cells (DCs) are equally important. DCs are professional antigen-presenting cells having a key regulatory role in the maintenance of tolerance to self-antigens and in the activation of innate and adaptive immunity against foreign antigens.

The immune system can also produce undesirable effects. For example, autoimmune disorders are characterized by the loss of tolerance against autoantigens (self-antigens), activation of lymphocytes reactive against autoantigens, and pathological damage in target organs.

Current therapies for autoimmune disorders mainly concentrate on symptomatic response and on mitigating the immune system as a whole. Antigen-specific therapy has recently emerged as a potential therapy for autoimmune disorders, but eliciting appropriate immune responses has proven difficult and these therapies have met with limited success.

There is thus a need for an effective therapy of autoimmune diseases.

Summary

It was the object of the present disclosure to provide agents for a therapy of autoimmune diseases. This object is achieved according to the disclosure by the subject matter of the claims.

The present disclosure encompasses compositions, methods and uses for inducing tolerance to an autoantigen. According to the disclosure, autoantigens against which an immune response is characteristic of autoimmune diseases are administered in the form of non-immunogenic RNA encoding a peptide or polypeptide comprising the autoantigen or a fragment thereof, or a variant of the autoantigen or fragment. The RNA to be administered in one preferred example is rendered non-immunogenic by incorporating into the RNA modified nucleotides suppressing RNA-mediated activation of innate immune receptors and removing double-stranded RNA (dsRNA). It is demonstrated according to the disclosure that immunization of mice with non-immunogenic RNA completely blocked any signs of the autoimmune disease. It is demonstrated also that administration of a single disease-driving epitope can be sufficient.

In one aspect, the present disclosure provides for a use of a pharmaceutical composition in the manufacture of a medicament for treating or preventing autoimmune diseases, said composition comprising non-immunogenic RNA encoding a peptide or polypeptide comprising an autoantigen, wherein the non-immunogenic RNA is rendered non-immunogenic by the incorporation of 1-methylpseudouridines and by the removal of dsRNA. One example of the disclosure relates to a method of treating an autoimmune disease in a subject, comprising administering to the subject non-immunogenic RNA encoding a peptide or polypeptide comprising an autoantigen or a fragment thereof, or a variant of the autoantigen or fragment.

In one example, the autoimmune disease is a T cell-mediated autoimmune disease.

One example of the disclosure relates to a method of inducing tolerance to autoreactive T cells in a subject, comprising administering to the subject non-immunogenic RNA encoding a peptide or polypeptide comprising an autoantigen or a fragment thereof, or a variant of the autoantigen or fragment.

In one example, the subject has an autoimmune disease. In one example, the autoimmune disease is a T cell-mediated autoimmune disease. In one example, the T cells are autoreactive with the autoantigen or cells expressing and preferably presenting the autoantigen.

In one example of the methods of the disclosure, the autoimmune disease is an autoimmune disease of the CNS. In one example of the methods of the disclosure, the autoimmune disease is Multiple Sclerosis.

In one example of the methods of the disclosure, the non-immunogenic RNA when administered does not result in activation of dendritic cells, activation of T cells and/or secretion of IFN-alpha.

In one example of the methods of the disclosure, the non-immunogenic RNA is rendered non-immunogenic by the incorporation of modified nucleotides and the removal of dsRNA. In one example, the modified nucleotides suppress RNA-mediated activation of innate immune receptors. In one example, the modified nucleotides comprise a replacement of one or more uridines with a nucleoside comprising a modified nucleobase. In one example, the modified nucleobase is a modified uracil. In one example, the nucleoside comprising a modified nucleobase is selected from the group consisting of 3-methyl-uridine (m3U), 5-

