Claims

1. A method for monitoring immune tolerance status of a subject undergoing immune tolerizing treatment for a disease or condition, the method comprising the steps of

(a) determining the immune tolerance status of the subject by assaying one or more biological sample(s) obtained from the subject prior to administration of treatment,

(b) determining the immune tolerance status of the subject by assaying one or more biological sample(s) obtained from the subject after administration of treatment, and

(c) determining the immune tolerance status of the subject by assaying one or more biological sample(s) obtained from the subject at regular intervals after administration of treatment,

wherein if an immune tolerance signature indicates a weakening, and/or loss of immunological tolerance, the tolerizing treatment is to be re-administered.

2. The method of claim 1, wherein the disease or condition is selected from the group consisting of an inflammatory condition, an autoimmune condition, an allergy, an abnormal immune response, a lysosomal storage disease, an enzyme deficiency, a protein deficiency, a genetic disorder, and/or is a transplant recipient.

3. The method of claim 2, wherein the autoimmune condition is selected from the group consisting of atopic dermatitis, multiple sclerosis, autoimmune myelitis, myelitis, transverse myelitis, neuromyelitis optica (NMO), neuromyelitis optica spectrum disorder (NMSOD), type-1 diabetes (T1D), type-2 diabetes (T2D), Celiac Disease (CD), Grave's Disease, Myasthenia Gravis, acute disseminated encephalomyelitis, Addison's Disease, alopecia, rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, autoimmune myocarditis, autoimmune neutropenia, autoimmune skin disease, autoimmune uveitis, ballus pemphigoid, Behcet's Syndrome, cerebral degeneration, chronic neuropathy, cicatrical pemphigoid, pemphigus vulgaris, Crohn's Disease, Inflammatory Bowel Disease (IBD), Inflammatory Bowel Syndrome (IBS), cryopathy, dermatitis hyperformis, Eaton Lambert's Disease, encephalomyelitis, epidermolysis bullosa acquisita, erythema nodosa, glomerulonephritis, Goodpasture's Disease, granulomatosis, Guillain-Barre Syndrome, Hashimoto Disease, Kawasaki Disease, hemolytic anemia, hypersensitivity vasculitis, lupus erythematosus, mixed connective tissue disease, mixed essential cryoglobulinemia, multifocal motor neuropathy, opsonoclonus-myoclonus, paraneoplastic pemphigus, pemphigoig gestationis, pemphigus

folaceus, pernicious anemia, peripheral biliary cirrhosis, polyangiitis overlap syndrome, polyarteritis nodosa, polyglandular failure, polyglandular syndrome, polymyositis/dermatomyositis, psoriasis, eczema, retinopathy, Reynaud's Syndrome, sarcoidosis, Scleroderma Type 1, sclerosis cholangitis, Sjogren's Syndrome, Stiffman's syndrome, Takayasu arteritis, termporal arteritis, thyroiditis, ulcerative colitis, immune thrombocytopenia purpura, thrombotic thrombocytopenia purpura, autoimmune hepatitis, primary biliary cholangitis (PBC), ANCA diseases, Granulamatosis with Polyangiitis, and Microscopic Polyangiitis.

4. The method of claim 1 or 2, wherein the immune tolerizing treatment comprises an antigen selected from the group consisting of one or more autoimmune antigens, allergens, enzyme replacement therapies, protein therapies, transplant antigens, and gene therapy vectors.

5. The method of claim 4, wherein the one or more autoimmune antigens are selected from the group consisting of myelin basic protein, acetylcholine receptor, endogenous antigen, myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP), proteolipid protein (PLP), myelin associated glycoprotein (MAG), cyclic nucleotide phosphohydrolase, pancreatic beta-cell antigen, insulin, proinsulin, islet-specific glucose-6-phophatase catalytic subunit-related protein (IGRP), glutamic acid decarboxylase (GAD), collagen type 11, human cartilage gp39, fp130-RAPS, fibrillarin, small nucleolar protein, thyroid stimulating factor receptor, histones, glycoprotein gp70, pyruvate dehydrogenase dehydrolipoamide acetyltransferase (PCD-E2), hair follicle antigen, aqua porin 4, Desmoglein 1, Desmoglein 3, nicotinic acetylcholine receptor, gliadin, ADAMTS13, GPIIb/GPIIIa, CYP2D6, BP180, NC16, BP230, Ro60, MPO, thyroid stimulating hormone receptor, and human tropomyosin isoform 5.

