycan.

CLAIMS:

1. A neoglycoconjugate vaccine or an adaptive immune response-triggering composition comprising a synthetic neoglycoconjugate and a pharmaceutically acceptable excipient and/or an adjuvant, wherein the synthetic neoglycoconjugate comprises one or more carbohydrate antigens (CA) conjugated to one or more amine groups of a carrier protein or peptide (CP-NH) via a linker, the synthetic neoglycoconjugate having the structure:



,

wherein p is 1 to 50 and corresponds to the total number of carbohydrate antigens conjugated to the carrier protein or peptide at said one or more amine groups.

2. The neoglycoconjugate vaccine or an adaptive immune response-triggering composition of claim 1, wherein the carrier protein or peptide is a protein or peptide that was engineered to add one or more further lysine residues.

3. The neoglycoconjugate vaccine or an adaptive immune response-triggering composition of claim 1 or 2, wherein the carrier protein or peptide is, is from, or comprises: cross-reacting material 197 (CRM197), Tetanus Toxoid (TT), Diphtheria Toxoid (DT), Meningococcal Outer Membrane Protein Complex (OMPC), H. Influenzae Protein D (HiD), a cytokine, an immunogenic peptide, Tetanus Toxin 831-844 (**SEQ ID NO: 1 or 2**), albumin, keyhole limpet hemocyanin (KLH), or an immunogenic fragment thereof.

4. The neoglycoconjugate vaccine or an adaptive immune response-triggering composition of claim 1 or 2, wherein the carrier protein or peptide is, is from, or comprises CRM197, TT, or an immunogenic fragment thereof.

5. The neoglycoconjugate vaccine or an adaptive immune response-triggering composition of any one of claims 1 to 4, which is a prophylactic vaccine.

6. The neoglycoconjugate vaccine or an adaptive immune response-triggering composition of any one of claims 1 to 4, which is a therapeutic vaccine.

7. The neoglycoconjugate vaccine or an adaptive immune response-triggering composition of any one of claims 1 to 6, wherein the carbohydrate antigen is *Thomsen-Friedenreich* (TF), Tn, S-Tn, (2,3)-S-TF, (2,6)-S-TF, Globo H, PSA, GD2, GD3, GM2, GM3, N-glycolyl-GM3, Fucosyl GM1, Le^a, sLe^a, Le^x, sLe^x, Le^y, or any combination thereof.

8. The neoglycoconjugate vaccine or an adaptive immune response-triggering composition of any one of claims 1 to 7, wherein the one or more carbohydrate antigens comprises unsialylated TF antigen, unsialylated Tn antigen, or analogs thereof.

9. The neoglycoconjugate vaccine or an adaptive immune response-triggering composition of any one of claims 1 to 8, wherein the carbohydrate antigen is or comprises a tumor associated carbohydrate antigen (TACA).

10. The neoglycoconjugate vaccine or an adaptive immune response-triggering composition of any one of claims 1 to 6, wherein the carbohydrate antigen is or comprises a viral polysaccharide antigen.

11. The neoglycoconjugate vaccine or an adaptive immune response-triggering composition of any one of claims 1 to 6, wherein the carbohydrate antigen is or comprises a bacterial capsular polysaccharide (CPS).

12. Use of the neoglycoconjugate vaccine or an adaptive immune response-triggering composition as defined in any one of claims 1 to 11 in the manufacture of a medicament for immunizing, vaccinating, or treating a subject having a disease.

13. The neoglycoconjugate vaccine or an adaptive immune response-triggering composition as defined in any one of claims 1 to 11 for use in therapy.

14. Use of the neoglycoconjugate vaccine or an adaptive immune response-triggering composition as defined in any one of claims 1 to 11 in detecting the presence of an antibody that specifically binds to the neoglycoconjugate *in vitro*.

15. Use of the neoglycoconjugate vaccine or an adaptive immune response-triggering composition as defined in claim 9 in the manufacture of a medicament for immunizing, vaccinating, or treating a subject having a cancer that expresses said TACA.

16. Use of the neoglycoconjugate vaccine or an adaptive immune response-triggering composition as defined in claim 10 in the manufacture of a medicament for immunizing or vaccinating a subject against a virus that expresses said viral polysaccharide antigen.

17. Use of the neoglycoconjugate vaccine or an adaptive immune response-triggering composition as defined in claim 11 in the manufacture of a medicament for immunizing or vaccinating a subject against a bacteria that expresses said CPS.