methoxy-uridine (mo5U), 5-aza-uridine, 6-aza-uridine, 2-thio-5-aza-uridine, 2-thio-uridine (s2U), 4-thio-uridine (s4U), 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxy-uridine (ho5U), 5-aminoallyl-uridine, 5-halo-uridine (e.g., 5-iodo-uridineor 5-bromo-uridine), uridine 5-oxyacetic acid (cmo5U), uridine 5-oxyacetic acid methyl ester (mcmo5U), 5-carboxymethyl-uridine (cm5U), 1-carboxymethyl-pseudouridine, 5-carboxyhydroxymethyl-uridine (chm5U), 5-carboxyhydroxymethyl-uridine methyl ester (mchm5U), 5-methoxycarbonylmethyl-uridine (mcm5U), 5-methoxycarbonylmethyl-2-thio-uridine (mcm5s2U), 5-aminomethyl-2-thio-uridine (nm5s2U), 5-methylaminomethyl-uridine (mnm5U), 1-ethyl-pseudouridine, 5-methylaminomethyl-2-thio-uridine (mnm5s2U), 5-methylaminomethyl-2-seleno-uridine (mnm5se2U), 5-carbamoylmethyl-uridine (ncm5U), 5-carboxymethylaminomethyl-uridine (cmnm5U), 5-carboxymethylaminomethyl-2-thio-uridine (cmnm5s2U), 5-propynyl-uridine, 1-propynyl-pseudouridine, 5-taurinomethyl-uridine (τm5U), 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-uridine (τm5s2U), 1-taurinomethyl-4-thio-pseudouridine, 5-methyl-2-thio-uridine (m5s2U), 1-methyl-4-thio-pseudouridine (m1s4ψ), 4-thio-1-methyl-pseudouridine, 3-methyl-pseudouridine (m3ψ), 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydrouridine (D), dihydropseudouridine, 5,6-dihydrouridine, 5-methyl-dihydrouridine (m5D), 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxy-uridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 3-(3-amino-3-carboxypropyl)uridine (acp3U), 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine (acp3 ψ), 5-(isopentenylaminomethyl)uridine (inm5U), 5-(isopentenylaminomethyl)-2-thio-uridine (inm5s2U), α-thio-uridine, 2′-O-methyl-uridine (Um), 5,2′-O-dimethyl-uridine (m5Um), 2′-O-methyl-pseudouridine (ψm), 2-thio-2′-O-methyl-uridine (s2Um), 5-methoxycarbonylmethyl-2′-O-methyl-uridine (mcm5Um), 5-carbamoylmethyl-2′-O-methyl-uridine (ncm5Um), 5-carboxymethylaminomethyl-2′-O-methyl-uridine (cmnm5Um), 3,2′-O-dimethyl-uridine (m3Um), 5-(isopentenylaminomethyl)-2′-O-methyl-uridine (inm5Um), 1-thio-uridine, deoxythymidine, 2′-F-ara-uridine, 2′-F-uridine, 2′-OH-ara-uridine, 5-(2-carbomethoxyvinyl) uridine, and 5-[3-(1-E-propenylamino)uridine. In one example, the nucleoside comprising a modified

nucleobase is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In one example, the nucleoside comprising a modified nucleobase is 1-methyl-pseudouridine.

In one example of the methods of the disclosure, the non-immunogenic RNA is mRNA. In one example of the methods of the disclosure, the non-immunogenic RNA is *in vitro* transcribed RNA.

In one example of the methods of the disclosure, the autoantigen is associated with an autoimmune disease. In one example of the methods of the disclosure, the autoantigen is a T cell-antigen. In one example of the methods of the disclosure, the autoantigen is CNS-derived. In one example of the methods of the disclosure, the autoantigen is a myelin antigen. In one example of the methods of the disclosure, the autoantigen is Myelin Oligodendrocyte Glycoprotein (MOG). In one example of the methods of the disclosure, the peptide or polypeptide comprising an autoantigen or a fragment thereof, or a variant of the autoantigen or fragment comprises amino acids 35 to 55 of Myelin Oligodendrocyte Glycoprotein (MOG).

In one example of the methods of the disclosure, the non-immunogenic RNA is transiently expressed in cells of the subject.

In one example of the methods of the disclosure, the non-immunogenic RNA is delivered to dendritic cells. In one example, the dendritic cells are immature dendritic cells.

In one example of the methods of the disclosure, the non-immunogenic RNA is formulated in a delivery vehicle. In one example, the delivery vehicle comprises particles. In one example, the delivery vehicle comprises a lipid. In one example, the lipid comprises a cationic lipid. In one example, the lipid forms a complex with and/or encapsulates the non-immunogenic RNA. In one example of the methods of the disclosure, the non-immunogenic RNA is formulated in liposomes.

One example of the disclosure relates to a pharmaceutical composition comprising non-immunogenic RNA encoding a peptide or polypeptide comprising an autoantigen or a fragment thereof, or a variant of the autoantigen or fragment.

In one example, the non-immunogenic RNA when administered does not result in activation of dendritic cells, activation of T cells and/or secretion of IFN-alpha.