6. The method of claim 4, wherein the one or more allergens is selected from the group consisting of Bahia grass pollen (BaGP), peach allergen, milk allergens, celery allergens, nut allergens, bovine serum albumin, Hazelnut allergens, ovalbumin, egg allergen, peanut allergens, fish allergens, shellfish allergens, and Japanese cedar pollen.

7. The method of any one of claims 1-6, wherein said immune tolerizing treatment is antigen specific.

8. The method of any one of claims 1-7, wherein the immune tolerizing treatment is selected from the group consisting of oral immunotherapy (OIT), subcutaneous immunotherapy (SCIT), sublingual immunotherapy (SLIT), and immune tolerizing nanomedicine.

9. The method of claim 8, wherein the immune tolerizing nanomedicine comprises tolerizing immune modifying particles (TIMPs).

10. The method of any one of claims 1-9, wherein the one or more biological samples are selected from the group consisting of whole-blood, peripheral blood, peripheral blood mononuclear cells (PBMCs), serum, plasma, urine, cerebrospinal fluid (CSF), stool, a tissue biopsy, and a bone-marrow biopsy.

11. The method of any one of claims 1-10, wherein said assay of the one or more biological samples is selected from the group consisting of analyzing cells, cell-surface proteins, extracellular proteins, intracellular proteins, nucleic acids, metabolites, and combinations thereof.

12. The method of claim 11, wherein the cells are

i) immune cells and/or non-immune cells;

ii) immune cells that are innate immune cells and/or adaptive immune cells; or

iii) non-immune cells selected from the group consisting of epithelial cells, stromal cells, endothelial cells, fibroblasts, pericytes, adipocytes, mesenchymal stem cells, hematopoietic stem cell, hematopoietic progenitor cells, liver sinusoidal endothelial cells (LSECs), and/or Kupffer cells.

13. The method of claim 12, wherein the immune cells are selected from the group consisting of monocytes, macrophages, neutrophils, granulocytes, dendritic cells, mast cells, eosinophils, basophils, T-cells, B-cells, NK cells, and NK-T cells, optionally wherein the T cells are T1 cells, T2a cells, Treg cells, Tr1 cells and/or Teff cells.

14. The method of any one of claims 1-13, wherein the assay of one or more biological samples in Step (a) is used to determine the immune tolerance status of the subject at Baseline.

15. The method of claim 14, wherein

i) the immune tolerance status of the subject at Baseline is determined by comparing the results from the assay of one or more biological samples in Step (a) to the assay of samples obtained from a healthy subject;

ii) the immune tolerance status of the subject at Baseline is used to determine whether the subject is to be administered a tolerizing treatment; and/or

iii) the immune tolerance status of the subject at Baseline indicates weak immune tolerance or absence of immune tolerance.

16. The method of claim 14 or 15, wherein the subject is to be administered treatment if the immune tolerance status of the subject at Baseline indicates weak immune tolerance or absence of immune tolerance.

17. The method of any one of claims 1-16, further comprising (d) comparing the results from the assay of one or more biological samples in step (c) with the results of steps (a) and/or (b) to generate the immune tolerance signature.

18. The method of any one of claims 1 to 17, wherein the immune tolerance signature of a subject is generated using one or more of the following parameters assayed from one or more biological samples obtained from the subject and stimulated ex vivo:

a. proportion of effector T cells in the total T cell population,
b. proportion of Treg cells in the total T cell population,
c. proportion of effector B cells in the total B cell population,
d. levels and/or ratios of specific IgG, IgA, IgM, and/or IgE,
e. levels of inflammatory cytokines and chemokines,
f. levels of anti-inflammatory cytokines and chemokines,
g. levels of inflammatory metabolites, and/or
h. levels of anti-inflammatory metabolites.

19. The method of claim 18, wherein the immune tolerance signature is indicative of maintenance of immune tolerance if 2, 3, 4, 5, 6, 7, or 8 parameters listed in (a)-(h) indicate maintenance of immune tolerance.

20. The method of claim 19, wherein the subject is determined to not require treatment with TIMPs if at least 2/8 parameters listed in (a)-(h) above indicate maintenance of immune tolerance.