In one example, the non-immunogenic RNA is rendered non-immunogenic by the incorporation of modified nucleotides and the removal of dsRNA. In one example, the modified nucleotides suppress RNA-mediated activation of innate immune receptors. In one example, the modified nucleotides comprise a replacement of one or more uridines with a nucleoside comprising a modified nucleobase. In one example, the modified nucleobase is a modified uracil. In one example, the nucleoside comprising a modified nucleobase is selected from the group consisting of 3-methyl-uridine (m3U), 5-methoxy-uridine (mo5U), 5-aza-uridine, 6-aza-uridine, 2-thio-5-aza-uridine, 2-thio-uridine (s2U), 4-thio-uridine (s4U), 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxy-uridine (ho5U), 5-aminoallyl-uridine, 5-halo-uridine (e.g., 5-iodo-uridineor 5-bromo-uridine), uridine 5-oxyacetic acid (cmo5U), uridine 5-oxyacetic acid methyl ester (mcmo5U), 5-carboxymethyl-uridine (cm5U), 1-carboxymethyl-pseudouridine, 5-carboxyhydroxymethyl-uridine (chm5U), 5-carboxyhydroxymethyl-uridine methyl ester (mchm5U), 5-methoxycarbonylmethyl-uridine (mcm5U), 5-methoxycarbonylmethyl-2-thio-uridine (mcm5s2U), 5-aminomethyl-2-thio-uridine (nm5s2U), 5-methylaminomethyl-uridine (mnm5U), 1-ethyl-pseudouridine, 5-methylaminomethyl-2-thio-uridine (mnm5s2U), 5-methylaminomethyl-2-seleno-uridine (mnm5se2U), 5-carbamoylmethyl-uridine (ncm5U), 5-carboxymethylaminomethyl-uridine (cmnm5U), 5-carboxymethylaminomethyl-2-thio-uridine (cmnm5s2U), 5-propynyl-uridine, 1-propynyl-pseudouridine, 5-taurinomethyl-uridine (τm5U), 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-uridine (τm5s2U), 1-taurinomethyl-4-thio-pseudouridine, 5-methyl-2-thio-uridine (m5s2U), 1-methyl-4-thio-pseudouridine (m1s4ψ), 4-thio-1-methyl-pseudouridine, 3-methyl-pseudouridine (m3ψ), 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydrouridine (D), dihydropseudouridine, 5,6-dihydrouridine, 5-methyl-

dihydrouridine (m5D), 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxy-uridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 3-(3-amino-3-carboxypropyl)uridine (acp3U), 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine (acp3 ψ), 5-(isopentenylaminomethyl)uridine (inm5U), 5-(isopentenylaminomethyl)-2-thio-uridine (inm5s2U), α-thio-uridine, 2′-O-methyl-uridine (Um), 5,2′-O-dimethyl-uridine (m5Um), 2′-O-methyl-pseudouridine (ψm), 2-thio-2′-O-methyl-uridine (s2Um), 5-methoxycarbonylmethyl-2′-O-methyl-uridine (mcm5Um), 5-carbamoylmethyl-2′-O-methyl-uridine (ncm5Um), 5-carboxymethylaminomethyl-2′-O-methyl-uridine (cmnm5Um), 3,2′-O-dimethyl-uridine (m3Um), 5-(isopentenylaminomethyl)-2′-O-methyl-uridine (inm5Um), 1-thio-uridine, deoxythymidine, 2′-F-ara-uridine, 2′-F-uridine, 2′-OH-ara-uridine, 5-(2-carbomethoxyvinyl) uridine, and 5-[3-(1-E-propenylamino)uridine. In one example, the nucleoside comprising a modified nucleobase is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In one example, the nucleoside comprising a modified nucleobase is 1-methyl-pseudouridine.

In one example of the pharmaceutical composition of the disclosure, the non-immunogenic RNA is mRNA. In one example of the pharmaceutical composition of the disclosure, the non-immunogenic RNA is *in vitro* transcribed RNA.

In one example of the pharmaceutical composition of the disclosure, the autoantigen is associated with an autoimmune disease. In one example of the pharmaceutical composition of the disclosure, the autoantigen is a T cell-antigen. In one example of the pharmaceutical composition of the disclosure, the autoantigen is CNS-derived. In one example of the pharmaceutical composition of the disclosure, the autoantigen is a myelin antigen. In one example of the pharmaceutical composition of the disclosure, the autoantigen is Myelin Oligodendrocyte Glycoprotein (MOG). In one example of the pharmaceutical composition of the disclosure, the peptide or polypeptide comprising an autoantigen or a fragment thereof, or a variant of the autoantigen or fragment comprises amino acids 35 to 55 of Myelin Oligodendrocyte Glycoprotein

(MOG).

In one example of the pharmaceutical composition of the disclosure, the non-immunogenic RNA is transiently expressed in cells of a subject to whom the pharmaceutical composition is administered.

In one example of the pharmaceutical composition of the disclosure, the non-immunogenic RNA is delivered to dendritic cells of a subject to whom the pharmaceutical composition is administered. In one example, the dendritic cells are immature dendritic cells.

In one example of the pharmaceutical composition of the disclosure, the non-immunogenic RNA is formulated in a delivery vehicle. In one example, the delivery vehicle comprises particles. In one example, the delivery vehicle comprises a lipid. In one example, the lipid comprises a cationic lipid. In one example, the lipid forms a complex with and/or encapsulates the non-immunogenic RNA. In one example of the pharmaceutical composition of the disclosure, the non-immunogenic RNA is formulated in liposomes.

One example of the disclosure relates to the pharmaceutical composition of the disclosure for use in the methods of the disclosure.

Other features and advantages of the instant disclosure will be apparent from the following detailed description and claims.

Detailed description of the invention

Although the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodologies, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and

Claims:

1. Use of a pharmaceutical composition in the manufacture of a medicament for treating or preventing autoimmune diseases, said composition comprising non-immunogenic RNA encoding a peptide or polypeptide comprising an autoantigen, wherein the non-immunogenic RNA is rendered non-immunogenic by the incorporation of 1-methylpseudouridines and by the removal of dsRNA.

2. The use of claim 1, wherein the non-immunogenic RNA to be administered does not result in activation of dendritic cells, activation of T cells and/or secretion of IFN-alpha.

3. The use of claim 1 or 2, wherein the non-immunogenic RNA is *in vitro* transcribed RNA.

4. The use of any one of claims 1 to 3, wherein the autoantigen is associated with an autoimmune disease and wherein the autoantigen is preferably a T cell-antigen.

5. The use of any one of claims 1 to 4, wherein the autoantigen is a myelin antigen, wherein the autoantigen is preferably Myelin Oligodendrocyte Glycoprotein (MOG) or a fragment thereof comprising amino acids 35 to 55 of Myelin Oligodendrocyte Glycoprotein (MOG).

6. The use of any one of claims 1 to 5, further comprising an immune inhibiting compound or a non-immunogenic RNA encoding said immune inhibiting compound, wherein said immune inhibiting compound is selected from the group consisting of transforming growth factor beta (TGF-β), interleukin 10 (IL-10), interleukin 1 receptor antagonist (IL-1RA), interleukin 4 (IL-4), interleukin 27 (IL-27), interleukin 35 (IL-35), programmed death-ligand 1 (PD-L1), inducible T-cell co-stimulator ligand (ICOSL), B7-H4, CD39, CD73, FAS, FAS-IL, indoleamine 2,3-dioxygenase 1 (IDO1), indoleamine 2,3-dioxygenase 2 (IDO2), acetaldehyde dehydrogenase 1 (ALDHl)/retinaldehyde dehydrogenase (RALDH), arginase 1

(ARG1), arginase 2 (ARG2), nitrous oxide synthase (NOS2), galectin-1, galectin-9, semaphorin 4A.

7. The use of any one of claims 1 to 6, wherein the use comprises that tolerance is to be induced to autoreactive T cells.

8. The use of any one of claims 1 to 6, wherein the autoimmune disease is a T cell-mediated autoimmune disease.

9. The use of any one of claims 1 to 6, wherein the autoimmune disease is an autoimmune disease of the CNS.

10. The use of any one of claims 1 to 6, wherein the autoimmune disease is multiple sclerosis.

11. The use of any one of claims 1 to 10, wherein the non-immunogenic RNA to be administered does not result in activation of dendritic cells, activation of T cells and/or secretion of IFN-alpha.

12. The use of any one of claims 1 to 11, wherein the modified nucleotides suppress RNA-mediated activation of innate immune receptors.

13. The use of any one of claims 1 to 12, wherein the use comprises that an immune inhibiting compound, or a non-immunogenic RNA encoding the immune inhibiting compound is to be provided to the subject that is treated, wherein the immune inhibiting compound is selected from the group consisting of transforming growth factor beta (TGF-β), interleukin 10 (IL-10), interleukin 1 receptor antagonist (IL-1RA), interleukin 4 (IL-4), interleukin 27 (IL-27), interleukin 35 (IL-35), programmed death-ligand 1 (PD-L1), inducible T-cell co-stimulator ligand (ICOSL), B7-H4, CD39, CD73, FAS, FAS-IL, indoleamine 2,3-dioxygenase 1 (IDO1), indoleamine 2,3- dioxygenase 2 (IDO2), acetaldehyde dehydrogenase 1 (ALDHl)/retinaldehyde dehydrogenase (RALDH), arginase 1 (ARG1), arginase 2 (ARG2), nitrous oxide synthase (NOS2), galectin-1, galectin-9, semaphorin 4A.