21. The method of any one of claims 1-20, wherein the subject is to be re-administered treatment if immune tolerance is weakened and/or lost by about 5%-100% in at least one parameter of immune tolerance.

22. The method of claim 21 wherein the at least one parameter of immune tolerance is selected form the group consisting of:

a. proportion of effector T cells in the total T cell population,

b. proportion of Treg cells in the total T cell population,

c. proportion of effector B cells in the total B cell population,

d. levels and/or ratios of specific IgG, IgA, IgM, and/or IgE,

e. levels of inflammatory cytokines and chemokines,

f. levels of anti-inflammatory cytokines and chemokines,

g. levels of inflammatory metabolites, and/or

h. levels of anti-inflammatory metabolites,

assayed from one or more biological samples obtained from the subject and stimulated *ex vivo*.

23. The method of any one of claims 1-20, wherein the subject is to be re-administered treatment if the immune tolerance signature indicates weakening and/or loss of immune tolerance by about 2-100-fold in at least one parameter of immune tolerance.

24. The method of claim 23 wherein the at least one parameter of immune tolerance is selected from the group consisting of:

a. proportion of effector T cells in the total T cell population,

b. proportion of Treg cells in the total T cell population,

c. proportion of effector B cells in the total B cell population,

d. levels and/or ratios of specific IgG, IgA, IgM, and/or IgE,

e. levels of inflammatory cytokines and chemokines,

f. levels of anti-inflammatory cytokines and chemokines,

g. levels of inflammatory metabolites, and/or

h. levels of anti-inflammatory metabolites,

assayed from one or more biological samples obtained from the subject and stimulated *ex vivo*.

25. Use of TIMPs in the manufacture of a medicament for use in the method of any one of claims 1-24, wherein the tolerizing treatment to be administered is TIMPs.

26. The use of claim 25, wherein the TIMPs are to be administered at a dose level of between about 0.1 to 12 mg/kg, optionally wherein the TIMPs are to be administered at a dose level of about 0.1 mg/kg, 0.25 mg/kg, 0.5 mg/kg, 0.75 mg/kg, 1.0 mg/kg, 1.25 mg/kg, 1.5 mg/kg, 2.0 mg/kg, 2.5 mg/kg, 3 mg/kg, 3.5 mg/kg, 4.0 mg/kg, 4.5 mg/kg, 5 mg/kg, 5.5 mg/kg, 6 mg/kg, 6.5 mg/kg, 7 mg/kg, 7.5 mg/kg, 8.0 mg/kg, 8.5 mg/kg, 9 mg/kg, 9.5 mg/kg, 10 mg/kg, 10.5 mg/kg, 11 mg/kg, 11.5 mg/kg, or 12 mg/kg.

27. The use of claim 25, wherein the TIMPs are to be administered at a dose level of between about 50 mg to 800 mg, optionally wherein the TIMPs are to be administered at a dose

level of about 50 mg, 75 mg, 100 mg, 125 mg, 150 mg, 175 mg, 200 mg, 225 mg, 250 mg, 275 mg, 300 mg, 325 mg, 350 mg, 375 mg, 400 mg, 425 mg, 450 mg, 500 mg, 525 mg, 550 mg, 575 mg, 600 mg, 625 mg, 650 mg, 675 mg, 700 mg, 725 mg, 750 mg, 775 mg, or 800 mg.

28. The use of any one of claims 25-27, wherein the TIMPs are to be administered in a single dose or multiple doses.

29. The use of any one of claims 25-28, wherein the TIMPs are to be administered once weekly, once every two weeks, once every three weeks, once every 4 weeks, once every two months, once every three months, once every 6 months, or once per year.

30. The use of any one of claims 25-29, wherein the TIMPs are to be administered intravenously, subcutaneously, intramuscularly, intraperitoneally, intranasally, or orally.

31. The method of any one of claims 1-24, or the use of any one of claims 25-30, wherein said tolerance status is determined by assay of one or more biological samples after one or more stimuli.

32. The method or use of claim 31, wherein the stimuli are provided ex vivo.

33. The method or use of claim 31 or 32, wherein the one or more stimuli are selected from the group consisting of one or more antigens, allergens, and activating agents.